UNIVERSITY OF OXFORD

COLONISATION AND INFECTION WITH ANTIBIOTIC-RESISTANT ORGANISMS IN PATIENTS IN THE ADULT INTENSIVE CARE UNIT, HOSPITAL FOR TROPICAL DISEASES, HO CHI MINH CITY, VIETNAM

DUONG BICH THUY

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Colonisation and Infection with Antibiotic-Resistant Organisms in Patients in the Adult Intensive Care Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

Duong Bich Thuy

Exeter College

Clinical medicine

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ABSTRACT

In Vietnam, data about colonisation and subsequent infections with antibiotic-resistant organisms (AROs) are limited, particularly from intensive care units (ICUs). A prospective longitudinal study was conducted in Adult ICU, Hospital for Tropical Diseases, Vietnam from 10th November 2014 to 14th January 2016 to characterize colonisation, identify risk factors for colonisation and understand its relationship with infections by *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp..

63.1% (529/838) of patients admitted to ICU were colonised with AROs. 61.3% (223/364) of patients with an ICU stay >48 hours acquired AROs colonisation in ICU. Increased Charlson Comorbidity Index score and receipt of antimicrobial therapy on ICU admission were significant risk factors for AROs acquisition. The proportion of patients with hospital-acquired infections (HAIs) was 23.4% (85/364), and AROs accounted for 41.5% (44/106) of pathogens causing HAIs. Vascular catheterization was identified as a risk factor for ICU-acquired bloodstream infection.

Of 67 *Staphylococcus aureus* isolates identified by conventional microbiology, 6 isolates (9%) were sequenced as *Staphylococcus argenteus*. Multilocus sequence typing (MLST) analysis indicated matching sequence types of infecting and previously colonizing isolates in 90% (9/10) and 94.4% (17/18) patients with HAIs by *Staphylococcus aureus* and *Klebsiella pneumoniae*, respectively. Phylogenetic analysis confirmed that ICU patients became infected with their previously colonizing *Staphylococcus aureus* ST188 (4 patients) or *Klebsiella pneumoniae* ST17, ST23 and ST86 (6 patients). Whole-genome sequencing helped to disprove 3 patient-to-patient transmission events (1 *Staphylococcus aureus* and 2 *Klebsiella pneumoniae*) that were suggested by MLST, and identified one additional *Staphylococcus aureus* transmission which was missed by other methods.

This thesis provides evidence of the high burden of antimicrobial resistance in Vietnam along with high rate of colonisation and infections with AROs. Therefore, the implementation of infection control measures and antimicrobial stewardship programs is urgently needed in Vietnam.

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DECLARATION

I, Duong Bich Thuy can confirm that the majority of work presented in this thesis is my own and was conducted under the supervision of Professor Guy E. Thwaites at OUCRU in Vietnam. Microbiology work was run in the lab of Microbiology Group at OUCRU in Vietnam. *Staphylococcus aureus* and *Klebsiella pneumoniae* sequencing was carried out in collaboration with my colleagues from Enteric Group at OUCRU in Vietnam. The performance of MLST, identification of antimicrobial resistance genes and virulence factors, phylogenetic analysis of *Staphylococcus aureus* and *Klebsiella pneumoniae* sequencing data was conducted with the support of Dr Chung The Hao. The results presented in Chapter 3 and Chapter 4 have been published in the PLOS ONE journal in 2017 and 2018. This thesis have not been submitted for any degree or other qualification elsewhere.

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First author

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Co-author

Vu Dinh Phu, Behzad Nadjm, Nguyen Hoang Anh Duy, Dao Xuan Co, Nguyen Thi Hoang Mai, Dao Tuyet Trinh, James Campbell, Dong Phu Khiem, Tran Ngoc Quang, Huynh Thi Loan, Ha Son Binh, Quynh-Dao Dinh, **Duong Bich Thuy**, Huong Nguyen Phu Lan, Nguyen Hong Ha, Ana Bonell, Mattias Larsson, Hoang Minh Hoan, Đang Quoc Tuan, Hakan Hanberger, Hoang Nguyen Van Minh, Lam Minh Yen, Nguyen Van Hao, Nguyen Gia Binh, Nguyen Van Vinh Chau, Nguyen Van Kinh, Guy E. Thwaites, Heiman F. Wertheim, H. Rogier van Doorn and C. Louise Thwaites (2017). **Ventilator-associated respiratory infection in a resource-restricted setting: impact and etiology.** *Journal of Intensive Care* 5:69. doi: 10.1186/s40560-017-0266-4.

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ABBREVIATIONS

95% CI	95% confidence interval
AFLP	Amplified fragment length polymorphism
AMR	Antimicrobial resistance
ANSORP	Asian Network for Surveillance of Resistant Pathogens
APACHE II	Acute Physiology and Chronic Health Evaluation II
AROs	Antibiotic-resistant organisms
BSI	Bloodstream infection
CAIs	Community-acquired infections
CDC	Center for Disease Control and Prevention
CLSI	Clinical Laboratory Standard Institute
CPO	Carbapenemase-producing organisms
CSF	Cerebral spinal fluid
DIC	Department of Infection Control
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EPIC	European Prevalence of Infection in Intensive Care
ESBL	Extended-spectrum-β lactamases
ETA	Endotracheal aspirate
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GARP	Global Antibiotic Resistance Partnership
GDP	Gross domestic product
HAIs	Hospital-acquired infections
HCAIs	Healthcare-associated infections
HICs	High-income countries

HIV/AIDS	Human immunodeficiency virus / Acquired immune deficiency syndrome
HTD	Hospital for Tropical Diseases
ICC	Infection Control Committee
ICN	Infection Control Network
ICU	Intensive Care Unit
INICC	International Nosocomial Infection Control Consortium
IQR	Interquartile range
KPC	*K. pneumoniae* carbapenemase
LMICs	Low- and middle-income countries
MALDI-TOF MS	Matrix assisted laser absorption ionization-time of flight mass spectrometry
MBL	Metallo-β-lactamase
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NHSN	National Healthcare Safety Network
OUCRU	Oxford University Clinical Research Unit
PCR	Polymerase chain reaction
PBP2a	Penicillin-binding protein 2a
PFGE	Pulsed-field gel electrophoresis
RAPD	Randomly amplified polymorphic DNA
SBP	Spontaneous bacterial peritonitis
SENIC	Study on the Efficacy of Nosocomial Infection Control
SHEA	Society for Healthcare Epidemiology of America
SNP	Single nucleotide polymorphism

SSI	Surgical site infection
SSTI	Skin and soft tissue infection
ST	Sequence type
UK	the United Kingdom
USA	the United States of America
UTI	Urinary tract infection
VAP	Ventilator-associated pneumonia
VRE	Vancomycin-resistant *Enterococcus*
WGS	Whole-genome sequencing
WHO	World Health Organization

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Chapter 1. BACKGROUND

1.1 GENERAL INTRODUCTION ON INFECTIONS IN ICUs

1.1.1 ICU setting

An ICU setting is an organized system providing critical care and life support for critically-ill patients with immunosuppression, major trauma, and other life-threatening conditions (eg. myocardial infarction, strokes, gastrointestinal bleeding and multi-organ dysfunction) [1]. Although ICUs constitute <10% of total beds in most hospitals, over 20% of all HAIs are acquired in ICUs [2,3]. Most ICUs are found in high-income countries (HICs). However, they are increasingly a feature of healthcare systems in low- and middle-income countries (LMICs). Despite an increasing number of ICUs in these areas, the mortality and morbidity of critical illness is disproportionally higher than in HICs. ICU staff in LMICs perceived lack of training, scarcity of nurses and low wages as major barriers to ICU functioning [4]. ICUs in LMICs also face additional challenges due to fewer therapeutic interventions available, deficits in quantity and quality of medical equipment, drugs and disposables, as well as more severe infectious diseases, especially antimicrobial-resistant infections compared to ICUs in HICs [5].

1.1.2 ICU patients

It has been reported that the risk of HAIs in ICU patients is about 3 - 10 times higher than those in general wards [6-8]. There are numerous predisposing factors of HAIs, such as the impaired host defenses of patients because of aging and severe underlying diseases, invasive monitoring and procedures, exposure to multiple antimicrobials, colonisation by AROs, stress ulcer prophylaxis and increasing length of ICU stay [9,10]. In HICs, about 30% of ICU patients are affected by at least one episode of HAIs, whereas the incidence of ICU-acquired infections among adult patients in LMICs ranged from 4.4% up to 88.9% [11].

1.1.3 Definitions of terms

Since the 1970s, bacterial infections in patients of all age-groups have been categorized as to whether they were acquired in the community or hospital [12]. Until 1988, criteria for nosocomial infections and specific types of infection have been defined for surveillance purposes by the Center for Disease Control and Prevention (CDC) in the United States of America (USA) [12]. According to the CDC 1988 guidelines, infections that are acquired in hospital and become evident after hospital discharge, should be categorized as nosocomial infections, and those that are not nosocomial are considered to be community-acquired by default. In the other words, the term "nosocomial infections" applies only to illnesses in hospitalized patients; it excludes patients in nursing homes or rehabilitation centers and those receiving dialysis or chemotherapy in outpatient clinics. 20 years later, the CDC 2008 guidelines were published, which considered the previous term ''nosocomial infections'' less clear definition, and was replaced by the term ''healthcare-associated infections'' (HCAIs) [13]. According to the World Health Organization (WHO) 2002 definition, nosocomial infections, also called "hospital-acquired infections" or HAIs, are defined as infections occurring in a patient during treatment in a hospital or other healthcare facility, which are not present or incubating at the time of admission. This includes infections acquired in hospital, but appearing after discharge, and also occupational infections among staff of the facility [14]. Until 2011, the term HCAIs has been used by WHO to replace "nosocomial" or "hospital" infections, as evidence has shown that this event can affect patients in any settings where they receive care [11]. Thus, HAIs represent a subset of HCAIs.

Mostly, HAIs only appear in patients hospitalized for ≥48 hours, which results in the use of the 48-hour criterion in several epidemiological surveillance systems [15]. The 48-hour cut-off is based on the average time required by a bacterium in a human host to develop from initial infection to detection with a positive diagnostic test. Friedman et al.

have proposed HCAIs as a distinct category defined as "infections occurring in patients at the time of hospital admission or within 48 hours of admission if the patient received specific home care (such as intravenous therapy, wound care or specialized nursing care) or attended a hospital or hemodialysis clinic in the 30 days before the infection, if the patient was hospitalized ≥2 days in the 90 days before infection, or if the patient resided in a nursing home or a long-term care facility" [16]. Community-acquired infections (CAIs) are infections occurring at the time of hospital admission or within 48 hours of admission for patients who did not fulfill any of the above criteria for HCAIs [16]. Consistent with the concepts introduced by Friedman et al., infections are considered as ICU-acquired if they occur in the ICU after 48 hours. Similarly, ICU-acquired infections represent a subset of HAIs.

1.1.4 Pathogenesis of HAIs

Over 90% of reported HAIs are bacterial, whereas viral, fungal or protozoal agents are less common. This thesis deals with bacterial pathogens only, since they are by far the major causes of HAIs. Patients may acquire HAIs from organisms belonging to their own normal flora on skin and mucous membranes (self-infection), or acquiring from other patients, healthcare workers, and the hospital environment (cross-infection) [17].

1.1.4.1 Self-infection (endogenous source of microorganisms)

The normal flora may become invasive after removal of the natural protective barriers against infections in human, following surgery, vascular insertion, endotracheal tubes or urethral catheters. For example, *Escherichia coli* (*E. coli*) and other enteric Gram-negative bacteria are able to colonise other body sites due to impaired natural host barriers and frequently cause surgical site infections (SSI) after abdominal surgery or urinary tract infection (UTI) in patients with indwelling urinary catheters [14]. Some organisms can form tough biofilms around catheters, such as coagulase-negative *Staphylococci*, *Pseudomonas* spp. and *Acinetobacter* spp.. Extraluminal migration of

these organisms is the major route of infection in central line-associated bloodstream infection (BSI). Moreover, treatment with broad-spectrum, long-term or repeated courses of antimicrobials can cause selective pressure on patients' normal flora, which may facilitate a conquest of potentially pathogenic microorganisms originating from the normal flora, or patients become infected with more resistant species coming from the other patients and environment [17,18]. Moreover, as a consequence of severe underlying diseases and/or acute illness at the time of admission with possibly impaired host defenses, and in the presence of risk factors (as described below), critically-ill patients are particularly susceptible to a rapid colonisation by endemic pathogens of the hospital flora, from which HAIs are assumed to arise [19].

1.1.4.2 Cross infection (exogenous source of microorganisms)

The major exogenous source of microorganisms causing HAIs arises from flora from other colonised or infected patients, healthcare workers' contaminated hands, contaminated items/equipment and environment. The most importance route of cross infection from patient to patient, especially for methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) by indirect contact via healthcare workers' hands when hand hygiene is neglected or performed inadequately by healthcare workers, has been documented widely [20,21]. Casewell and Phillips showed that nurses could contaminate their hands with *Klebsiella* spp. during "clean" activities such as lifting patients, taking the patient's pulse, blood pressure or oral temperature [22]. Other studies have shown that healthcare workers can also contaminate their hands with Gram-negative bacteria, *Staphylococcus aureus* (*S. aureus*), *Enterococci*, or *Clostridium difficile* (*C. difficile*) by performing clean procedures or touching intact areas of skin of hospitalized patients [23,24]. Transmission may also occur by direct contact between patients if they are placed in the same ward, and by contact with contaminated surfaces and surroundings. Last but not least, cross infection may occur

via equipment and utensils which have not been decontaminated adequately before being used, and through drugs and blood products [17].

1.1.4.3 Pathogens associated with HAIs

The distribution of bacterial pathogens responsible for HAIs varies over years among different patient populations, healthcare settings and countries. In the early antimicrobial era, HAIs were dominated by *staphylococcal* infections and well controlled by penicillin. Then, as *staphylococci* became β-lactamase producers, β-lactamase stable compounds were used to manage Gram-positive infections. Then MRSA and Gram-negative bacteria emerged as agents responsible for HAIs. In the late 1960s, resistant *Enterobacteriaceae* (*Klebsiella* spp., *Serratia* spp.) became increasingly involved in HAIs. In 1975 - 1980, the emergence of multi-resistant Gram-negative bacteria (*Pseudomonas* spp., *Acinetobacter* spp.) was observed in most European and American surveys, presenting difficult therapeutic problems [25,26]. More recent surveys have indicated the re-emergence of Gram-positive *cocci* including coagulase-positive and -negative *staphylococci* and *streptococci*, whereas *Enterobacteriaceae* tended to decrease for *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*). Additionally, all surveys report the increasing incidence or simultaneous persistence of *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter* spp., and emergence of newer nosocomial Gram-negative organisms such as *Burkholderia cepacia* and *Stenotrophomonas maltophilia* (*S. maltophilia*) [18,27,28]. The role of *Candida* spp., mainly in the form of nosocomial candidaemia, with increasing prevalence of *non-albicans* species called *Candida auris* was underlined in recent surveys with an excess mortality [29].

1.1.5 Epidemiology of HAIs

The rates and types of HAIs, the distribution of pathogens, and the pattern of antimicrobial resistance (AMR) vary across geographic areas among different ICUs and hospitals.

1.1.5.1 HAIs in HICs

HAI surveillance as part of a broad-based prevention and control strategy has received more attention from HICs. In 2010, the European Centre for Disease Prevention and Control (ECDC) reported that 14/28 (50%) European countries participated in the surveillance of HAIs in ICUs, 23 (82.1%) in the pilot point prevalence survey of HAIs and antimicrobial use in acute-care hospitals, and 25 (89.3%) in the first European Union-wide point prevalence survey of HAIs and antimicrobial use in long-term care facilities [30]. In 2012, over 11,000 healthcare facilities in all 50 states of the USA regularly reported data on ICU-acquired infections to the National Healthcare Safety Network (NHSN), established by the CDC [31]. Other HICs, such as Australia, France, Germany and Japan, have coordinated surveillance at national or state level.

According to the European Prevalence of Infection in Intensive Care study (EPIC-I) conducted in 17 Western European countries in 1992, the overall rate of HAIs was 44.8%, and ICU-acquired infections were recorded in 20.6% of patients. Pneumonia (46.9%), UTI (17.6%) and BSI (12%) were the most frequent types of ICU-acquired infections reported. Most frequently isolated organisms were *Enterobacteriaceae* (34.4%), *S. aureus* (30.1% [60% resistant to methicillin]), *P. aeruginosa* (28.7%), coagulase-negative *staphylococci* (19.1%) and fungi (17.1%) [32]. 15 years after the EPIC-I study, EPIC-II was conducted and extended to 75 countries from 7 geographical regions: North America, Central and South America, Western Europe, Eastern Europe, Asia, Oceania and Africa [33]. On the day of the study, 51% of the patients were classified as infected. The lung was the most common site of infection (64%), followed by the

abdomen (20%), the bloodstream (15%) and the renal tract/genitourinary system (14%). Patterns of infecting organisms were similar to those in previous studies, with predominant organisms being *S. aureus*, *Pseudomonas* spp., *Enterobacteriaceae* (mainly *E. coli*) and fungi.

A literature review of national or multicenter studies published from 1995 to 2008 found that the prevalence of hospitalized patients with at least one HAI in developed countries varies between 5.1% and 11.6% (Figure 1.1) with a pooled prevalence of 7.6% [11]. Over 4 million patients affected by HAIs every year in Europe, 1.7 million affected patients in the USA, and approximately 30% of ICU patients suffer from at least one episode of HAI. In the USA and Europe, UTI was the most frequent type of infection hospital-wide (36% and 27%, respectively). In the USA, this was followed by SSI (20%), BSI and pneumonia (both 11%). In Europe, the second most common type of infection was lower respiratory tract infection (24%), followed by SSI (17%) and BSI (10.5%). Moreover, the most frequently reported pathogens in ICU-acquired infections were: *S. aureus* (21.8%); *Enterobacteriaceae* (20.2%); *Pseudomonas* spp. (17.2%); *Enterococci* (10%); *E. coli* (9.1%); *Candida* spp. (8.8%); coagulase-negative *staphylococci* (7%) and *Acinetobacter* spp. (5.1%).

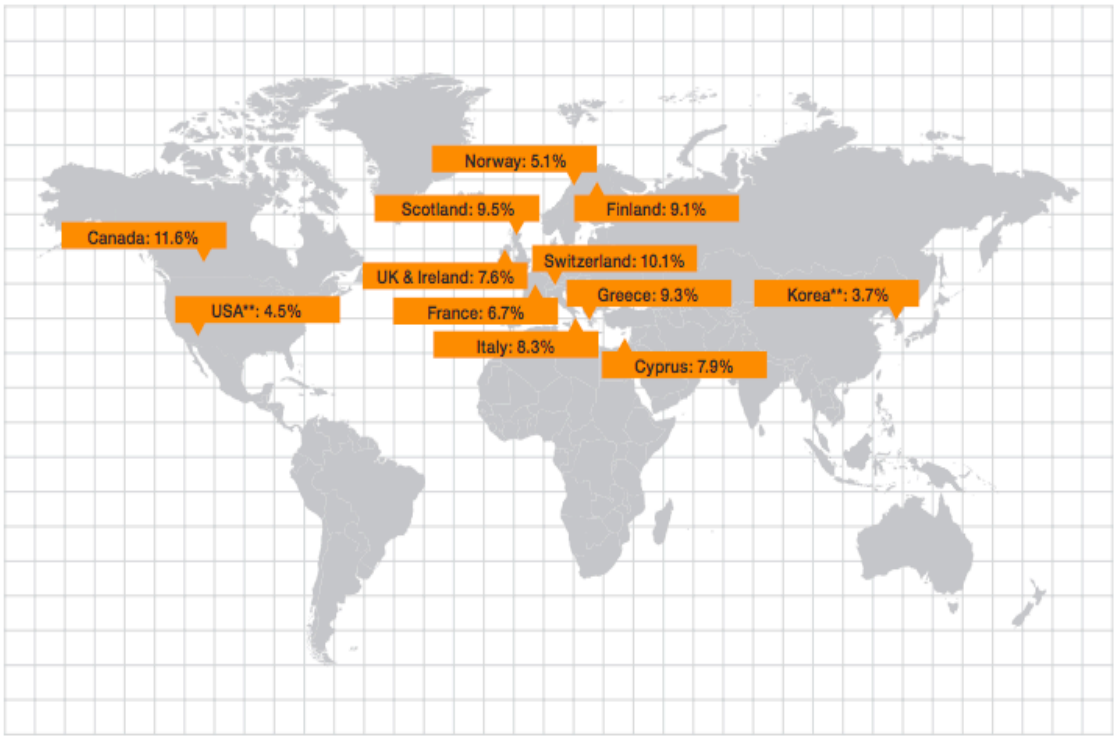


Figure 1.1 Prevalence of HAIs in HICs, 1995 - 2008 [11]

1.1.5.2 HAIs in LMICs

Few HAIs data are available from LMICs. According to a survey conducted by the WHO in 2010, only 23 LMICs (15.6%, 23/147) reported a functioning national surveillance system of HAIs, whereas there was no published data on HAIs endemic burden from 66% (97/147) of LMICs [11]. The lack of reliable estimates from LMICs is mainly because surveillance of HAIs expends time and resources, and needs expertise in study design, data collection, analysis, and interpretation that very few LMICs can afford.

The systematic review and meta-analysis conducted by Allegranzi et al. from 220 selected publications from 1995 to 2008 found that the prevalence of HAIs in LMICs varied from 5.7% to 19.1% (Figure 1.2) with a pooled prevalence of 10.1%, which was much higher than those reported from Europe (7.1%) and the USA (4.5%) [11,34]. Similarly, according to International Nosocomial Infection Control Consortium results (INICC), the rates of ICU-acquired infections in LMICs were 3 - 5 times higher than

those reported from the USA [35]. Moreover, the most frequent type of HAIs was SSI (29.1%), followed by UTI (23.9%), BSI (19.1%), and pneumonia (14.8%); and the rate of device-associated infections was 13 times higher than that of the USA [34]. Regard to HAIs pathogens, Gram-negative bacteria represented the most common nosocomial isolates in both mixed populations and high-risk patients (burn, transplant and ICU patients). *S. aureus* was the most common causative agent of both SSI and BSI, and *Acinetobacter* spp. for ventilator-associated pneumonia (VAP) [34].

What are the important determinants of HAIs in LMICs? With available evidence, this question is difficult to answer, particularly since information on risk factors is scanty (data not shown). However, some potential determinants have been suggested: inadequate environmental hygienic conditions; poor infrastructure; insufficient equipment; understaffing; overcrowding; paucity of knowledge and application of basic infection-control measures; prolonged and inappropriate use of invasive devices and antimicrobials; scarcity of local and national guidelines and policies, low hygiene compliance and reuse of equipment (including needles and gloves) [34,36].

Figure 1.2 Prevalence of HAIs in LMICs, 1995-2008 [11,34]

1.1.6 Risk factors of HAIs

In general, the risk of acquiring a HAI is dependent on intrinsic and extrinsic factors. The intrinsic factors are associated with patient's characteristics, such as age, sex, race or body mass index; and patient's conditions already presenting at the time of admission, like underlying disease, immunosuppression or severity of disease. These endogenous factors are hard to influence by preventive methods. The extrinsic factors involve all measures related to patient treatment during hospitalization, for example: invasive procedures (endotracheal tubes, intravascular catheters or indwelling devices), antimicrobial therapy, length of stay, admission to a general ward or ICU [37]. The other risks of HAIs also include crowding (mixed ICU), animate reservoirs (colonised or infected patients) and infection control practices (hand hygiene compliance) [7,8].

There is a need for global investigation into which factors are frequently presented by patients and the relationship between these factors and HAIs. In the EPIC-I study, seven

risk factors for ICU-acquired infections were identified: increasing length of ICU stay, mechanical ventilation, diagnosis of trauma, central venous or pulmonary artery or urinary catheterization and stress ulcer prophylaxis [32]. However, information about risk factors of HAIs in LMICs is scarce. A recent systematic review and meta-analysis conducted in North America, Europe, Latin America and the Caribbean from 2009 to 2016 has summarized some major risk factors associated with HAIs, inluding diabetes mellitus, immunosuppression, body temperature ≥38.5°C or ≤36°C, surgery time in minutes, reoperation, cephalosporin exposure, days of exposure to central venous catheter, ICU admission, ICU stay in days and mechanical ventilation [37].

1.1.7 Impact of HAIs

It is difficult to quantify impact of HAIs as most studies are observational ones and the risk of HAIs is associated with a vast of intrinsic and extrinsic factors (as described above). However, all studies have come out with the same results: HAIs are associated with morbidity and mortality in hospitals, and carry a high economic burden in both HICs and LMICs. Crude ICU mortality rates associated with ICU-acquired infections vary from 17% to 39%, while the corresponding figures for hospital mortality range from 3% to 18% [38].

In Europe, HAIs cause 16 million extra-days of hospital stay, 37,000 attributable deaths every year, and also annual financial losses of approximately €7 billion [11]. In the USA, approximately 2 million patients suffer from HAIs, and nearly 99,000 attributable deaths in 2002. Of these, approximately 36,000 were due to pneumonia; 31,000 to BSI; 13,000 to UTI; 8,200 to SSI and 11,062 to other infections [39]. The overall direct cost of HAIs to hospital ranges from $28 - 45 billion [40]. Information is again very scanty from LMICs and no data are available at national or regional levels. In India, an ICU-acquired infection was associated with significantly higher treatment cost, longer duration of ICU and hospital stay, but did not impact on hospital mortality [41]. In

Vietnam, ventilator-associated respiratory infection has limited impact on mortality, but associated with significantly increased patient costs, length of stay and antimicrobial use, particularly when caused by carbapenem-resistant bacteria [42].

Moreover, the increased use of drugs, the need for isolation, the use of additional laboratory and other diagnostic studies also contribute to costs. Critically, the widespread use of antimicrobials for treatment has promoted the emergence of multidrug-resistant bacteria which may become endemic in the hospital [14]. HAIs pathogens can be transmitted to the community through discharged patients, medical staff and visitors. If organisms are multi-resistant, they may cause significant disease in the community [14].

1.1.8 Management of HAIs

1.1.8.1 Prevention of HAIs

As previously described, the epidemiology and pathogenesis of HAIs are numerous and complex. However, since the Study on the Efficacy of Nosocomial Infection Control in 1985 (SENIC), it is estimated that at least 30% of HAIs could be prevented [43]. Obviously, most prevention measures are costly, however, in many cases, they are well below the cost of treating patients with HAIs. The interventions should focus on hand hygiene, antimicrobial stewardship and surveillance of HAIs.

The extreme importance of hand washing has been known since 1847, when Dr Ignaz Semmelweis discovered that washing hands before performing obstetric exams on pregnant women reduced childbirth-related infectious mortality from >10% to <1%. However, several studies have documented that adherence of healthcare workers to hand hygiene was poor, with mean rates of 5 - 81% (overall average 40%) [44]. In 2000, a landmark study by Pittet et al. demonstrated that implementing a multidisciplinary program to promote bedside, antiseptic hand-rubs led to increased compliance of

healthcare workers with hand hygiene, coinciding with a reduction of HAIs and MRSA transmission [45]. This finding is also consistent with results of other studies [21,44,46,47].

A meta-analysis evaluating the impact of antimicrobial stewardship showed that interventions intended to decrease excessive prescribing were associated with reduction in colonisation and infection with aminoglycoside- or cephalosporin-resistant Gram-negative bacteria, MRSA and VRE [48]. Other infection control measures should include safe injection practice, use of sterilized equipment and proper surface cleaning. A British retrospective ICU study reported that significantly higher rate of MRSA infections was associated with inadequate surface cleaning [49]. Moreover, selective digestive decontamination is among the few interventions in ICUs that has shown reduction in infection rates in critically-ill patients and improved outcome [50-52]. However, their use is limited to a minority of European ICUs [53].

HAIs are considered an indicator of the safety and quality of patient care [54]. Therefore, the development of a surveillance program to monitor this rate is essential [14]. HAIs surveillance helps to improve awareness of medical staff about HAIs, so they appreciate the need for preventive action. This program is also helpful to monitor trends of HAIs to understand which factors increase a patient's risk of infection, and to identify which local problems and priorities should be focused in future intervention program.

1.1.8.2 Antimicrobials selection guidelines for HAIs

The changing epidemiology of HAIs and emerging resistance mechanisms have resulted in evolving empirical antimicrobial therapy in patients at risk. The choice of empirical antimicrobials for HAIs before bacterial agent is available requires: regular surveillance data on local microbial ecology, surveillance of resistance patterns of prevalent organisms, and identification of HAIs outbreaks. Critically, the early identification of infection site, based on clinical symptoms and initial laboratory tests will help clinical

physicians to make a rapid decision concerning resuscitation measures and antimicrobial coverage of potential nosocomial pathogens.

In general, a conventional combination of a 3rd-generation cephalosporin (ceftriaxone, cefotaxime) plus aminoglycoside (amikacin, gentamycin) offers broad spectrum of antibacterial activity for most Gram-negative infections. The association of an anti-pseudomonal penicillin (ticarcillin, piperacillin) with an aminoglycoside, or an anti-pseudomonal cephalosporin (ceftazidime, cefepime) plus a fluoroquinone (ciprofloxacin) have been for long the initial regimen recommended officially for hospital-acquired *P. aeruginosa* infections. However, in situations suggestive of Gram-positive organisms such as MRSA, the addition of a glycopeptide forms part of empiric therapy. During outbreaks of HAIs with endemic multi-resistant organisms such as *P. aeruginosa* and *A. baumannii*, carbapenems (imipenem, meropenem) in combination with an aminoglycoside or a fluoroquinolone should be recommended [55].

All empirical therapy should be always re-assessed 2 or 3 days after the initiation of therapy. This is an important stage in the follow-up of the treatment, and should be readjusted on the basis of the microbiology (available on day 2 or 3), on the clinical response of patient, on the improved results of white blood cell count, C-reactive protein or procalcitonin (if available), on the potential choice of more suitable combination therapy, or on the potential switch to less expensive and toxic antimicrobials when the clinical status of patient suggests to do so.

1.2 GENERAL INTRODUCTION ON BACTERIAL COLONISATION

1.2.1 Definitions of terms

There are many clinical situations when an organism is isolated but it is not causing disease. A common mistake that doctors may make in their daily practice is giving antimicrobials to treat bacteria that are not actually causing infection. We are living in a sea of microbes where there are approximately 10 times as many bacterial cells in the human flora as there are human cells in the body [56]. Therefore, it is not surprising that an organism might be present in a clinical sample and colonise almost all body surfaces [57]. Distinguishing colonisation from infection is an important factor in making the correct diagnosis and treatment, as well as developing preventive measures.

What is colonisation? Colonisation is the presence of an organism on/in a body surface (the nares, trachea, skin folds, rectum, or in a decubitus ulcer) with growth and multiplication to a sufficiently high concentration of bacteria that can be detected, but does not invade tissues or cause damage or make the patient sick [58]. For better understanding about the chronological development of colonisation, most researchers come up with "initial colonisation" and "acquired colonisation". Initial colonisation, also known as admission colonisation or community-acquired colonisation, is the isolation of organisms on/in a host at the time of admission or within 48 hours of hospitalization [59-63], whereas acquired colonisation (or hospital-acquired colonisation) is the acquisition of microorganisms on/in a host after 48 hours of admission [60,62,63].

1.2.2 Pathogenesis of colonisation

How can bacteria colonise the host, despite the presence of multiple host defense mechanisms? It has been demonstrated that bacterial adherence is a crucial step for establishment of colonisation. Most bacteria can colonise a specific tissue or site because they can adhere to that tissue or site in a lock-and-key type fashion (Figure 1.3). Specific adherence involves biochemical interactions between bacterial surface

components (ligands or adhesins) and host cell molecular receptors (Table 1.1). The bacterial components that provide adhesins are molecular parts of their capsules, cell walls, fimbriae or pilli. The receptors on human cell or tissue surface are usually glycoprotein molecules, and different epithelial surfaces contain different types of receptors. Therefore, each mucosal site of the body has its own unique normal and colonizing flora. However, O'Fallon et al. found that simultaneous colonisation with more than one organism is common, and duration of colonisation is prolonged [64].

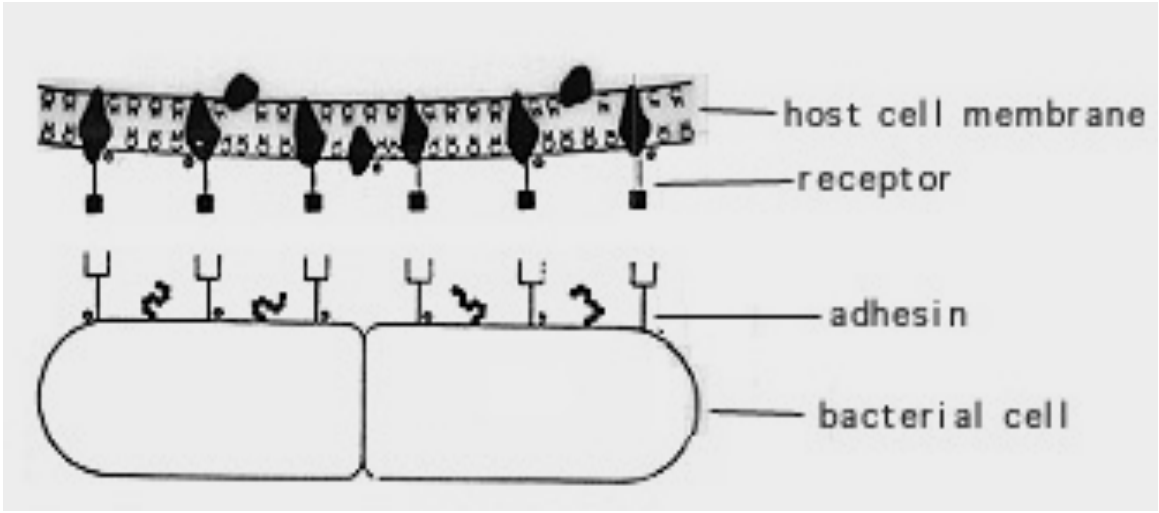


Figure 1.3 Colonisation with a bacterium [65]

Table 1.1 Examples of bacterial specific adherence to host cells or tissue

Bacterium	Bacterial adhesion	Attachment site
S. aureus [66]	Cell wall teichoic acid	Nasal epithelial cells
E. coli [67]	Type-1 fimbriae	Intestinal epithelial cells
Klebsiella spp. [68]	Type-I and type-III fimbriae	Tracheal and pulmonary epithelial cells
Acinetobacter spp. [69]	Fibronectin binding proteins	Tracheal and pulmonary epithelial cells
Pseudomonas spp. [70]	Type-IV fimbriae and flagella	Tracheal and pulmonary epithelial cells

Once firmly attached to host cell surface, some of the bacteria replicate forming micro colonies that eventually mature into biofilms, then secrete carbohydrate slime (exopolymer) that imbed the bacteria and attract other organisms to the biofilms for protection or nutritional advantages [71]. In addition to adhesins, a wide range of other bacterial surface factors with adhesive properties have been described to facilitate the binding of bacteria to host cell surfaces, such as integrins, cadherins, collagen, fibronectin, laminin or elastin [72].

1.2.2.1 Nasal colonisation

Nasal epithelial cells are an important site for colonisation, multiplication, and dissemination of several bacteria, namely *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *P. aeruginosa*. In contrast, *Streptococcus viridans* and *K. pneumoniae* exhibited feeble or no adherence to nasal mucosal cells [73]. *S. aureus* seems to be the predominant colonizing bacteria in the nasal cavity because its primary habitat is moist squamous epithelium of the anterior nares. Adherence of *S. aureus* to nasal epithelial cells is mediated by cell wall teichoic acid, which governs direct interactions with nasal epithelial surfaces [66].

1.2.2.2 Respiratory tract colonisation

The development of respiratory tract colonisation depends on the ability of bacteria to bind successfully to epithelial cells or mucus. A facilitating condition leading to chronic airway colonisation is the impairment of mucociliary clearance which has prolonged mucosal contact with bacteria. Moreover, tracheal cells are likely to bind the organism more avidly than buccal cells can (defining a tropism, or preference of *P. aeruginosa* for the lower respiratory tract). Thus, the patient with chronic bronchitis is commonly colonised by *Haemophillus influenzae* and *Mycoplasma catarrhalis*, whilst the patient with cystic fibrosis or bronchiectasis is commonly colonised by *P. aeruginosa* [74,75].

1.2.2.3 Gastrointestinal colonisation

From a bacterial perspective, successful colonisation of the digestive tract represents a formidable task. First, bacteria must pass through the esophagus, survive the low pH of the stomach, then locate to a suitable niche within the intestine. To establish colonisation, bacteria are required to multiply to a sufficient population density in order not to be swept away by intestinal motility and secretion. During this process, bacteria must incessantly compete with the endogenous flora for nutrients, growth factors, binding sites, etc [76]. Few studies have directly addressed the adherence to intestinal cells or mucus structures as a prerequisite for colonisation, and existing studies are limited to *E. coli* [77]. *E. coli* strains adhere to gastrointestinal mucosa via certain specific adhesins. For intestinal bacteria other than *E. coli*, information on adhesins and their capacity to bind to mucosal structures is at the best scanty.

1.2.2.4 Organisms associated with colonisation

The precise sources of bacteria causing colonisation remain unclear. In general, the three major potential sources of colonizing bacteria are the patient himself (endogenous), other patients and the inanimate environment (exogenous).

The endogenous flora. A human first becomes colonised by a normal flora at the moment of birth. In utero, the fetus is sterile, but when the baby passes through the birth canal, colonisation of the body surfaces occurs. Handling and feeding of the infant after birth leads to establishment of a stable endogenous flora on the skin, oral cavity and intestinal tract similar to that seen in adults. The predominant bacteria on the surfaces of the human body are listed in Table 1.2 [58]. These endogenous bacteria are important for host defense because they provide protection against colonisation by exogenous microorganisms and limit the concentration of endogenous potentially pathogenic microorganisms. This defense mechanism is termed “colonisation resistance” [78]. Antimicrobial agents may influence the endogenous flora by disrupting the ecological

balance of the microbial flora. This may result in colonisation by exogenous pathogenic organisms and the outgrowth of endogenous pathogenic ones. So, pathogenic organisms may be part of the endogenous flora without causing infection. They may cause infection, however, if the resistance against infection is decreased (as in neutropenic patients) or if they reach high numbers (as after antimicrobial therapy). Moreover, antimicrobial use may provide these bacteria with a selective growth advantage, allowing them to colonise other body sites. Schwartz et al. found that colonisation of the oropharynx preceded colonisation of the trachea in 75% of patients [79], while Hélène et al. noted that ESBL-producing *Enterobacteriaceae* digestive colonisation is a predictor of ESBL-producing *Enterobacteriaceae* respiratory colonisation [80].

Cross transmission. During hospital stay, the bacterial microbiota in patients are replaced with the hospital microbiota by means of indirect patient-to-patient transmission through the hands of healthcare workers or, less commonly, by direct transmission from colonised healthcare workers [18]. Casewell and Phillips showed that 10-10^3 *Klebsiella* spp. of the same serotypes as those colonizing patients could be found on the hands of nurses after simple activities like touching patients or taking blood pressure [22]. Moreover, a prospective surveillance study conducted by Antonella et al. suggested that ICU personnel and environment served as reservoirs for cross-transmission of *P. aeruginosa* [81]. Therefore, strict adherence to hand hygiene or gloves during patient contact is mandatory to minimize the transmission of pathogenic organisms from patient to patient.

Table 1.2 Examples for frequent natural reservoirs and sites of colonisation, transmission paths and types of infections

Name of bacteria	Natural reservoirs and sites of colonisation	Transmission paths	Types of infections
S. aureus	Skin, anterior nares, throat, perineum, vagina	Contact, aerosols / droplets (inhalation)	Skin and soft tissue infection (SSTI), endocarditis, sepsis
E. coli	Gastrointestinal tract	Contact	Sepsis, UTI, intra-abdominal infections
Klebsiella spp.	Gastrointestinal and respiratory tract; water storage tanks, surface water, sinks/drainage	Contact, ingestion of contaminated water, bathing, aerosols / droplets (inhalation)	Sepsis, UTI, pneumonia, intra-abdominal infections, SSTI, meningitis/brain abscess
Acietobacter spp.	Skin and mucous membranes; sinks / drainage; soil; warm and humid environments, but also able to survive in a dry environment	Contact, ingestion of contaminated water, aerosols/droplets	Suppurative infections in any organ system, dominating respiratory infections, and soft tissue infections; pancreatic and liver abscesses, sepsis
Pseudomonas spp.	Skin, upper respiratory tract, gastrointestinal tract; moist environments including water (sinks/drainage), soil	Contact, ingestion of contaminated water, aerosols / droplets	Usually do not cause infections in healthy people; infections in any organ system, dominating sepsis, lung infections

The environment. When patients enter a hospital, they arrive with complex and dynamic microbial assemblages that will be shaped not only by the interactions they have with medical staff or other patients, but also by the treatment they receive. Contaminated medical devices have been shown repeatedly to be exogenous sources for colonisation and infections. A review of 2,250 HAIs obtained via contaminated substances reported that the most commonly involved items were disinfection materials (622 patients), heparin solutions (451), red blood cells, clotting factors and other blood products (333), albuterol inhalers (143), total parenteral nutrition (109), propofol (53), rantidine (50) and ultrasound gel (36) [82]. Furthermore, contaminated environmental surfaces with biofilms containing viable AROs, such as a sterile supply box, privacy curtain, venetian blind cord, see-through ward entrance door and rubber from around a sink also remain an important route of colonisation because bacteria within biofilm are more resistant to desiccation, removal by detergents and inactivation by disinfectants [83]. Another study showed a positive correlation between patient colonisation and environmental spread of *A. baumannii* (bedrail, bed sheet, cabinet, monitor, ventilator, feeding pump and infusion pump) [84]. There was also a significant correlation between the isolation of *P. aeruginosa* and *A. baumannii* from tap water faucet aerators and the prevalence of colonisation in ICU patients [85].

With regard to colonisation with AROs, especially MRSA, VRE, and cephalosporin-resistant *Enterobacteriaceae*, the mechanisms are much more complicated. Firstly, these strains may enter the hospital upon the admission or readmission of patients already colonised with resistant organisms. Secondly, susceptible bacteria may develop resistance due to genetic mutations or through the transfer of resistance genes during hospitalization. Thirdly, resistance may emerge through the induction of genes that are already present in susceptible bacterial subpopulations [60].

1.2.3 Epidemiology of colonisation

1.2.3.1 Method of screening

There is currently no consensus method for the active screening of bacteria: moist swabs or sponges, coupled with transport medium or not, which one has a higher sensitivity? Moreover, recommendation about screening sites and bacterial identification method (culture-based phenotypic or genotypic methods) vary a lot between national guidelines.

For *S. aureus* (methicillin-sensitive *S. aureus* (MSSA) and MRSA), the standard method for identifying colonisation is nasal swab. Other swabs from the throat, groin, wounds, skin breaks, catheters, catheter insertion sites, tracheostomy, sputum and urine are also recommended, but are more controversial [86-88]. By using nasal swab culture, almost 80% of MRSA-colonised patients were positive for the organism in the nostrils, but less than 40% of patients who had clinical infection with MRSA had a positive nasal culture at the same time [89]. The addition of a second screening method has been suggested. The sensitivity of MRSA detection from nasal swabs alone were 48% and 62% by culture or by rapid polymerase chain reaction (PCR) test, respectively. These percentages increased to 79% and 92% with the addition of groin swabs, and to 96% and 99% with the addition of groin and throat swabs. This study concludes neither by culture nor by rapid PCR test is nose sampling alone sufficient for MRSA detection. Additional anatomical sites should include at least the groin and throat [90]. However, Baker et al. reported that extra-nasal MRSA colonisation was rare and strongly associated with nasal MRSA colonisation, which suggests that nasal-only screening is not missing a large number of MRSA-colonised patients [88], whereas Bignardi and Lowes claimed that throat swabs identified more cases than nose swabs [91].

As the gastrointestinal tract is an important source of enteric bacteria, rectal swab is a widely used technique for detection of ESBL- or carbapenemase-producing

Enterobacteriaceae within the hospital or ICU. However, weekly rectal swab collections for long periods may cause embarrassment and discomfort to patients. Some studies indicate that perirectal swabs are as sensitive and precise as rectal swabs but with greater patient acceptance [92]. A recent study show that the inguinal region can be considered an alternative for screening multidrug-resistant enteric bacteria because the inguinal swab technique is sensitive and specific for assessing AROs, less embarrassing for patients, and simple to implement in hospital practice [93].

For *Acinetobacter* spp., the screening sites include the forehead, nostrils, buccal mucosa, axilla, antecubital fossa, groin, and toe webs. The sensitivity ranges between 69.6% and 82.6% by using sterile sponges, and 21.7% to 52.2% for swab cultures [94]. The sponge and swab sites with the best sensitivity are the leg and the buccal mucosa, respectively (82.6% and 52.2%). The combined sensitivity for the upper arm and leg with a sponge is 89.1% [94]. In another study, a combination of tracheal aspirate and rectal specimens identified 75% of patients colonised with carbapenem-resistant *A. baumannii*, suggesting the role of these 2 sites as screening sites to detect carbapenem-resistant *A. baumannii* [95].

For *Pseudomonas* spp., a study aimed at evaluating the effectiveness of testing multiple samples (urine, stool, and pharyngeal swabs) for screening multidrug-resistant *P. aeruginosa*. The data suggested that urine culture was the most effective in detecting multidrug-resistant *P. aeruginosa* colonisation, particularly for indwelling patients. However, sample cultures from all 3 sites increased the detection rate of multidrug-resistant *P. aeruginosa*. Therefore, the use of a urine culture in combination with either a stool and/or a pharyngeal swab culture is recommended [96].

1.2.3.2 Active surveillance cultures

Most organisms which colonise on/in a host surface are harmless commensals and are best left alone. However, it is important to remember that most pathogenic organisms set up colonisation first and switch to an invasive mode if they have an opportunity (which will be introduced in latter paragraphs). It is critical to pinpoint exactly when colonisation turns to invasion, but challenging, especially in the era of AMR. Therefore, active surveillance cultures have been considered an important component of control program for AROs colonisation allowing early detection of emerging pathogens, monitoring of epidemiological trends, and verification of the effectiveness of control interventions. However, the efficacy and cost-effectiveness of universal screening remain controversial [97-99]. Numerous different matters must be addressed when considering a screening program: who is to be screened, what method is to be employed, what sites should be sampled, when and how often should the screening be performed, who is going to pay for the screening, and perhaps most importantly, how are screening results to be communicated to healthcare providers and what kind of interventions are best undertaken based on the results?

In December 2010, the English Department of Health introduced mandatory MRSA screening of all elective and emergency admissions to high-risk specialties (vascular, renal/dialysis, neurosurgery, cardiothoracic surgery, hematology/oncology/bone marrow transplant, orthopedics/trauma, and all ICUs). However, there were no randomized controlled trials to provide evidence on the most effective and cost-effective screening strategies [86]. At this time, a study was conducted to estimate the effectiveness and cost-effectiveness of this policy. Overall results show that screening all admissions is unlikely to be cost-effective, so targeted MRSA screening should be modified [100]. In northern European countries (Denmark, Finland and the Netherlands) where MRSA is well-controlled with a low prevalence of 0.5 - 1%, active screening is an integral part of

their approach [92]. In the USA, legislation aimed at controlling MRSA and VRE through the use of active surveillance cultures to screen hospitalized patients has been introduced in at least 2 states. However, the Association of Professionals in Infection Control and Epidemiology (APIC) and the Society for Healthcare Epidemiology of America (SHEA) support the continued development, validation, and application of efficacious and cost-effective strategies for the prevention of infections caused by MRSA, VRE, and other antimicrobial-resistant pathogens rather than the legislation to mandate use of active surveillance cultures to screen for these pathogens [101]. Again, there is a paucity of information on MRSA surveillance system and control policies in LMICs. In a Nigerian teaching hospital, only 52% of healthcare workers were aware of MRSA, and 87.9% reported that there were no MRSA control measure in their respective work stations [102].

For resistant Gram-negative bacteria (such as ESBL or carbapenemase-producing bacteria), there is currently a lack of consensus about the value of screening cultures. Though some studies indicate that universal or admission screening may be cost-effective [103], other evidence suggests that targeted screening has similar sensitivity to universal screening [104] and that it may be an effective strategy when combined with other control measures, particularly in non-critical care settings [105]. In Ontario, Canada, screening, testing and surveillance for AROs (MRSA, resistant *Enterobacteriaceae*) were introduced to all healthcare settings since March 2007 [106]. Healthcare settings are encouraged to work towards these best practices in an effort to improve quality of care, which are also practical for other clinical settings all over the world.

1.2.4 Risk factors of colonisation

A variety of factors, including host factors, medical interventions, and hospital environment, influence the development of bacterial colonisation. Intrinsic or host factors may alter the distribution of colonizing organisms, eg. underlying disease (chronic bronchitis, cystic fibrosis or bronchiectasis). Extrinsic factors include the use of invasive devices or procedures that disrupt the human defence system (eg. intravascular catheters or surgery), and exposures that may facilitate a conquest of pathogenic organisms (eg. antimicrobials, steroids or immunosuppressive therapy) [58]. Those things are especially true for ICU patients, where the invasiveness of treatment, high circulation of AROs and reduced immunity of the patient will predispose them to an increased number of colonisation and infection [18].

It is hoped that increased understanding of bacterial colonisation will lead to new therapeutic strategies to prevent bacterial invasion, especially with AROs. Therefore, studies about risk factors for bacterial colonisation among ICU patients have been conducted in some areas around the world. A study undertaken in North India revealed that co-morbidities, a hospital stay >48 hours, use of >3 groups of antimicrobials and mechanical ventilation >48 hours before transfer to ICU was associated with risk of colonisation with ESBL- and metallo-β-lactamase (MBL)-producing Gram-negative bacteria in patients on ICU admission [61]. Prior hospitalization >48 hours was also identified as a risk factor for colonisation with *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter* spp. and MRSA on admission to a Spanish ICU [59]. The use of antimicrobials and pre-hospital length of stay were significant risk factors for colonisation with VRE, ESBL-producing *E. coli* and *Klebsiella* spp., *Acinetobacter* spp., *P. aeruginosa* and *Candida* species among Turkish patients within 48 hours of ICU admission [2].

1.2.5 Impact of colonisation

Prior bacterial colonisation may increase the risk of subsequent HAIs. This causal relationship has been well-established and will be introduced in detailed in the later part of this chapter. Many studies have also proved that bacterial colonisation, especially with AROs resulted in extended length of ICU and hospital stay, and broad-spectrum antimicrobial use in case of subsequent infections. This will enhance the colonisation pressure, the antimicrobial selection pressure, the risk of cross-transmission and local spread of AROs. Therefore, more interventions should be taken to prevent the transmission of AROs in healthcare settings, such as improved adherence to standard precautions, introduction of contact precautions for colonised and infected patients, and the placement of those patients in isolation areas. Additional measures, including rapid detection of asymptomatically colonised patients, and topical antimicrobial treatments are sometimes mandatory practices. Not surprisingly, these activities are very time, labor and money-consuming, and not all methods are helpful.

Recently, in a cluster-randomized trial conducted in ICU, some authors suggested that surveillance for MRSA and VRE colonisation as well as the expanded use of barrier precautions was not effective in reducing the transmission of these two AROs [98]. Contact isolation of infected or colonised patients with AROs has been associated with reduced frequency of healthcare workers' interventions and interfere with patient monitoring [107,108], increased rate of depression and anxiety symptoms, decreased patient satisfaction with care [107], higher rates of medical errors and preventable adverse events [108]. Errors in anticoagulant prescription, hypoglycemia, hyperglycemia and VAP caused by AROs remain more frequent in isolated patients than non-isolated patients [108].

Additionally, readmission of colonised patients, especially with AROs, is an important factor in the hospital dynamics of AROs. It creates a 'feedback loop' where pathogens are reintroduced into the ward and can colonise or infect new patients. However, little is

known about duration of colonisation with AROs after hospital discharge. Data from a cluster-randomized trial in Europe (2008 - 2011) describes that when all AROs were considered together, the median duration of carriage was 4.8 months; in comparison, the median duration of carriage of MRSA, highly resistant *Enterobacteriaceae* and VRE when considered separately were 0.4, 1.4 and 1.5 months, respectively [109]. Simultaneously, discharge of colonised patients also poses a huge threat to the community where unregulated antimicrobial use and lack of universal precautions will trigger the circulation of AROs among population. Therefore, knowledge about the time until clearance of AROs is of great importance for understanding hospital dynamics and for predicting effects of interventions.

1.2.6 Prevention of colonisation

As previously described, the establishment of colonisation is a multi-factor process. Prevention of colonisation, therefore, relies on a multifaceted approach to infection control measures (as mentioned below).

1.2.6.1 Preventing bacterial adherence

The interaction between adhesins and cell receptors could be prevented theoretically either by soluble receptors that bind competitively to adhesins or, conversely, by soluble adhesins that competitively adhere to cell receptors. In another theoretical approach, anti-adhesin or anti-receptor antibodies may prevent the interaction between adhesins and cell receptors [110]. Finally, adherence could be modified by altering bacterial surface proteins of either adhesins or cell receptors. This may be the mechanism by which aminoglycosides and ceftazidime, when used in sub-inhibitory concentrations, inhibited adherence of *P. aeruginosa* and *E. coli* to tracheal and buccal cells in vitro [111].

1.2.6.2 Modulation of the colonizing bacteria

Modulation of the bacterial flora, as a matter of infection prevention, may be performed at present only by application of antimicrobial agents (applied systemically or topically) or by maintenance of the physiological mucosal environment [110].

Systemic antimicrobials. Systemic antimicrobials have been used to prevent the progression from colonisation to infection more than by modulating colonisation. Leukopenic patients often receive broad-spectrum antibiotics, such as fluoroquinolones, orally for both gut decontamination and systemic prophylaxis. The increasing prevalence of AROs within hospitals justifies a rationale for restrictive use of these agents, and prophylaxis seems to be justified in leukopenic patients only [110].

Topical nasal decontamination. The most favorable results are reported with the use of mupirocin, which is active against staphylococci, including MRSA, streptococci, and some gram-negative bacteria. Nasal mupirocin has been demonstrated to eradicate carriage of *S. aureus* in healthcare workers [112] and to control MRSA outbreaks in veterans' nursing homes, ICUs, and neonatal nurseries [113]. Moreover, nasal mupirocin provides a cost-effective adjunct to routine infection control measures in the containment of MRSA epidemics. However, resistance to mupirocin identified so far is remarkably low, probably because of its unique structure and mode of action. Thus, prolonged and inappropriate use of mupirocin should be avoided to maintain its efficacy for eradicating MRSA.

Selective digestive decontamination. Selective digestive decontamination has been studied extensively in ICU patients, which uses non-absorbable oral and enteric antibiotics and parenteral antibiotics to prevent or eradicate the oropharyngeal and intestinal abnormal carriage of pathogenic organisms, such as *E. coli*, *K. pneumoniae*, *P. aeruginosa* and MRSA. The final parts of selective digestive decontamination consist of optimal hygiene in the ICU to prevent acquisition of hospital pathogens, and the

performance of surveillance cultures of rectal and respiratory tract samples, to monitor decontamination efficacy and the emergence of selective digestive decontamination-resistant pathogens [114]. The major concerns with regard to selective digestive decontamination in ICUs are about the increase in Gram-positive colonisation and infection, selection of AROs, and to date a lack of demonstrable reduction in mortality or duration of hospital stay [110].

Maintaining the physiological mucosal environment. The introduction of modulation of the colonizing bacterial flora by using topical, oral or systemic antimicrobials, is stymied by AMR. Therefore, studies that address the maintenance of the physiological mucosal environment using non-antimicrobial agents are warranted to avoid this pitfall. For example, gastric colonisation rates were less frequent among patients with a median intra-gastric pH <4 [115], in the semi-recumbent position (45-degree angle) [116], without nasogastric tubes [117], with intermittent enteral feeding [118] and with jejunal tube feeding [119].

1.2.6.3 Adherence to standard precautions

This is a cornerstone in all strategies to minimize and eliminate colonisation in hospitalized patients. Many studies have demonstrated the spread of bacteria from patient to patient via hands of healthcare workers, and the incidence of cross-colonisation among patients has been reduced by enforcing hand washing between patient contacts [21]. Additionally, non-sterile gloves and gowns can be used to protect medical staff from contamination with potentially infectious secretions, excretions and antimicrobial-resistant skin flora from patients [98]. Sterile gloves should be used by healthcare workers to protect patients against acquiring colonisation during invasive procedures, such as suctioning intubated patients or touching open wounds [44,47]. Contact isolation measures for limiting the spread of AROs should be designed according to the individual risk and collective benefit to ensure the benefits outweigh the risks.

1.3 RELATIONSHIP BETWEEN COLONISATION AND INFECTIONS

It has been suggested that infection represents merely the tip of an iceberg, and that colonisation reflects the submerged part. The causal relationship between bacterial colonisation and subsequent infections has been well studied all over the world, especially in HICs. *S. aureus* nasal carriage has been identified as a risk factor for the development of HAIs in general hospital populations [120], orthopaedic surgical patients [121], thoracic surgical patients [122], patients on haemodialysis [123] or peritoneal dialysis [124], and patients with liver cirrhosis [125]. For ICU patients, nasal colonisation with *S. aureus* was significantly associated with ICU-acquired *S. aureus* infection including SSI, BSI, pneumonia and UTI [126-129]. Recently, molecular methodology has been used widely to investigate the staphylococcal outbreaks and transmission events in clinical settings [130]. Whole-genome sequencing (WGS) was employed successfully to confirm the transmission of MRSA and MSSA among patients during wound care in a healthcare center in eastern Ghana [131], or to "rule in" the transmission of MRSA via deceased donor liver transplantation in the USA when the donor and recipient MRSA isolates are genetically 100% identical [132]. WGS is also an ideal method to "rule out" a suspected outbreak of MRSA-related SSI in a rural hospital in the USA where the MRSA strain from a colonised surgical team member was identified not to be the source of the SSI cases and no evidence of transmission occurred among the patients with SSI [133]. Additionally, in a multicenter study of *S. aureus* bacteremia conducted in Germany, the blood isolates were clonally identical to those obtained from nasal swabs in 82.2% of patients (180/219) [134]. In another multicenter study conducted in the USA, colonizing MRSA isolates belonged to the same clonal types as the strains causing SSTI in most cases (73.7%) [135]. In Korea, concordance rates by methicillin susceptibility and sequence type (ST) between colonizing and clinical *S. aureus* isolates obtained from children were 90.3% and 84%, respectively among the 31 pairs of healthcare-associated

S. aureus, and 100% concordance was observed by methicillin susceptibility and ST for 6 pairs of community-associated *S. aureus* [136]. Similarly, rectal carriage of multidrug-resistant *Enterobacteriaceae* is a major reservoir of subsequent bloodstream, lung, urinary tract, and central venous catheter infection in ICU [137,138]. The 2013 *Klebsiella* Acquisition Surveillance Project at Alfred Health (Australia) confirmed that *K. pneumoniae* colonisation is a significant risk factor for infection in ICU, and genome comparison indicated matching carriage and infection isolates in 80% (12/15) of isolate pairs [139]. A 2016 study at the University of Michigan Health System (the USA) reported a significant association between rectal *K. pneumoniae* colonisation and subsequent infections (pneumonia, UTI and BSI). Moreover, there was high concordance among colonizing and infecting isolates, particularly for pneumonia and UTI, as measured by genome analyses [140]. Many studies about the associations between colonisation and infection with non- *Enterobacteriaceae* bacteria have been conducted, for example *P. aeruginosa* and *A. baumannii*. A study undertaken in Spain found that among 77 ICU patients who developed *P. aeruginosa* pneumonia, 69 (89.6%) had prior *P. aeruginosa* rectal colonisation, and 60 (87%) of these paired rectal and clinical isolates exhibited genotyping concordance [141]. Another study of 189 consecutive ICU patients in Spain found that 20 (10.6%) patients were colonised with multidrug-resistant *A. baumannii* upon ICU admission, and 57 (30.2%) additional patients acquired colonisation, mostly during the first week of ICU admission. Rectal colonisation was associated with increased ICU-acquired infections with multidrug-resistant *A. baumannii*, such as respiratory tract infections, BSI, SSI, peritonitis, osteoarthritis and meningitis [142]. Understanding how these colonizing organisms become invasive and infected will contribute to the epidemiology and pathogenesis of colonisation, which is necessary to successfully combat subsequent infections.

1.4 MICROBIOLOGICAL DIAGNOSIS

1.4.1 Bacterial identification using biochemical tests

The commonly used biochemical tests are mentioned below (Table 1.3).

Table 1.3 Common biochemical tests for bacterial identification [143]

Name of test	Purpose	Common application
Catalase	Detect the catalase enzyme that releases oxygen from H_2O_2	Differentiate between *staphylococci* (catalase positive) and *streptococc*i (catalase negative)
Coagulase	Detect the coagulase enzyme that converts fibrinogen (soluble) to fibrin (insoluble)	Differentiate between *S. aureus* (coagulase positive) and other *staphylococci* (coagulase negative)
Oxidase	Detect the cytochrome oxidase enzyme that converts a colourless reagent to a dark purple product	Differentiate between *Pseudomonas* spp. (oxidase positive) and other Gram-negative bacteria (*E. coli*, *Klebsiella* spp.: oxidase negative)
Indole	Detect the ability to degrade the amino acid tryptophan and produce indole	Distinguish *Enterobacteriaceae* spp. (*E. coli*: indole positive; *Klebsiella* spp.: indole negative)
Citrate	Detect the ability to use citrate as carbon and energy source, which is demonstrated by the colour change of a pH indicator	Distinguish *Enterobacteriaceae* spp. (*E. coli*: citrate negative; *Klebsiella* spp.: citrate positive)
Urease	Detect the ability to hydrolyse urea and produce ammonia and carbon dioxide	Distinguish *Enterobacteriaceae* spp. (*E. coli*: urease negative; *Klebsiella* spp.: urease positive)

1.4.2 Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS has emerged as an advance tool for accurate microbial identification and diagnosis. The principle of MALDI-TOF MS is that bacterial cells are ionized into charged molecules, then ratio of their mass-to-charge is measured and analysed by a mass spectrometer. Every bacterial genus/species has a distinctive protein spectra which can be compared with a database software so that nearest organism can be identified [144–146]. The process is rapid, sensitive and economical in terms of both labor and costs involved. The limitation of the technology is that MALDI-TOF MS is unable to give identification if the bacterial spectrum is not yet in the database and cannot identify between very closely related organisms such as *E. coli* and *Shigella.*

1.4.3 Genotypic bacterial typing methods

1.4.3.1 Genotyping

Genotyping is the process of determining differences in the genotype of an individual by examining the individual's deoxyribonucleic acid (DNA) sequence using biological assays and comparing it to another individual's sequence or a reference sequence. Genotyping allows for the identification of bacterial isolates to the strain level and provides basic information about the evolutionary biology, population biology, taxonomy, ecology, and genetics of bacteria. The main purpose of genotyping is epidemiological surveillance [147].

There are several methods for bacterial genotyping, but pulsed-field gel electrophoresis (PFGE) and MLST are the most commonly used [147]. PFGE is a laboratory technique that allows different sizes of DNA fragments from 50kb to 10Mb to be separated by using electrophoresis. Although PFGE used to be a prevalent typing method for investigation of bacterial pathogen and outbreaks in many public health laboratories [148,149], it has been superseded by better techniques (eg. WGS). MLST is a genetic

method with a high resolution power based on sequencing fragments of 7 housekeeping genes. MLST detects variations at each of the seven loci and each allele is compared with the known alleles included in the MLST database (www.mlst.net/databases/) and is assigned the allele number. For each isolate, the allele number at each of the seven loci defined the allelic profile which corresponded to its ST [150]. Due to its high value in epidemiology surveillance, MLST has been applied to study several bacterial epidemics such as meningococcal disease, outbreaks of typhoid, MRSA, and multidrug-resistant *P. aeruginosa.*

1.4.3.2 Whole genome sequencing

WGS is the process of determining the complete DNA sequence of an organism's genome at a single time. More recently, the invention of high-throughput 'next-generation' sequencing technology, with relatively simple bench-top technology and efficient library preparation protocols, has significantly improved the capacity to perform low-cost, efficient WGS, and has made it a powerful tool for clinical microbiology. Currently, there are four main potential applications of WGS for bacterial pathogen characterization in the diagnostic microbiology laboratory: identification, typing, resistance detection and virulence gene detection. Moreover, the emergence of WGS has unveiled its potential as a powerful tool for epidemiological surveillance and outbreak investigation to inform infection control procedures [151]. For example, WGS allows *S. aureus* isolates to be compared with each other and with reference sequences across time and space, down to a resolution of a single nucleotide polymorphism (SNP) difference [152]. When two isolates are indistinguishable it does not mean that they are identical, WGS makes it possible to determine whether sequences are really identical, if not, to state exactly by how much they differ. Furthermore, a pangenome-wide association study was performed to look for associations between gene repertoire and disease potential/outcome and to identify distinct sets of accessory genes associated

with virulence traits of *K. pneumoniae* in humans worldwide [153]. This enhances our knowledge about the molecular characteristics of some organisms of interest like *S. aureus* and *K. pneumoniae*, the evolutionary history of lineages, and the potential for an outbreak investigator by determining unambiguously the relatedness of isolates. Combined with epidemiological data (eg. patient-stay data), it is then possible to draw inferences about the probability that a transmission event occurred or not to direct better targeting of infection control resources [130,154,155]. These properties give WGS the potential to replace conventional typing methods, and to enhance infection control practice on local, national and international scales.

1.4.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing is a laboratory method to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates, which is vital for patient care. Antimicrobial susceptibility testing was interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines 2015 [156].

1.4.5 Common resistant mechanism

1.4.5.1 Extended-spectrum-β lactamase (ESBL)

ESBL is an enzyme capable of conferring bacterial resistance to penicillins, 1st-, 2nd- and 3rd-generation cephalosporins, aztreonam (but not cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic acid [157]. ESBL is frequently plasmid encoded which carry genes encoding resistance to other drugs (eg. aminoglycosides). The CLSI has proposed disk diffusion methods and dilution antimicrobial susceptibility tests for screening ESBL production.

1.4.5.2 AmpC β-lactamase

AmpC β-lactamase is an enzyme that mediates resistance to cephalothin, cefazolin, cefoxitin, most penicillins and β-lactamase inhibitor- β-lactam combinations. In many

bacteria, AmpC is inducible and can be expressed at high levels. Over-expression confers resistance to broad-spectrum cephalosporins (cefotaxime, ceftazidime and ceftriaxone). AmpC is encoded by both chromosomal and plasmid genes are also evolving to hydrolyze broad-spectrum cephalosporins more efficiently. Techniques to identify AmpC-producing isolates are available but are still evolving, and are not yet optimized for the clinical laboratory, which probably now underestimates this resistance mechanism [158].

1.4.5.3 Carbapenem-resistant *Enterobacteriaceae*

Carbapenem-resistant *Enterobacteriaceae* or carbapenemase-producing *Enterobacteriaceae* are Gram-negative bacteria that are resistant to the carbapenem class of antimicrobials, a drug of last resort for such infections. They are resistant by producing carbapenemases (β-lactamases capable of hydrolyzing carbapenems). There are many types of carbapenemase enzymes, such as *K. pneumoniae* carbapenemase (KPC), OXA enzymes, the MBLs group (comprised of IMP, VIM and NDM enzymes). However, there is no perfect test to detect all enzyme types [159].

1.4.5.4 Methicillin-resistant *Staphylococcus aureus* (MRSA)

Virtually all MRSA produce an additional penicillin-binding protein 2a (PBP2a) which confers resistance to oxacillin, methicillin, and all currently available β-lactam agents, including cephalosporins and carbapenems. PBP2a is encoded by the *mecA* gene [160]. Due to the high percentage of infections associated with MRSA, the CLSI proposed the routine testing of MRSA from all *S. aureus* isolates [161]. The detection methods for MRSA include the cefoxitin disk screen test, oxacillin disk screen test, latex agglutination test for PBP2a and PCR for the *mecA* gene [162,163].

1.5 ANTIMICROBIAL RESISTANCE: AN INCREASING GLOBAL HEALTH PROBLEM

Prior to the 1990s, AMR was never considered a threat to the management of infectious diseases. Until gradually treatment failures were increasingly being seen in healthcare settings against first-line drugs and second-line drugs or more, the threat of AMR is growing at an alarming pace and knows no boundaries [164]. AMR is the ability of a microorganism to resist the action of one or more antimicrobial agents [165]. Development of AMR is a natural phenomenon caused by mutations in bacterial genes, or acquisition of exogenous resistance genes that can spread horizontally between bacteria. A bacterium can acquire several different resistance mechanisms and therefore be resistant to several antimicrobial agents, which may severely limit the available treatment alternatives for the infection. The major drivers behind the occurrence and spread of AMR are: suboptimal use of antimicrobials for prophylaxis and treatment of infection; non-compliance with infection control practices; prolonged ICU and hospital stays; multiple comorbidities in hospitalized patients; increased use of invasive medical devices, transfer of colonised patients from hospital to hospital; grouping of colonised patients in long-term-care facilities; antibiotic use in agriculture and household chores; and increasing national and international travel. However, it is now accepted that antimicrobial use is the single most important factor responsible for increased AMR [164-166].

It is estimated that by 2050, 10 million lives and a cumulative $100 trillion are at risk due to resistant infections if no solutions are applied now to slow down the rise of AMR [166]. In general, there are four core actions that will help fight these deadly infections: preventing infections, increasing surveillance, improving antimicrobial stewardship, and developing new drugs and diagnostic tests. Among all of the bacterial resistance problems, Gram-negative pathogens are particularly worrisome, because they are

becoming resistant to nearly all drugs that would be considered for treatment. This is true as well, but not to the same extent, for some of the Gram-positive infections (*Staphylococcus* and *Enterococcus*). The most serious Gram-negative infections are healthcare-associated, and the most common pathogens are *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp..

1.5.1 Current status of AMR in HICs

In Europe, antimicrobial-resistant infections were responsible for about 25,000 deaths and at least €1.5 billion for medical costs and productivity losses annually [165]. Over the last four years (2012 - 2015), important resistance trends for Gram-negative bacteria are recognizable. The resistance to 3rd-generation cephalosporins in *E. coli* and *K. pneumoniae* increased significantly in combination with ESBL production. This has lead to an increased use of carbapenems, as a result further favouring the emergence and spread of carbapenem-resistant bacteria. Carbapenem resistance in *K. pneumoniae* is about 0 - 61.9% between countries. Significant increases in carbapenem resistance and multidrug resistance are also reported in *P. aeruginosa* and *Acinetobacter* spp. More seriously, *K. pneumoniae* with polymyxin resistance has been detected in some European countries [165,167].

In the USA, over 2 million people acquire antimicrobial-resistant infections annually and at least 23,000 people die each year as a direct result [167]. The total economic cost of AMR is as high as $20 billion in excess direct medical costs, with additional costs to society for lost productivity as high as $35 billion a year [167]. Both carbapenem and multidrug resistance were reported in >60% of *Acinetobacter* spp. among most HAIs types [168]. The most significant overall increase in carbapenem resistance was observed for *Klebsiella* spp. (from 1.6% to 10.4%) [169].

In Canada, annually over 18,000 hospitalized patients acquire antimicrobial-resistant infections and the loss to gross domestic product (GDP) ranges from 0.4% to 1.6%.

There has been an 8-fold increase in the rate of MRSA infections among hospitalized patients in 1995 - 2012. In 2012, 30% of MRSA infections identified in hospitalized patients were acquired in the community, compared to 10% in 1995 [170]. The overall rate of carbapenem-resistant *Enterobacteriaceae* infections in Canada decreased from 0.013 cases to 0.007 cases per 10,000 patient-day from 2011 to 2014, and slightly increased to 0.008 cases per 10,000 patient-day in 2015 [171].

1.5.2 Current status of AMR in LMICs

From the early stages of AMR, the problem was identified more severely in LMICs compared to the HICs. The causes of AMR in LMICs are complex and may be rooted in practices of healthcare professionals and patients' behavior towards the use of antimicrobials, as well as supply chains of antimicrobials in the population. Some of these factors may include inappropriate prescription practices, inadequate patient education, limited diagnostic facilities, unauthorized sale of antimicrobials, lack of appropriate functioning drug regulatory mechanisms, and non-human use of antimicrobials such as in animal production (Figure 1.4) [164].

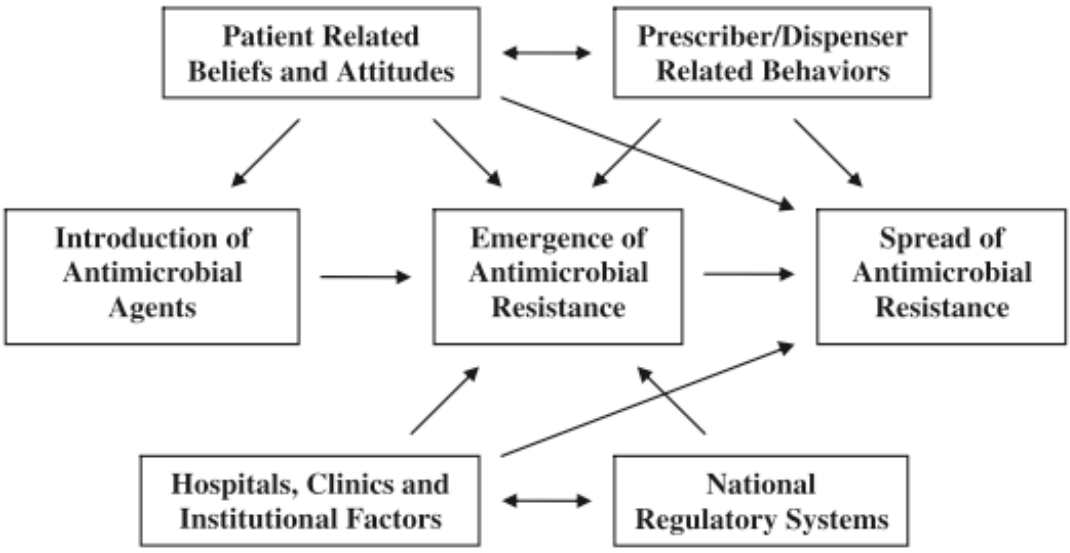


Figure 1.4 Determinants of antimicrobial resistance [164]

A surveillance study conducted by the Asian Network for Surveillance of Resistant Pathogens (ANSORP, 2004 - 2006) confirmed that MRSA infections have been increasing in Asian countries: MRSA accounted for 25.5% of community-acquired *S. aureus* infections and 67.4% of HAIs [172]. The percentage of MRSA among hospital-acquired *S. aureus* infections was relatively low in India (22.6%) and the Philippines (38.1%) due to the data collection from only one ANSORP hospital in each country, whereas Sri Lanka (86.5%), Korea (77.6%) and Vietnam (74.1%) showed very high rates of MRSA. In terms of community-acquired *S. aureus* infections, the proportion of MRSA varied by country: Sri Lanka (38.8%); Taiwan (34.8%); the Philippines (30.1%); Vietnam (30.1%); Korea (15.6%); Hong Kong (8.5%); India (4.3%) and Thailand (2.5%). Moreover, ESBL-producing *Enterobacteriaceae* are being found with increasing frequency. The ESBL rates in India for *E. coli*, *K. pneumoniae* and *K. oxytoca* were 79%, 69.4% and 100%, respectively [173]. In Taiwan, the prevalences of *Enterobacteriaceae* isolates with ESBL were 26% in *K. pneumoniae* and 14% in *E. coli*. In the Asia-Pacific region, 29.8% of *P. aeruginosa* and 73% of *A. baumannii* isolates were not susceptible to at least one carbapenem, whereas the majority of *Enterobacteriaceae* (97.2%) were susceptible to all carbapenems [174].

1.6 WHAT DO WE KNOW AND DO NOT KNOW ABOUT HAIs, COLONISATION AND AMR IN VIETNAM

1.6.1 HAIs in Vietnam

There is little information on infection control, prevention, and surveillance in Vietnam**.** The Vietnamese Ministry of Health has actively worked to develop a national infection control program with its first infection control regulation in 1997. It designated responsibilities to all hospitals throughout the country for establishment of Infection Control Committee (ICC), Department of Infection Control (DIC), and Infection Control Network [175]. By 2002, 56% (184 hospitals) and 41% (135 hospitals) had established ICCs and DICs, respectively. By 2005, around 93% of hospitals had established ICCs and 98% DICs. Over the past 20 years since the establishment of Vietnamese national infection control program, however, there have been no national estimates of the rates and burden of HAIs, and data about HAIs in ICUs are sparse in Vietnam. The reason is that firstly, Vietnamese infection control system has struggled with the different levels of professional knowledge and staff skills that have made infection control activities difficult. Secondly, the scale of HAIs between Vietnamese hospitals is largely not quantified. Thirdly, the infrastructure for basic infection control is minimal and the application of universal precautions has been limited in patient care areas and laboratories due to a shortage of resources. Moreover, increased workload, severe overcrowding, and lack of finance are big barriers to training and research activities about HAIs.

The few small studies performed only some include ICUs, but have reported HAIs prevalence ranging from 5.2% to 29.5% [175-178]. A recent point prevalence survey conducted in 15 adult ICUs across Vietnam (2012 - 2013) observed a wide range of HAI prevalence from 5.6% to 60.9% between ICUs. Pneumonia was the most common HAI (79.4%), followed by BSI (4.4%) and SSI (4.2%). In multivariate analysis,

significant risk factors for ICU-acquired infections were endotracheal intubation, urinary catheter, no involvement of a family member in patient care and surgery after admission. Gram-negative bacteria were the most frequently pathogens cultured, namely *A. baumannii* (24.4%), *P. aeruginosa* (13.8%) and *K. pneumoniae* (11.6%), with high carbapenem resistance rates of 89.2%, 55.7% and 14.9%, respectively [178].

1.6.2 Colonisation in Vietnam

Little is known about bacterial colonisation in Vietnam. There are several reasons for this, but include a lack of routine screening which is rarely available or affordable. A study conducted in a tetanus ICU in Ho Chi Minh City from 2004 to 2006 reported average daily prevalence rates for MRSA, ESBL-producing *Enterobacteriaceae*, *P. aeruginosa*, gentamicin-resistant *K. pneumoniae*, and amikacin-resistant *Acinetobacter* species of 34%, 61.3%, 53.4%, 65.7% and 57.1%, respectively. The combination of simple infection control measures and antimicrobial mixing was highly effective in reducing the prevalence of MRSA by 69.8% (to 10.3%), but not of Gram-negative bacteria [179].

In the community, *S. aureus* nasopharyngeal colonisation is present in about one-third (303/1,016, with approximately 25% MRSA) of participants in Dong Da (urban) and Ba Vi (rural) districts of Hanoi. Pharyngeal carriage is more common than nasal carriage. Risk factors for *S. aureus* (including MRSA) colonisation are younger age, living in an urban area, and antimicrobial use [180]. The prevalence of ESBL-producing *E. coli* in healthy people was 46.2% [181]. The colonisation rate of *K. pneumoniae* was 14.1%, which was found to be independently associated with old age, smoking, rural living location and moderate to heavy weekly alcohol consumption [182].

1.6.3 AMR in Vietnam

In Vietnam, a national resistance surveillance program existed until 2005, but was stopped due to lack of donor funding [183]. The Global Antibiotic Resistance Partnership

(GARP) Vietnam and Oxford University Clinical Research Unit (OUCRU) collaborated with the Vietnamese Ministry of Health to set up a new surveillance program for both antimicrobial consumption and resistance since 2009 [184]. A community-based study undertaken in Vietnam, in 2007, found that 78% of antimicrobials were purchased in retail pharmacies without prescriptions and healthcare providers often dispensed antimicrobials unnecessarily for viral diseases such as the common cold and cough [184]. Approximately one-third of hospitalized Vietnamese patients (1,573/5,104) received inappropriate antimicrobials [185]. The significantly positive correlations were established between cephalosporin consumption and cefuroxime-resistant *E. coli*, and carbapenem use and ceftazidime-resistant *Acinetobacter* spp [183]. To sum up, the lack of knowledge about appropriate antimicrobial use has contributed to the appearance and spread of AMR in Vietnam which threatens the management of individual patients and healthcare policy generally [164].

In Vietnam, AMR was common in Gram-negative bacteria, including: *E. coli*, *Klebsiella* spp., *Acinetobacter* spp. and *Pseudomonas* spp.. Data from the 15 participating hospitals across Vietnam showed about 30 - 70% of the Gram-negative bacteria are resistant to 3rd and 4th-generation cephalosporins, approximately 40 - 60% to aminoglycosides and fluoroquinolones. Up to 40% of *Acinetobacter* spp. showed decreased susceptibility to imipenem [183]. A point prevalence survey conducted in 15 adult ICUs across Vietnam (2012 - 2013) revealed that the most common pathogens were *A. baumannii* (24.4%), *P. aeruginosa* (13.8%) and *K. pneumoniae* (11.6%) with carbapenem resistance rates of 89.2%, 55.7% and 14.9%, respectively [178]. Additionally, a prospective observational study in 4 ICUs (2013 - 2015) demonstrated that most ventilator-associated respiratory infection was caused by Gram-negative bacteria: *A. baumannii* (43.8%), *K. pneumoniae* (35.6%) and *P. aeruginosa* (32.9%); and 58.8% of patients with positive cultures for these had carbapenem-resistant isolates [42]. The 2010

Comparative Activity of Carbapenem Testing (COMPACT) II study also highlighted the prevalence of carbapenem resistance of 5.6% for *Enterobacteriaceae* spp., 46.7% for *P. aeruginosa* and 89.5% for *A. baumannii* in Vietnam. Notably, the overall rate of carbapenem-resistant Gram-negative bacteria in Vietnam was 35% which was higher than prevalence of carbapenem resistance reported in New Zealand (11.7%), the Philippines (18.9%), Singapore (22.1%) and Thailand (22.2%) [174]. However, resistance rate of *S. aureus* varied among Vietnamese hospitals. In 2009, up to 63.8% of *S. aureus* isolated from Hue hospital located in central Vietnam were resistant to oxacillin, whereas the rate was about 30% in Bach Mai hospital (northern), and approximately 40% in Hospital for Tropical Diseases (HTD, southern) [183]. The Asian Network for Surveillance of Resistant Pathogens (ANSORP) study also showed that in Vietnam, MRSA accounted for 74.1% of hospital-acquired *S. aureus* infections, and 30.1% of community-acquired *S. aureus* infections during 2004 - 2006 (data obtained from only one participating hospital) [186]. For *S. aureus* bacteremia in adults, the proportion of MRSA bacteremia was 19% in 2010 [187].

At the HTD, according to a recent microbiological report conducted from January 2015 to January 2018 (unpublished data), the most frequently isolated pathogens in Adult ICU were *S. aureus*, *E. coli, Klebsiella* spp.*, Pseudomonas* spp. and *Acinetobacter* spp.. Common bacteria involved in *hospital-acquired pneumonia* included *P. aeruginosa* (28%), *Acinetobacter* spp. (16.8%) and *K. pneumonia* (12%), with high carbapenem resistance rate of 57%, 72% and 16% respectively. For BSI, the two most common pathogens were *E. coli* (19%, of which were 70% ESBL) and *S. aureus* (12%, of which were 57% MRSA). For UTI, *E. coli* was most prevalent (36%) and 63% of *E. coli* isolates were ESBL-producing organisms.

1.7 HYPOTHESIS AND AIMS OF THE THESIS

Colonisation and its relationship to subsequent infections in ICUs are important problems around the world. Data suggest that they may be even more serious in LMICs due to the appearance and spread of AROs. However, there are sparse and poor quality data regarding the epidemiology, management and prevention of colonisation and HAIs in Vietnam. Since 2009, the antimicrobial use and resistance surveillance program lead by the Vietnamese Ministry of Health has paid more attention to *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *Acinetobacter* spp. and *S. aureus* because they are key common nosocomial pathogens in all Vietnamese hospitals. A better understanding of the components of colonisation and HAIs is vital for targeting appropriately methods to reduce the burden of these problems and to combat the threat of AMR in Vietnam.

Therefore, this thesis "Colonisation and infection with antibiotic-resistant organisms in patients in the Adult ICU, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam" aims at improving human knowledge about the epidemiology, management and prevention of colonisation and HAIs, as well as providing high quality data to inform future policy in Vietnam and potentially other low-resource settings.

1.7.1 Hypotheses

- A high proportion of Vietnamese adult patients are colonised or infected with AROs, namely MRSA, 3rd-generation cephalosporin-resistant *E. coli*, 3rd-generation cephalosporin-resistant *Klebsiella* spp., ceftazidime-resistant *Pseudomonas* spp., carbapenem-resistant *E. coli*, carbapenem-resistant *Klebsiella* spp., carbapenem-resistant *Acinetobacter* spp. and carbapenem-resistant *Pseudomonas* spp. on admission to ICU.
- A high proportion of Vietnamese adult patients acquire colonisation and infections with the above-mentioned AROs during ICU stay.
- Colonisation is associated with infections by the same organism.

- There are multiple clinical genotypes of colonizing and infecting *S. aureus* or *K. pneumoniae* isolates co-circulating in ICU. For CAIs or HCAIs, colonizing and infecting *S. aureus* or *K. pneumoniae* strains obtained from the same ICU patients genetically clustered. In case of HAIs, ICU patients became infected with their previously colonizing *S. aureus* or *K. pneumoniae* isolates.

1.7.2 Aims of the study

The following research questions are addressed:

- Question 1: What are the characteristics of bacterial colonisation and infections among adult patients within 48 hours of ICU admission?
- Question 2: What are the characteristics of hospital-acquired bacterial colonisation and infections among adult patients during ICU stay?
- Question 3: What are the molecular characteristics of *S. aureus* colonisation and infections among ICU patients?
- Question 4: What are the molecular characteristics of *K. pneumoniae* colonisation and infections among ICU patients?

Chapter 2. MATERIALS AND METHODS

2.1 SETTING

2.1.1 Vietnam

Vietnam is a Southeast Asian country with a total land area of 310,070 km^2 and a population of 96,3 million as of 2018, based on the latest United Nations estimates [188]. According to the Ministry of Health, the disease patterns in Vietnam are currently in a transitional period with multiple burdens from infectious diseases, and a number of emerging epidemic diseases. The percentage of hospital admissions due to infectious diseases dropped from 55.5% in 1976 to 19.8% in 2010, but increased to 25.3% in 2013 [189]. Since 1998, the CDC has partnered with the Vietnamese government to provide life-saving care and treatment for people living with HIV/AIDS, and to prevent the spread of infectious diseases such as influenza and other health threats [190]. Currently, the problem of AMR is particularly pressing in Vietnam with high morbidity, mortality and health cost. Since 2009, the GARP Vietnam and OUCRU collaborated with the Vietnamese Ministry of Health to set up a new surveillance program for both antimicrobial consumption and resistance [183]. This program reported a significant positive correlation between antimicrobial consumption and resistance and has resulted in a call for prompt and effective interventions to improve the quality of health care.

2.1.2 Hospital for Tropical Diseases (HTD)

The HTD is a tertiary referral hospital for infectious diseases in southern Vietnam under the direction of the Ho Chi Minh City Service of Health and the Vietnamese Ministry of Health. Originally founded in 1862, the hospital has 550 beds and receives more than 2,500 outpatients daily. Over 70% of patients are citizens of Ho Chi Minh City and the remaining patients are from the nearby provinces. The hospital has 14 clinical wards including two ICUs (adult ICU and paediatric ICU), one ward for infection of the central nervous system, one HIV ward, six general adult wards, and four general

paediatric wards. The HTD has been working in partnership with OUCRU since 1991. The collaborative research focuses on the following core areas: malaria, dengue, influenza, typhoid, central nervous system infections (viral encephalitis, pyogenic and tuberculous meningitis), opportunistic infections related to HIV and tetanus, with additional interest in diphtheria, shigella, fascioliasis, and *Streptococcus suis* infections.

2.1.3 Adult ICU

Adult ICU is a 20-bed ward, but usually admits additional patients. Adult ICU is dedicated to management of critically-ill patients with severe infectious diseases like tetanus, Dengue infection, influenza, sepsis or septic shock, and end-stage cirrhosis. There are approximately 24 - 28 patients per day and about 1,000 - 1,200 admissions per year. The nurse-to-patient ratio is about 1:3, and varying numbers of nursing students may also be present and take part in clinical activities. The ICU is divided into 4 small blocks (about 4 - 8 patients per block). The standard infection control measures are in place, including personal protective equipment for routine patient care (head coverings, mask, and gloves); daily patient bathing with 2% chlorhexidine gluconate; healthcare worker education and adherence monitoring with a focus on hand hygiene. Hand washing audits are performed periodically with mean compliance rates of 70 - 80% (monthly report from Infection Prevention and Control Department, unpublished data). Anios Special DJP SF (Laboratoires Anios ^{TM}, France) is used for disinfection of ICU surfaces by air, and Surfa'Safe (Laboratoires Anios ^{TM}, France), detergent disinfectant foam for non-invasive medical device surfaces after patient discharge. The surveillance of HAIs has been mandatory in Adult ICU since 2015, with mean rate of 5 - 10%. The most frequent types of HAIs are pneumonia, UTI and BSI. According to annual report from Microbiology Department (unpublished data), common bacteria involved in hospital-acquired pneumonia included *P. aeruginosa* (28%), *Acinetobacter* spp. (16.8%) and *K. pneumonia* (12%), with high carbapenem

resistance rate of 57%, 72% and 16% respectively. For BSI, the two most common pathogens were *E. coli* (19%, of which were 70% ESBL) and *S. aureus* (12%, of which were 57% MRSA). For UTI, *E. coli* was most prevalent (36%) and 63% of *E. coli* isolates were ESBL-producing organisms. Active screening for patients with AROs is not available in Adult ICU. Therefore, contact isolation or geographic separation of colonised or infected patients with AROs is rarely applied.

2.2 ETHICAL APPROVAL

The conduct of this study was consistent with the principles of the Declaration of Helsinki. The protocol was reviewed and approved by the Ethics Committee of the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam (HTD Approval: 59/HDDD - QD and HTD Study code CS/ND/14/13), and the Oxford University Tropical Research Ethics Committee, England (OxTREC Reference: 54-14). Written informed consent was obtained from all patients or their legal representatives.

2.3 METHODS

2.3.1 Methods for Chapter 3 and Chapter 4

2.3.1.1 Study design and aims

A prospective, longitudinal study was conducted in Adult ICU of the HTD from 10th November 2014 to 14th January 2016. The study aim of Chapter 3 is to characterize bacterial colonisation and infections among adult patients within 48 hours of ICU admission, and that of Chapter 4 is to characterize hospital-acquired colonisation and infections among adult patients during ICU stay.

2.3.1.2 Inclusion and exclusion criteria

Patients were eligible for this study if they were ≥15 years of age and admitted to Adult ICU of the HTD during the study period. I excluded patients who were readmitted to Adult ICU within 90 days because these patients may have a risk of colonisation and infections with the same bacterial profile that had been isolated in the previous 90 days.

2.3.1.3 Sample size

As this was a prospective, observational study, the sample size was dependent on the number of patients admitted to Adult ICU according to the inclusion and exclusion criteria (as described above) during the study period.

2.3.1.4 Study procedure

The study procedure is shown in Figure 2.1.

Sample collection: Nasal swab, rectal swab, and/or endotracheal aspirate (ETA, in case of intubation or tracheostomy) were taken for detecting initial and acquired colonisation with *S. aureus*, *E. coli*, *Klebsiella* spp., *Acinetobacter* spp., *Pseudomonas* spp.. Swabs were taken within 48 hours of ICU admission and repeated twice a week (on Monday and Thursday) until patients were discharged from ICU. Other clinical specimens (blood, urine, sputum or pus sample, etc.) being indicated for clinical purposes by Adult ICU doctors were also collected for analysis.

Data collection: Demographic data (age, sex and gender), medical history (underlying diseases, prior hospitalization, prior antimicrobial use, steroid use, immunosuppressive therapy), Charlson Comorbidity Index score, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, treatment (antimicrobial therapy, intubation or tracheostomy, mechanical ventilation, hemodialysis/hemofiltration, central venous catheter, arterial catheter, urinary catheter, etc.), reason for ICU admission, diagnosis and outcomes (CAIs, HCAIs, HAIs, discharge, transfer or death) were collected for analysis.

Data entry: Relevant data were recorded onto a case record form and checked for accuracy before data entry onto an electronic database (CliRes, OUCRU, Vietnam).

2.3.1.5 Definitions

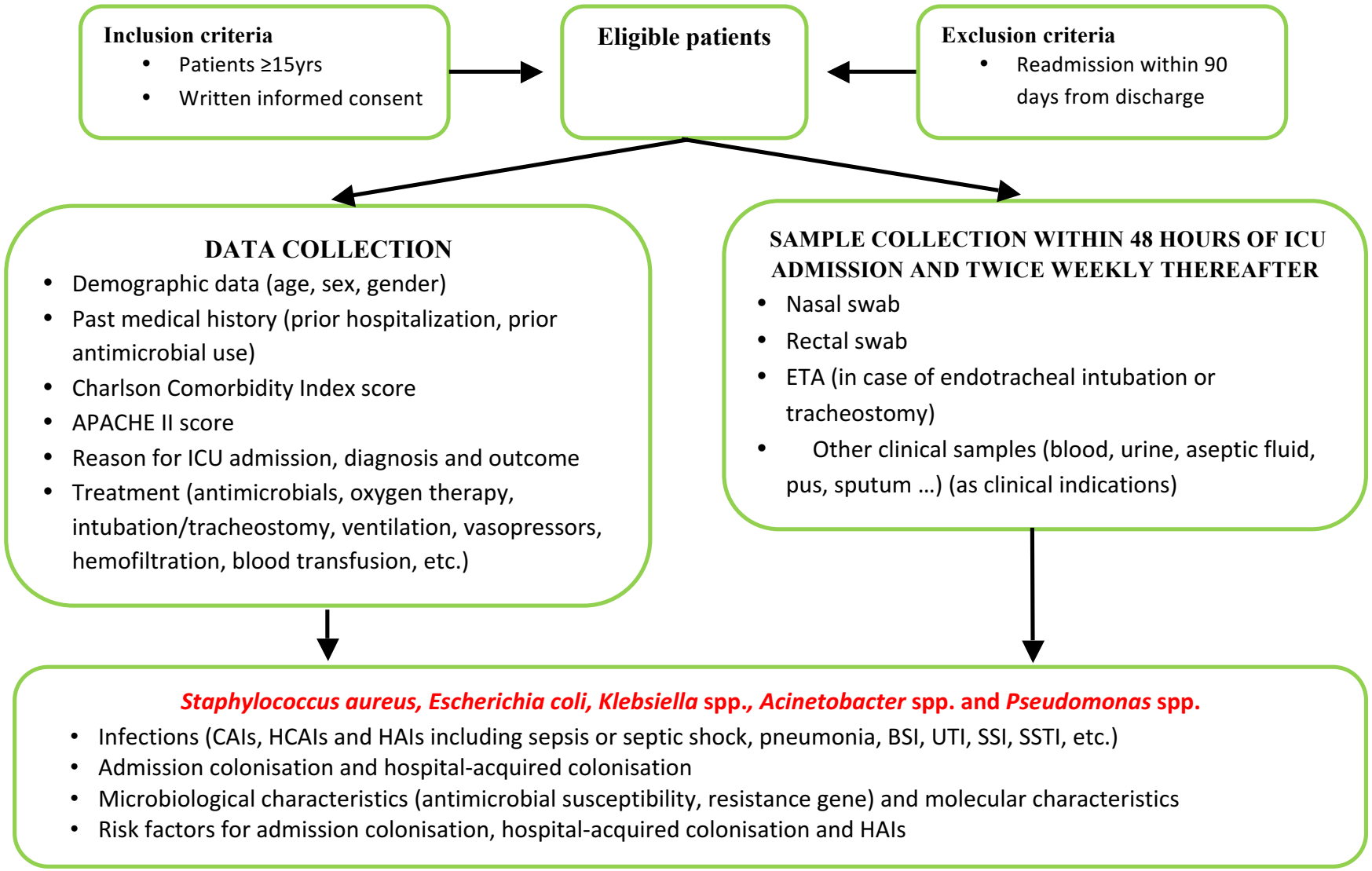
For the purposes of investigation, admission colonisation was defined as a positive culture of either nasal swab, rectal swab or ETA with *S. aureus*, *E. coli, Klebsiella* spp.,

Pseudomonas spp., *Acinetobacter* spp. and/or AROs taken within 48 hours of ICU hospitalization. Acquired colonisation was defined by negative swab cultures on ICU admission or during ICU stay followed by a later positive swab culture; or new positive swab culture with differently specified bacteria or with the same organisms but different antimicrobial resistance pattern compared to previous positive swab cultures. AROs were defined as MRSA, 3rd-generation cephalosporin-resistant *E. coli*, 3rd-generation cephalosporin-resistant *Klebsiella* spp., ceftazidime-resistant *Pseudomonas* spp., carbapenem-resistant *E. coli*, carbapenem-resistant *Klebsiella* spp., carbapenem-resistant *Acinetobacter* spp. and carbapenem-resistant *Pseudomonas* spp.

ICU patients were assigned to one of three groups: those with CAIs, those with HCAIs and those with HAIs. HCAIs were defined as infections detected on ICU admission or within the first 48 hours after ICU hospitalization, which fulfill one of the following criteria: (i) receiving specific home care (such as intravenous therapy, wound care or specialized nursing care), (ii) attending a hospital or hemodialysis clinic in the 30 days before the infection, (iii) being hospitalized ≥2 days in the 90 days before the infection, (iv) residing in a nursing home or a long-term care facility [16]. CAIs were defined as infections occurring on ICU admission or within the first 48 hours after ICU hospitalization without fulfilling any of the above criteria. HAIs were defined as infections developing after ≥2 days of ICU admission.

The ascertainment of the source of infection was performed by Adult ICU doctors according to the modified CDC definitions [191], including pneumonia, UTI, BSI, SSTI, SSI, spontaneous bacterial peritonitis (SBP), gastrointestinal tract infection, bacterial meningitis, bacterial pleural effusion, cholangitis and endocarditis (Appendix 1). The classification of sepsis and septic shock was based on Sepsis-3 [192]. Antimicrobial-resistant infections are infections caused by AROs.

Figure 2.1 Flowchart of study procedure



Tetanus is clinically diagnosed on the presence of lockjaw, muscular rigidity, and/or spasm (without specific lab tests) [193]. Dengue infection is confirmed by the detection of NS1 antigen or IgM antibodies for Dengue virus [194,195]. Scrub typhus is clinically diagnosed with persistent fever, low platelet count, high liver enzymes and the presence of an eschar [196]. Hepatitis B flare is characterized by elevated aminotransferase levels ≥5 times the upper limit of normal and HBV DNA $>10^5$ copies/ml if HBeAg positive and $>10^4$ copies/ml if HBeAg negative [197,198]. Hepatic encephalopathy is a brain dysfunction caused by liver insufficiency and it manifests as a wide spectrum of neurological or psychiatric abnormalities ranging from subclinical alterations to coma [199]. Melioidosis is diagnosed with the help of positive blood culture for detection of *Burkholderia pseudomallei* (*B. pseudomallei*) [200]. The criterion standard for serologic identification of leptospirosis is microscopic agglutination testing (MAT) [201].

Prior hospital stay was defined as hospitalization in the last 3 months (90 days) or hospital stay of ≥48 hours before ICU admission. Antimicrobial use was considered to be present when antibiotics (not including antifungal and antiviral treatments) were taken 24 hours before ICU admission. Data on antimicrobial use was recorded according to antibiotic family: fluoroquinolones, carbapenems, aminoglycosides, antipseudomonal and non-antipseudomonal cephalosporins, and antipseudomonal and non-antipseudomonal penicillins from 3 months until the day of ICU admission. Inappropriate antimicrobial treatment was defined as use of antimicrobials to which bacterial isolates were detected to be resistant in vitro.

Charlson Comorbidity Index is a method of categorizing comorbid disease of patients which indicates that the higher score, the more likely predicted outcome will result in mortality [202]. APACHE II score is a severity of disease classification system that uses a point score based on initial values of 12 routine physiologic measurements, age, and previous health status. It is a validated tool to predict mortality for patients in the ICU

[203]. For comorbidities, diabetes mellitus is diagnosed based on either hemoglobin A1C (HbA1c) ≥6.5% or the fasting plasma glucose ≥126 mg/dl (7 mmol/l) or in a patient with classic symptoms of hyperglycemia (polyuria, polydipsia, and unexpected weight loss) or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mmol/l) [204]. Corticosteroid therapy is classified as using glucocorticoids or mineralocorticoids within 3 months prior to ICU admission.

2.3.1.6 Microbiological methods

Bacterial identification

Nasal and rectal swab were taken by using Transwab® (Corsham, Wiltshire, England). For nasal swab, a small, soft-tipped, moist and sterile swab was inserted about 2 cm into the anterior part of the nasal cavity and rotated a few times against the nasal mucosa until it was covered in secretions. The rectal swab was collected by the same type of swab which was placed into the anal canal, beyond the anal verge about 3 cm, then rotated gently, and removed. For ETA, a sterile 500 mm, 14-gauge tracheal aspiration catheter (Embramed®, Brazil) was attached to a 20 ml syringe filled with 20 ml sterile saline. The distal end was introduced via the tracheostomy or endotracheal tube until significant resistance was felt (level of the carina in the trachea) and retracted approximately 2 cm. This was followed by the saline instillation over 10 seconds, then the release of the vacuum; and the suction catheter was slowly pull up and out for no more than 10 seconds, from which about 5 - 10 ml of secretion was aspirated into a sterile collection bottle (Zammi®, Brazil). No further aspiration was attempted during removal of the catheter to avoid contamination with tracheal secretions. ETA with >10 epithelial cells per low power field on a Gram stained slide of a direct smear was rejected as inadequate for culture.

To specifically isolate *S. aureus*, *E. coli*, *Klebsiella* spp., *Pseudomonas* spp. and *Acinetobacter* spp., nasal and endotracheal specimens were cultured on Blood agar

(BioMérieux) and MacConkey (BioMérieux), whereas Xylose Lysine Deoxycholate agar (BioMérieux) was added to culture rectal swabs. Suspected staphylococcal colonies were identified by colony morphology, Gram stain, and catalase and coagulase testing, plus checked for methicillin resistance using cefoxitin disc diffusion, and finally re-checked on MALDI-TOF MS (Bruker).

The identification of *E. coli*, *Klebsiella* spp., *Pseudomonas* spp. and *Acinetobacter* spp. was confirmed by MALDI-TOF MS. Appropriate organisms were screened using CHROMagar (CHROMagar, Paris, France) for ESBL production. The double disc diffusion method was then used to detect ESBL activity using both cefotaxime and ceftazidime, alone and in combination with clavulanate. ESBL activity is considered if there is a ≥5mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate compared to the zone diameter of the agent when tested alone. CHROMagar C3G (CHROMagar, Paris, France) was used as a screening agar for AmpC production. Then suitable colonies had an AmpC induction test to detect induced AmpC lactamase activity. A ceftazidime disc was placed near cefoxitin/imipenem. A flattening zone of 3rd-generation cephalosporin toward the inducer (cefoxitin/imipenem) indicates the inducible AmpC lactamase. Following detection of reduced susceptibility to meropenem in routine susceptibility tests, a modified carbapenem inactivation method (mCIM) was performed to identify the production of carbapenemase. If the test isolate produces a carbapenemase, the meropenem in the disk will be hydrolyzed and there will be no inhibition or limited growth inhibition of the meropenem-susceptible *E. coli* ATCC® 25922 (the indicator organism) [205].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted by the Kirby/Bauer disc diffusion method and interpreted using the CLSI guidelines 2015 [156]. Choice of antimicrobial disc sensitivity was dependent on the bacterial species. For *S. aureus*, we performed

antimicrobial susceptibility testing for the following drugs: penicillin, oxacillin, vancomycin, erythromycin, rifampicin, clindamycin, ciprofloxacin, trimethoprim-sulfamethoxazole and levofloxacin. For *S. aureus* bacteremia-associated isolates, linezolid and teicoplanin were added for testing. For *E. coli*, *Klebsiella* spp., *Pseudomonas* spp. and *Acinetobacter* spp., the following drugs were used, inluding cefriaxone, ceftazidime, cefepime, amoxcillin/clavulanic acid, gentamycin, ertapenem, imipenem, ciprofloxacin, ofloxacin, amikacin, piperacillin/tazobactam, ticarcillin/clavulanic acid and colistin.

Other clinical samples (blood, urine, aseptic fluid and wound drainage) were performed according to the local Standard Operating Procedures of the Microbiology Department of the HTD. All isolates were preserved by freeze drying and logged onto a data base giving date of freeze drying and position in the freeze dried ampoule bank.

2.3.1.7 Statistical analysis

Analysis of admission colonisation and infection

Descriptive analyses were performed, consisting of frequency (percentage) for categorical data, and median (95% confidence interval - 95% CI or interquartile range - IQR) for continuous data. Comparisons of percentage were performed using $\chi 2$ or Fisher's exact tests. Comparisons of means were performed using Student's t test or nonparametric tests, such as the Kruskal-Wallis test. Statistical analyses used the R 3.4.0 statistical software (R foundation, Vienna, Austria). P values <0.05 (two-sided) were considered statistically significant.

Analysis of risk factors for acquired colonisation

Risk factors for specifically acquired nasal/rectal/endotracheal colonisation with AROs during ICU stay were evaluated using Cox proportional cause-specific hazards regression, with discharge and death as competing events. We considered the following potential risk factors at admission: tetanus disease, Charlson Comorbidity Index score,

colonisation status (by anatomical sites), receipt of antimicrobial treatment, and intensive care procedures including nasogastric tube and respiratory support consisting of intubation, tracheostomy and mechanical ventilation. Respiratory support was tested for nasal and endotracheal (not for rectal) colonisation with AROs. Tetanus disease was included in the model because the pathogenesis of tetanus disease is related to the activity of a neuro-toxin released from *Clostridium tetani* [193], which is different from other illnesses, of which the pathology is related to the host response to an infectious or non-infectious agent leading to systemic inflammatory, organ dysfunction and organ failure [192,206]. Moreover, due to a high proportion of tetanus patients (>56%, 204/364) in this study, tetanus disease was likely to act as a confounding variable for the other risk factors. The other variables were selected based on literature review [125,207-214]. The continuous variable Charlson Comorbidity Index score was included as linear term. All other factors were binary variables. It was assumed that patients remained with acquired colonisation with AROs from detection of AROs acquisition until ICU discharge.

Analysis of risk factors for HAIs development during ICU stay

Cox proportional hazards regression was also used to determine the potential risk factors for specific types of HAI: pneumonia, UTI and BSI. However, multivariate Cox regression model was not performed for BSI due to a very low number of events. The following risk factors were considered: admission for tetanus disease, Charlson Comorbidity Index score, prior colonisation status (including initial colonisation on ICU admission and acquired colonisation during ICU stay per anatomical sites), and intensive care procedures on ICU admission (respiratory support consisting of intubation, tracheostomy and mechanical ventilation for pneumonia; urinary catheter for UTI; and vascular catheters including central venous, arterial and hemofiltration catheter for BSI). In this model, prior nasal/rectal/endotracheal colonisation is

considered as a time-dependent risk factor in subsequent HAIs. Time zero was the date of ICU admission, and the date of acquired colonisation was assumed to be at the midway point between the latest negative culture and the first positive surveillance culture. It was assumed that patients remained colonised from detection of colonisation on ICU admission or acquisition during ICU stay until ICU discharge.

Statistical analyses used the R 3.4.0 software (R foundation, Vienna, Austria), especially the R functions *coxph* from the *survival* package. P values <0.05 (two-sided) were considered statistically significant.

2.3.2 Methods for Chapter 5 and Chapter 6

2.3.2.1 Study design and aims

A prospective, observational and longitudinal study was conducted in Adult ICU of the HTD from 10th November 2014 to 14th January 2016. The study aim of Chapter 5 was to use WGS to determine whether or not ICU patients became infected with their previously colonizing *S. aureus* isolates, and that of Chapter 6 focused on *K. pneumoniae* strains.

2.3.2.2 Inclusion and exclusion criteria

ICU patients (≥15 years) with confirmed diagnoses of *S. aureus* or *K. pneumoniae* infections (including CAIs, HCAIs and HAIs) admitted to Adult ICU during the study period were eligible for analysis. Only the first episode of *S. aureus* or *K. pneumoniae* infection was included.

2.3.2.3 Sample size

As this was a prospective study, the sample size was dependent on the number of patients with confirmed diagnoses of *S. aureus* or *K. pneumoniae* infections admitted to Adult ICU of the HTD during the study period.

2.3.2.4 Sample collection

Consecutive, non-repetitive, colonizing and infecting isolates of *S. aureus* or *K. pneumoniae* obtained from ICU patients with confirmed diagnoses of *S. aureus* or *K. pneumoniae* infections were collected for molecular analysis. Infecting isolates of *S. aureus* or *K. pneumoniae* were recovered from clinical specimens, such as pus sample (in case of SSTI), sputum or ETA (in case of pneumonia), blood sample (in case of BSI), urine sample (in case of UTI) and peritoneal fluid sample (in case of SBP). For CAIs and HCAIs by *S. aureus* or *K. pneumoniae*, colonizing isolates of *S. aureus* or *K. pneumoniae* obtained from swab cultures (nasal swab, rectal swab and/or ETA) taken within 48 hours of ICU admission were collected for analysis. For HAIs by *S. aureus* or *K. pneumoniae*, only colonizing isolates of *S. aureus* or *K. pneumoniae* cultured from surveillance swabs (nasal swab, rectal swab and/or ETA) taken before the date of *S. aureus* or *K. pneumoniae* infections being diagnosed were collected for further analysis. The ascertainment of *S. aureus* or *K. pneumoniae* infections was performed by the Adult ICU doctors according to the CDC definitions [191].

2.3.2.5 Definitions

Admission colonisation with *S. aureus* or *K. pneumoniae* was defined as a positive culture of either nasal swab, rectal swab or ETA with *S. aureus* or *K. pneumoniae* taken within 48 hours of ICU hospitalization. Acquisition or acquired colonisation with *S. aureus* or *K. pneumoniae* was defined by a negative admission swab culture followed by a later positive swab culture by conventional method; or a positive admission swab followed by a later positive swab of distinct STs by MLST analysis. Patient-to-patient transmission in ICU was defined according to either MLST criteria by acquisition of *S. aureus* or *K. pneumoniae* with matching ST and identical antibiogram of a strain cultured previously from an ICU patient with overlapping ICU stay, or phylogeny analysis by forming a same clade of *S. aureus* or *K. pneumoniae* strains evolving

dependently (irrespective of overlapping patient stay). In addition, patient-to-patient transmission of *S. aureus* in ICU was also defined by WGS using SNP differences (irrespective of overlapping patient stay) with a SNP difference of >40 used to exclude a recent transmission [154,215].

2.3.2.6 Molecular methods

Bacterial DNA extraction

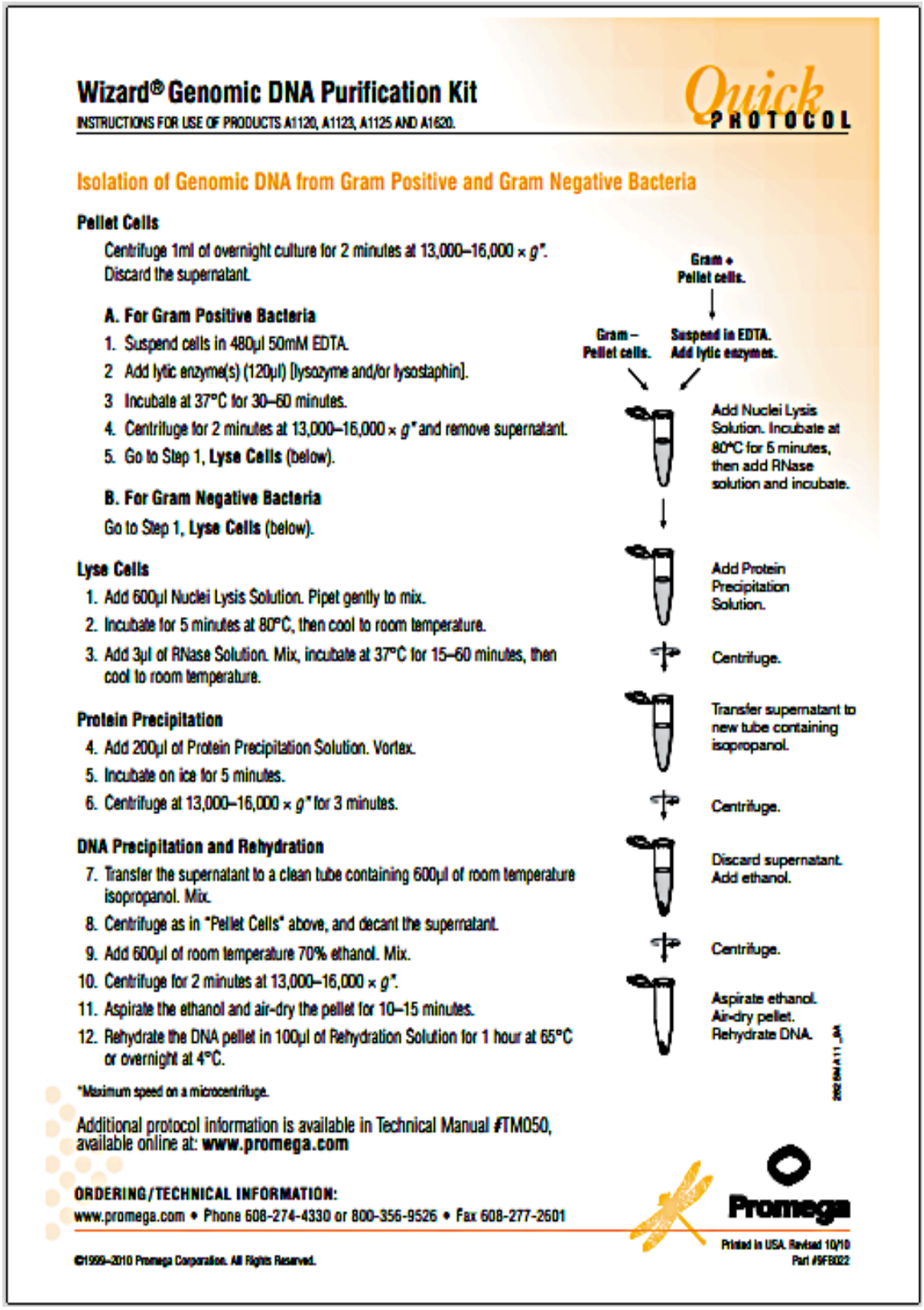
DNA from *S. aureus* or *K. pneumoniae* isolates was extracted using the Wizard ® Genomic DNA Purification Kit (Promega) following the manufacturer's instructions (Figure 2.2) [216]. The overnight culture was scraped (about a half of loop with isolates) and added into 1ml of sterile saline to form an even suspension. The suspension was centrifuged for 2 minutes at 13,000 rpm to pellet the cells, and then the supernatant was removed and discarded. 600µl of Nuclei Lysis Solution was added and mixed by gently pipetting until the cells were fully re-suspended. The solution was incubated for 5 minutes at 80°C to lyse the cells, and then allowed to cool at room temperature. 3µl of RNase solution was added to the cell lysates, mixed and incubated at 37°C for 5 minutes, and then allowed to cool to room temperature. 200µl of Protein Precipitation Solution was added and vortexed vigorously at high speed for 20 seconds. The sample was then incubated on ice for 5 minutes before centrifugation at 13,000 rpm for 3 minutes. The supernatant containing the DNA was transferred to a newly labeled microcentrifuge tube containing 600µl of Isopropanol and gently mixed by inversion until the thread like strands of DNA formed a visible mass. The suspension was centrifuged for 2 minutes at 13,000 rpm, and the supernatant was carefully poured off. 600µl of room temperature 70% ethanol was added and the microcentrifuge tube gently inverted several times to wash the DNA pellet. The suspension was centrifuged for 2 minutes at 13,000 rpm, the ethanol was removed and the DNA pellet was air-dried at room temperature for 10 - 15 minutes. The DNA pellet was rehydrated with 200µl of

Rehydration Solution, and incubated at 65°C for 1 hour, then stored at 4°C until required.

Bacterial DNA sequencing

WGS was performed on the Illumina MiSeq bench-top sequencer using the Nextera ® DNA Library Prep Reference (Illumina) to generate 250 bp paired-end reads [217]. The workflow using a Nextera DNA Library Prep Kit is illustrated in Figure 2.3. The Nextera DNA Library Prep Kit uses an engineered transposome to tagment genomic DNA of *S. aureus* and *K. pneumoniae*, which is a process that fragments DNA and then tags the DNA with adapter sequences in a single step. A limited-cycle PCR step uses the adapters to amplify the insert DNA. The PCR step adds index adapter sequences on both ends of the DNA, which enables dual-indexed sequencing of pooled libraries on Illumina sequencing platforms. Then AMPure XP beads are used to purify the library DNA and to remove short library fragments. To achieve the highest-quality data on Illumina sequencing platforms, and to create optimum cluster densities across every lane of the flow cell, the next step requires accurate quantification of DNA library templates by adjusting the DNA concentration at conversion formula of 1ng/µl = 3nM. The final step before sequencing is to normalize indexed libraries to 2nM in the Nextera Dilution Plate and then pool in equal volumes in the Nextera Pooled Plate.

Figure 2.2 Bacterial DNA extraction [216]



Wizard® Genomic DNA Purification Kit

INSTRUCTIONS FOR USE OF PRODUCTS A1120, A1123, A1125 AND A1620.

Quick PROTOCOL

Isolation of Genomic DNA from Gram Positive and Gram Negative Bacteria

Pellet Cells

Centrifuge 1ml of overnight culture for 2 minutes at 13,000–16,000 × *g**. Discard the supernatant.

A. For Gram Positive Bacteria

1. Suspend cells in 480µl 50mM EDTA.
2. Add lytic enzyme(s) (120µl) [lysozyme and/or lysostaphin].
3. Incubate at 37°C for 30–60 minutes.
4. Centrifuge for 2 minutes at 13,000–16,000 × *g** and remove supernatant.
5. Go to Step 1, **Lyse Cells** (below).

B. For Gram Negative Bacteria

Go to Step 1, **Lyse Cells** (below).

Lyse Cells

1. Add 600µl Nuclei Lysis Solution. Pipet gently to mix.
2. Incubate for 5 minutes at 80°C, then cool to room temperature.
3. Add 3µl of RNase Solution. Mix, incubate at 37°C for 15–60 minutes, then cool to room temperature.

Protein Precipitation

4. Add 200µl of Protein Precipitation Solution. Vortex.
5. Incubate on ice for 5 minutes.
6. Centrifuge at 13,000–16,000 × *g** for 3 minutes.

DNA Precipitation and Rehydration

7. Transfer the supernatant to a clean tube containing 600µl of room temperature isopropanol. Mix.
8. Centrifuge as in "Pellet Cells" above, and decant the supernatant.
9. Add 600µl of room temperature 70% ethanol. Mix.
10. Centrifuge for 2 minutes at 13,000–16,000 × *g**.
11. Aspirate the ethanol and air-dry the pellet for 10–15 minutes.
12. Rehydrate the DNA pellet in 100µl of Rehydration Solution for 1 hour at 65°C or overnight at 4°C.

*Maximum speed on a microcentrifuge.

Additional protocol information is available in Technical Manual #TM050, available online at: **www.promega.com**

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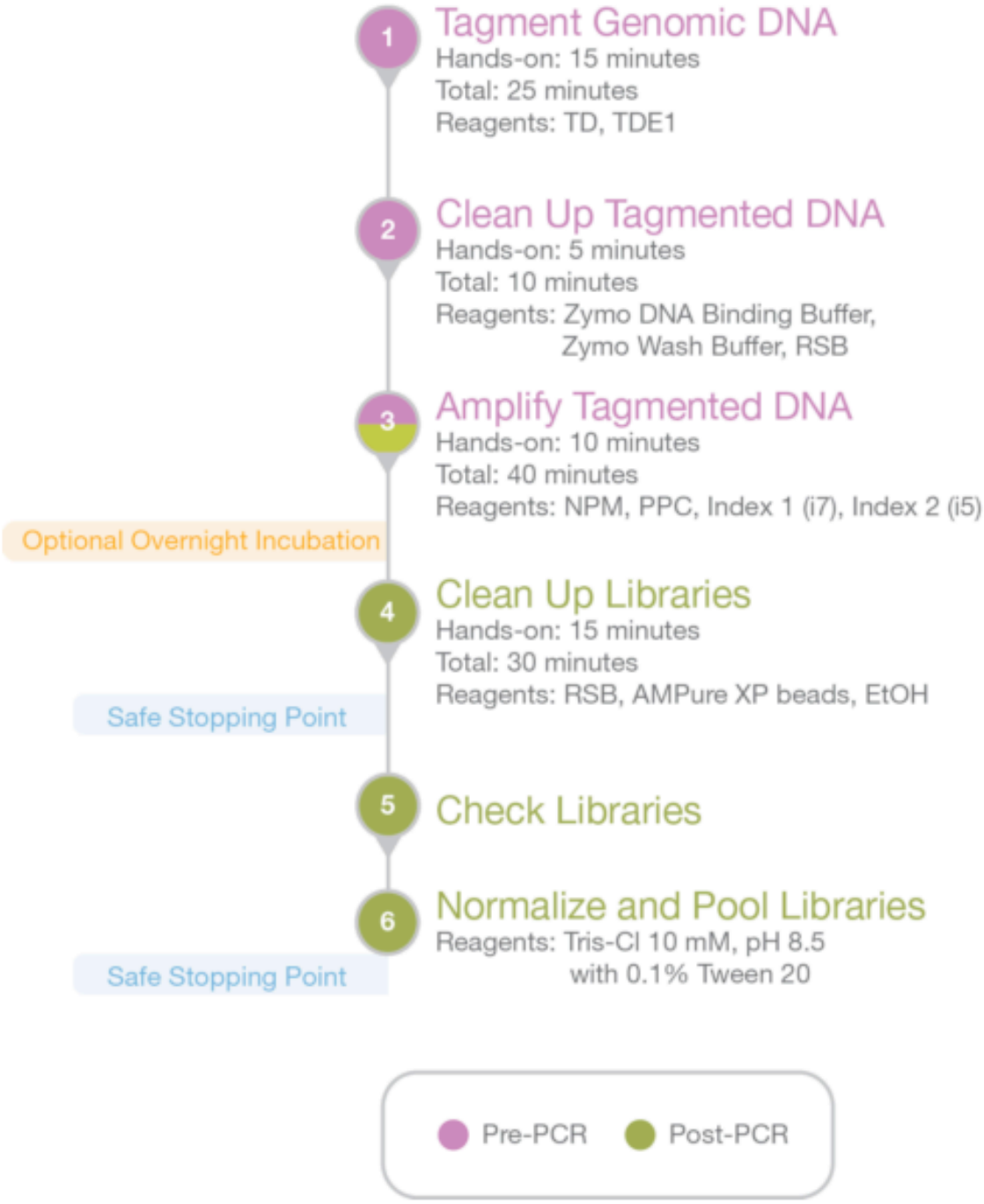
Figure 2.3 Nextera DNA Library Prep Workflow [217]



Genomic investigation

Identification of AMR genes

For *S. aureus*, the genes implicated in resistance to oxacillin (*mecA*), penicillin (*blaZ*), erythromycin (*ermA, ermB, ermC* and *msrA*), fluoroquinolones (*gyrA* and *norA*), and vancomycin (*vanA*) were detected using ARIBA [218]. This was done by mapping the short read sequences to the ResFinder database, the Center for Genomic Epidemiology (www.genomicepidemiology.org) [219].

For *K. pneumoniae*, only isolates of ST17, ST23 and ST86 were screened for the presence of a repertoire of virulence factors (siderophores and *rmpA*), capsular K antigen, and acquired resistance genes (*bla*ESBL and *bla*CARB) using Kleborate, with *de novo* assemblies serving as the input [220,221]. Crucially, siderophores are considered the key function of iron acquisition which enhances the ability of bacteria to survive and replicate within the host. Evidence is mounting that *K. pneumoniae* isolates carrying acquired siderophores are highly pathogenic and can cause invasive diseases [153,222-224]. Moreover, the "regulator of mucoid phenotype" *rmpA*, which can up-regulate capsule production, has also been identified as a virulence factor in *K. pneumoniae* [225]. Only *K. pneumoniae* ST17, ST23 and ST86 were chosen since they were cultured from ≥3 ICU patients with sufficient isolates for analysis (ST17: 13 isolates, 5 patients; ST23: 12 isolates, 4 patients; and ST86: 17 isolates, 3 patients).

Correlation between resistant phenotype and genotype

Intermediate phenotypes were counted as resistant in this analysis. Each interpretation of resistant or susceptible to a given antimicrobial agent was compared with the presence or absence of a known corresponding resistance gene(s). The correlation between resistant phenotype and genotype was demonstrated by sensitivity and specificity of the presence or absence of a resistance gene. Using the phenotypic results as the reference outcome, sensitivity was calculated by dividing the number of isolates

that were genotypically resistant by the total number of isolates exhibiting clinical resistance phenotypes. Specificity was calculated by dividing the number of isolates that were genotypically susceptible by the total number of isolates with susceptible phenotypes.

MLST analysis

MLST was performed for all sequenced *S. aureus or K. pneumoniae* isolates using ARIBA [218]. For *S. aureus*, the following seven housekeeping genes were used: carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*) and acetyl coenzyme A acetyltransferase (*yqiL*). For *K. pneumoniae*, the following seven housekeeping genes were performed: beta-subunit of RNA polymerase B (*rpoB*), glyceraldehyde 3-phosphate dehydrogenase (*gapA*), malate dehydrogenase (*mdh*), phosphoglucose isomerase (*pgi*), phosphoporine E (*phoE*), translation initiation factor 2 (*infB*) and periplasmic energy transducer (*tonB*). Isolates were defined by the alleles present at the seven loci (the allelic profile), and each unique allelic profile was assigned as a ST using the MLST database (http://saureus.mlst.net and www.pasteur.fr/mlst/Kpneumoniae.html). Isolates with the same ST, therefore, had identical sequences at all seven MLST loci and were considered to be members of a single clone. Based on ST, the program BURST was applied to divide isolates into clonal CCs, which were defined as groups of STs in which every ST shared at least five of seven identical alleles with at least one other ST in the group [226]. STs that did not correspond to any CC were defined as singletons. Alleles and STs that had not been previously described were assigned as new designations.

Phylogenetic analysis

The MLST results indicated that ST188 was the predominant ST of *S. aureus* in this study. So, only sequence reads of ST188 were mapped against the reference MSSA476 (accession number NC_002953) using bwa-mem [227]. This reference, which belongs to ST1, was selected because it was the most closely related complete reference for ST188. For *K. pneumoniae*, only three STs (ST17, ST23 and ST86) were included for further phylogenetic analysis since they were isolated from ≥3 ICU patients with sufficient isolates. ST23 and ST86 are also of great clinical importance since they have been associated with hypermucoviscous capsular type K1 and K2 respectively, which could cause highly fatal invasive diseases [228]. For each of these STs, short reads were mapped to appropriate references (XH209, accession number NZ_CP009461, for ST17; NTUH-K2044, accession number NC_012731, for ST23; and CG43, accession number NC_022566, for ST86) using bwa-mem [227]. These references were selected so that they belong to the same ST of the corresponding sampled isolates.

SNPs against the reference were detected and filtered using SAMtools (v1.3) and bcftools (v1.2), respectively [229]. Duplicate reads were removed by PICARD, and the package GATK was employed for indel realignment, as previously recommended [230]. SNPs were called using the 'consensus' option, and low quality SNPs were removed according to the following criteria: consensus quality <50, mapping quality <30, ratio of SNPs to reads at a position <90%, and read depth <4. This helps to create a set of high quality SNPs suitable for investigating patient-to-patient transmission. Mapping coverage at each position in the reference genome was summarized using bedtools (v2.24.0). A pseudo-sequence was created to incorporate the identified SNPs, regions of low mapping coverage, and invariant sites, using the vcf2fa python script (https://github.com/brevans/vcf2fa). Pseudo-sequences of the same ST were concatenated to create alignments suitable for phylogenetic reconstruction. Exclusion of

genomic regions pertaining to recombination or horizontal gene transfer (such as prophages, genomic islands) was performed using Gubbins, with ten iterations to ensure convergence [231]. Removal of invariant sites further generated an alignment of 1,005 SNPs, which served as an input for phylogenetic reconstruction of *S. aureus* ST188. Removal of invariant sites further generated SNP only alignments of following length: 781 bp for ST17, 468 bp for ST23 and 697 bp for ST86, and these alignments served as inputs for phylogenetic reconstruction of *K. pneumoniae*. Maximum-likelihood phylogeny was inferred using RAxML v8.1.3 with the GTRGAMMA model with 1,000 rapid bootstrap to assess the reliability of the resulting phylogenetic topology [232]. For all aforementioned STs, we separately constructed *de novo* assemblies for each read set using SPAdes v3.12.0 with default parameters [233]. Prior to assembly, each read set was processed using Trimmomatic to retain high quality read pairs of at least 50 bp [234]. In addition to assess diversity of *S. aureus* isolates, I calculated the median and maximum pairwise SNP differences between isolates within the same patient across different sampling sites and over time; and the minimum pairwise SNP differences between isolates obtained between patients across the whole study. *S. aureus* isolates were defined to be of the same subtype if they differed by no more than 40 SNPs [154,215,235].

2.3.2.7 Statistic analysis

By using R (R foundation, Vienna, Austria), descriptive analyses of the endpoints were performed, consisting of frequency (percentage) for categorical data, and median (95% CI or IQR) for continuous data supplemented by graphical displays where relevant.

Chapter 3. BACTERIAL COLONISATION AND INFECTIONS AMONG ADULT PATIENTS WITHIN 48 HOURS OF ICU ADMISSION

3.1 INTRODUCTION

Antimicrobials are among the most significant medical achievements of mankind. These drugs have saved millions of lives from the fatal consequences of severe infectious diseases. The success of antimicrobial therapy has led to a widespread use of antimicrobials all over the world. As a result, unrestricted access to antimicrobials has contributed to the appearance and spread of AMR which is one of the most serious threats to public health all over the world [164].

The impact of AMR may be more serious in LMICs than in HICs because of increased numbers of infections and unregulated antimicrobial use [185]. Moreover, these countries struggle to implement hospital infection control activities due to limited resources and consequently, poor infection control practices have contributed to the spread of AROs within healthcare settings. In addition, inpatients infected and colonised with AROs, such as MRSA and 3rd-generation cephalosporin-resistant *Enterobacteriaceae*, constitute an important reservoir of AROs in hospitals that can potentially ignite outbreaks of life-threatening infections [137,236]. ICU patients are particularly susceptible to infection, especially antimicrobial-resistant bacterial infection, due to prolonged hospitalization, invasive medical procedures, and decreased immune function [7,8,58,237]. However, little is known about colonisation and infection with AROs among ICU patients especially in less well-off countries, like Vietnam.

If we can better understand colonisation status, infection patterns, antimicrobial susceptibility and acquisition routes of AROs in ICU patients, we can design future interventions to control the appearance and spread of AROs and minimize the burden of AMR not only in hospitals but also in the community.

3.2 MATERIALS AND METHODS

The detailed methods of this chapter are reported in Chapter 2 “Materials and methods”.

3.3 RESULTS

3.3.1 Patient characteristics

During the study period, 873 patients were admitted to Adult ICU (Figure 3.1). Of these, 838 patients (96%) met the inclusion criteria and were enrolled in the study. There were 333 patients (39.7%) admitted directly to ICU from the community while 419 patients (50%) were transferred to ICU from other hospitals, and 86 patients (10.3%) entered ICU from other general wards at the HTD. 108 patients (12.9%) had a history of previous hospitalization within 90 days. 58 patients (6.9%) had previous antimicrobial therapy within 90 days, and that of within 24 hours prior to ICU admission were 181 patients (21.6%). The common antimicrobials used prior to ICU admission were 3rd-generation cephalosporins (cefpodoxime, cefotaxime, ceftriaxone, ceftazidime), quinolones (ciprofloxacin, levofloxacin) or carbapenems (ertapenem, imipenem, meropenem) which were given as monotherapy or combination therapy.

The median age of subjects was 43 years (IQR 29 - 59), and three quarters of patients were under 60 years of age. Male sex accounted for 59.3%. Nearly 40% of patients had underlying diseases with Charlson Comorbidity Index ≥1. The most common comorbid diagnoses were chronic liver disease (19.1%), and diabetes mellitus (11.1%). The clinical signs leading to ICU admission were respiratory distress (26.8%), narrow pule pressure (21.5%), lockjaw and/or muscle rigidity (14.4%), hypotension (11.3%), seizure and/or laryngeal spasm (11.2%), impaired consciousness (6.9%), and bleeding (5.5%). The median APACHE II score in this study was 8 (IQR 4 - 14). The percentage of patients with mild (<5), moderate (5 - 12) and severe (>12) APACHE II scores were 28.6%, 39.4% and 32%, respectively. Detailed information are shown in Table 3.1.

Figure 3.1 Patient characteristics upon ICU admission

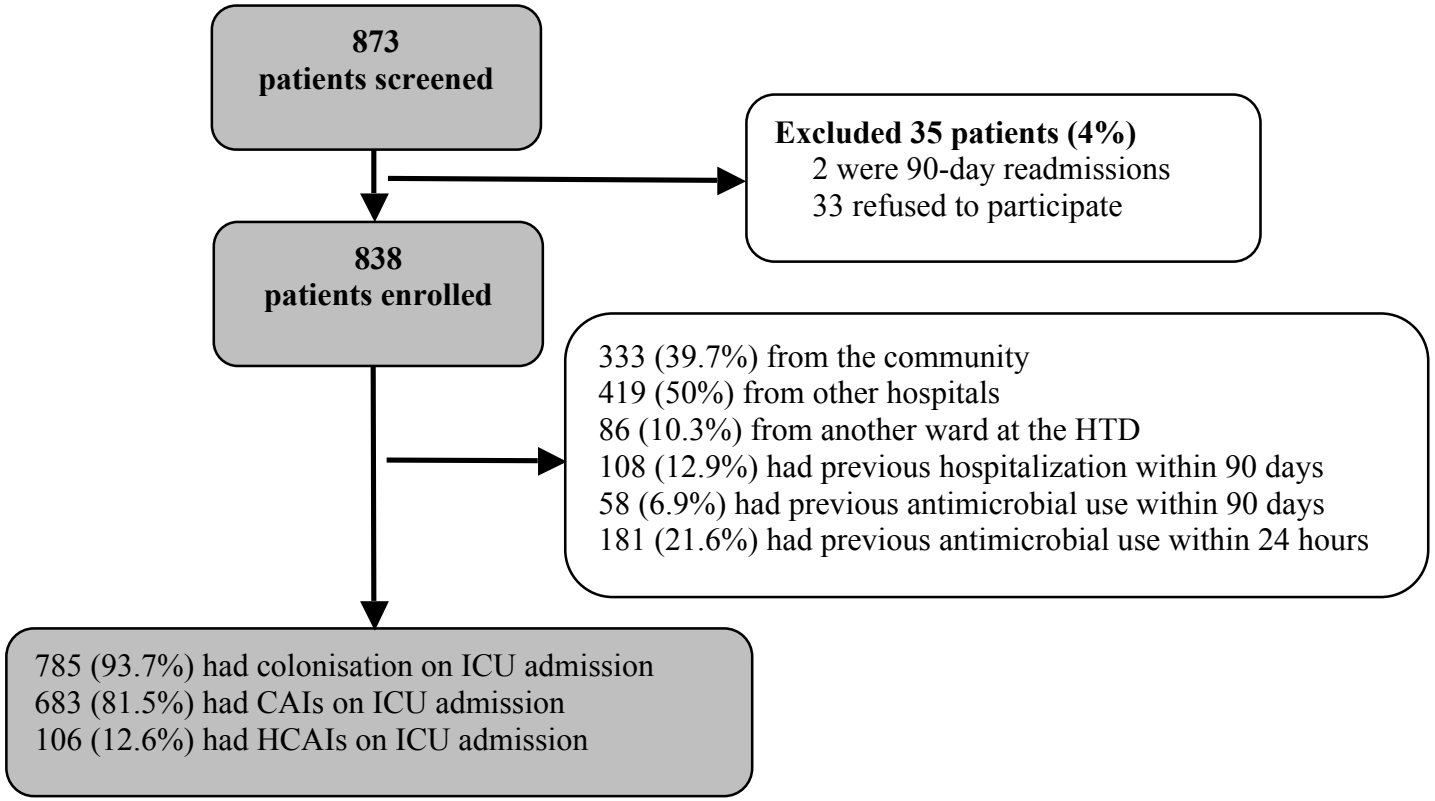


Table 3.1 Patient characteristics upon ICU admission

Age (yr) - median (IQR)	43 (29 - 59)
<60 - n (%)	634 (75.7)
≥60 - n (%)	204 (24.3)
Sex - n (%)	
Male	497 (59.3)
Female	341 (40.7)
Charlson Comorbidity Index - median (IQR)	0 (0 - 1)
No comorbidity (0) - n (%)	512 (61.1)
Mild (1 - 2) - n (%)	166 (19.8)
Moderate (3 - 4) - n (%)	108 (12.9)
Severe (≥5) - n (%)	52 (6.2)
Comorbidities * - n (%)	
Chronic liver disease	160 (19.1)
Diabetes mellitus	93 (11.1)
Corticosteroid therapy	39 (4.7)
HIV infection	29 (3.5)
Chronic lung disease	26 (3.1)
Cardiovascular disease	17 (2)
Reason for ICU admission # - n (%)	
Respiratory distress	225 (26.8)
Narrow pulse pressure (≤20 mmHg)	180 (21.5)
Lockjaw and/or muscle rigidity (tetanus)	121 (14.4)
Hypotension (blood pressure less than 90/60 mmHg)	95 (11.3)
Seizure and/or laryngeal spasm	94 (11.2)
Impaired consciousness (Glasgow Coma Score <15)	58 (6.9)
Bleeding	46 (5.5)
Severe organ injury @	26 (3.1)
Internal medicine diseases $	9 (1.1)
APACHE II score - median (IQR)	8 (4 - 14)
Mild (<5) - n (%)	240 (28.6)
Moderate (5 - 12) - n (%)	330 (39.4)
Severe (>12) - n (%)	268 (32)

* Some patients had >1 underlying disease. # Some patients admitted to ICU with >1 reason.

@ Liver enzymes ≥1.000 IU/L: 11 patients, kidney failure: 6 patients, myocarditis: 3 patients, myocardial infarction: 6 patients.

$ Atrial fibrillation: 3 patients, malignant hypertension: 2 patients, diabetic ketoacidosis: 2 patients, epilepsy: 2 patients.

3.3.2 Characteristics of infections on ICU admission

Out of 838 study patients, 683 (81.5%) suffered CAIs and 106 (12.6%) had HCAIs. Other patients were admitted to ICU with diagnosis of hepatic encephalopathy (24, 2.9%) or non-infectious diseases (25, 3%) (Table 3.2), which were not classified into CAIs or HCAIs. For CAIs, most frequently patients entered ICU for tetanus (215, 31.5%), Dengue infection (211, 30.9%), sepsis (80, 11.7%), septic shock (54, 7.9%) and pneumonia (66, 9.7%). Of HCAIs, sepsis was the most common, found in 40.6% of patients, followed by septic shock (28.3%) and pneumonia (19.8%).

The sites of infection associated with community-acquired sepsis included pneumonia (11, 13.7%), UTI (11, 13.7%), SBP (8, 10%), SSTI (7, 8.7%), gastrointestinal tract infection (4, 5%), bacterial meningitis (3, 3.8%), cholangitis (3, 3.8%), endocarditis (3, 3.8%) and temporal sinusitis (1, 1.3%) (Table 3.3). The specific infection and source of sepsis could not be identified in 36.3% of cases. Out of the 54 community-acquired septic shock cases, the common types of infection were pneumonia (20, 37%), SBP (6, 11.1%), UTI (4, 7.4%), gastrointestinal tract infection (4, 7.4%), bacterial meningitis (4, 7.4%), SSTI (2, 3.7%), liver abscess (1, 1.9%) and appendicitis (1, 1.9%). No focus of infection was found in 22.2% of septic shock cases.

The common causes of infection leading to healthcare-associated sepsis were pneumonia (11, 25.6%), UTI (6, 14%), SBP (6, 14%), gastrointestinal tract infection (1, 2.3%), cholangitis (1, 2.3%) and SSI (spinal operation) (1, 2.3%). One patient (2.3%) suffered UTI and SSTI concomitantly which could not be clarified which one was the primary source of infection leading to sepsis. However, the origin of sepsis could not be identified in 37.2% of cases. Out of 30 patients with healthcare-associated septic shock, the common primary sources of infection were pneumonia (8, 26.7%), SBP (7, 23.3%), UTI (3, 10%) and bacterial pleural effusion (1, 3.3%). No definitive source was found in about 36.7% of patients.

Table 3.2 Diagnosis on ICU admission

Diagnosis - n (%)	**CAIs** [π] **683 (81.5)**	**HCAIs 106 (12.6)**
Communicable diseases		
Tetanus	215 (31.5)	
Dengue infection	211 (30.9)	
Tuberculosis	35 (5.1)	
Scrub typhus	14 (2.1)	
Hepatitis B flare	9 (1.3)	
Melioidosis	5 (0.7)	
Leptospirosis	3 (0.4)	
Systemic infections		
Sepsis	80 (11.7)	43 (40.6)
Septic shock	54 (7.9)	30 (28.3)
Localized infections		
Pneumonia	66 (9.7)	21 (19.8)
Urinary tract infection	15 (2.2)	6 (5.7)
Spontaneous bacterial peritonitis	2 (0.3)	4 (3.8)
Skin and soft tissue infection	4 (0.6)	1 (0.9)
Bacterial pleural effusion	4 (0.6)	1 (0.9)
Other infections [!]	3 (0.4)	
Other diseases		
Hepatic encephalopathy	24 (2.9)	
Non-infectious diseases [∞]	25 (3)	

π Some patients suffered concomitantly 2 different diseases on ICU admission.

! Gastrointestinal tract infection (1 patient), liver abscess (1 patient), kidney abscess (1 patient).

∞ Myocardial infarction (7 patients), atrial fibrillation (4 patients), malignant hypertension (3 patients), chronic obstructive pulmonary disease (5 patients), asthma (2 patients), diabetes mellitus (2 patients), epilepsy (2 patients).

Table 3.3 Sources of infection leading to sepsis and septic shock

Types of infection - n (%)	CAIs		HCAIs	
	Sepsis (n = 80)	Septic shock (n = 54)	Sepsis (n = 43)	Septic shock (n = 30)
Pneumonia	11 (13.7)	20 (37)	11 (25.6)	8 (26.7)
Urinary tract infection	11 (13.7)	4 (7.4)	6 (14)	3 (10)
Spontaneous bacterial peritonitis	8 (10)	6 (11.1)	6 (14)	7 (23.3)
Skin and soft tissue infection	7 (8.7)	2 (3.7)		
Urinary tract infection + Skin and soft tissue infection			1 (2.3)	
Gastrointestinal tract infection	4 (5)	4 (7.4)	1 (2.3)	
Bacterial meningitis	3 (3.8)	4 (7.4)		
Bacterial pleural effusion				1 (3.3)
Liver abscess		1 (1.9)		
Cholangitis	3 (3.8)		1 (2.3)	
Endocarditis	3 (3.8)			
Temporal sinusitis	1 (1.3)			
Appendicitis		1 (1.9)		
Surgical site infection			1 (2.3)	
Unknown	29 (36.3)	12 (22.2)	16 (37.2)	11 (36.7)

Microbiologically-proven infections were found in 117 (35%) of 334 patients with clinically suspected bacterial infection (228 patients with CAIs and 106 patients with HCAIs). The rate of positive cultures stratified by the focus of infection was 40.1% (83/207) for sepsis and septic shock, 5.8% (8/137) for pneumonia, 50% (23/46) for UTI, 54.5% (18/33) for SBP, 46.7% (7/15) for SSTI and 11.1% (4/36) for other infections. A total of 150 bacterial isolates were identified (96 for CAIs and 54 for HCAIs). The bacteria were cultured from blood (85 isolates, 56.6%), urine (23 isolates, 15.3%), abdominal fluid (18 isolates, 12%), wound pus (10 isolates, 6.7%), ETA (9 isolates, 6%), cerebrospinal fluid (3 isolates, 2%), pleural fluid (1 isolate, 0.7%) and sputum (1 isolate, 0.7%). 31 (9.3%) patients were infected with more than two bacterial isolates. Gram-negative bacteria (117 isolates, 78%) were more common than Gram-positive bacteria (33 isolates, 22%).

Microbiological analyses found that 85 different bacterial isolates were responsible for the 207 cases of sepsis and septic shock. Of them, the most frequently involved bacteria were *E. coli* (33, 38.8%), *K. pneumoniae* (11, 12.9%), *Streptococcus suis* (9, 10.6%), *S. aureus* (8, 9.4%) and *B. pseudomallei* (5, 5.9%). Fungal isolates were not collected. Ten different bacterial isolates were detected as the cause of 137 pneumonia cases, of which *K. pneumoniae* and *B. pseudomallei* were the most common (2 isolates for each). For the 46 cases of UTI, 23 bacteria were isolated, including *E. coli* (17, 73.9%), *K. pneumoniae* (3, 13.1%), *Enterococcus faecalis* (2, 8.7%) and *Morganella morganii* (1, 4.3%). SBP (33 cases) was caused by 18 different bacteria, of which *E. coli* (9, 50%) and *K. pneumoniae* (5, 27.8%) were also the most common organisms. The pathogen profile was different for SSTI (15 cases), of which *E. coli* (4 isolates), *K. pneumoniae* (2), *S. aureus* (2), *Aeromonas caviae* (1) and *Vibrio vulnificus* (1) were the causative agents. In general, the most common bacterial causes of CAIs were *E. coli* (37/96 isolates, 38.5%), *K. pneumoniae* (14, 14.6%), *Streptococcus suis* (10, 10.4%), *S. aureus*

(8, 8.3%) and *B. pseudomallei* (7, 7.3%). For HCAIs, *E. coli* (26/54 isolates, 48.1%) and *K. pneumoniae* (10, 18.5%) were the most prevalent causative organisms. Of 26 *E. coli* causing HCAIs, 14 (53.8%) were ESBL-producing *E. coli* and 1 (3.8%) was AmpC-producing *E. coli*. Of 10 *K. pneumoniae* causing HCAIs, 2 were ESBL-producing *K. pneumoniae*, 2 were carbapenemase-producing *K. pneumoniae*, and 1 was ESBL and carbapenemase producing *K. pneumoniae*.

Antimicrobial-resistant infections were microbiologically confirmed in 35 patients (10.5% of 334), of which 19 were CAIs caused by 25 AROs and 16 were HCAIs with 23 AROs. In other words, antimicrobial-resistant infections accounted for 8.3% (19/228) of CAIs and 15.1% (16/106) of HCAIs. In case of CAIs, AROs isolated were ESBL-producing *E. coli* (20, 80% of 25 isolates), MRSA (3, 12%), AmpC-producing *E. coli* (1, 4%) and ESBL-producing *A. baumannii* (1, 4%). In case of HCAIs, AROs cultured were ESBL-producing *E. coli* (14, 60.9% of 23), ESBL-producing *K. pneumoniae* (3, 13.1%), carbapenemase-producing *K. pneumoniae* (2, 8.8%), ESBL and carbapenemase-producing *K. pneumoniae* (1, 4.3%), AmpC-producing *E. coli* (1, 4.3%), ESBL-producing *A. baumannii* (1, 4.3%) and AmpC-producing *Aeromonas hydrophilia* (1, 4.3%).

To sum up, AROs represented 32% of all bacterial isolates (48/150) and ESBL-producing *E. coli* was the most common bacteria (34 isolates, 22.7%). The rate of ESBL in *E. coli* and *K. pneumoniae* was 54% (34/63 *E. coli* isolates) and 12.5% (3/24 of *K. pneumoniae* isolates), respectively. 8.3% (2/24) of *K. pneumoniae* isolates were carbapenem resistant and 4.2% (1/24) was ESBL and carbapenemase-producing *K. pneumoniae*. All 3 resistant *K. pneumoniae* isolates were responsible for HCAIs (1 case of sepsis and 2 cases of UTI). MRSA accounted for 30% (3/10) of *S. aureus isolates* and they were also the causative organism of community-acquired sepsis. Detailed information are described in Table 3.4.

Table 3.4 Bacteria causing infections on ICU admission (including CAIs and HCAIs)

Microorganisms - n (%)		Pathogens for					
	All pathogens isolated (n = 150)	Sepsis and septic shock (n = 207)	Pneumonia (n = 137)	Urinary tract infection (n = 46)	Spontaneous bacterial peritonitis (n = 33)	Skin and soft tissue infections (n = 15)	Other infections (n = 36)
Gram-positive cocci	**33 (22)**						
Staphylococcus aureus	**10 (3 MRSA)**	8 (3 MRSA [*])				2	
Streptococcus anginosus	**2**	2					
Streptococcus gallolyticus	**3**	2			1		
Streptococcus mitis	**1**		1				
Streptococcus pasteurianus	**1**						1 [¥]
Streptococcus pneumoniae	**1**		1				
Streptococcus pyogenes	**1**	1					
Streptococcus salivarius	**1**	1					
Streptococcus suis	**10**	9					1 [¥]
Enterococcus faecalis	**2**			2			
Enterococcus faecium	**1**	1					
Enterobacteriaceae	**88 (58.7)**						
Enterobacter sakazaki	**1**				1		
Escherichia coli	**63 (34 ESBL, 2 AmpC)**	33 (19 ESBL [Ø], 1 AmpC [#])		17 (10 ESBL [@], 1 AmpC [*])	9 (3 ESBL [$])	4 (2 ESBL [∞])	
Klebsiella pneumoniae	**24 (3 ESBL, 2 CPO, 1 ESBL+CPO)**	11 (1 CPO [#])	2 (1 ESBL [#])	3 (1 ESBL [#], 1 CPO [#], 1 ESBL+CPO [#])	5	2 (1 ESBL [#])	1 [¥]

Nonenterobacterial gram-negative bacteria	**29 (19.3)**						
Acinetobacter baumannii	**3 (2 ESBL)**	2 (1 ESBL [*])			1 (1 ESBL)		
Aeromonas caviae	**1**					1	
Aeromonas hydrophilia	**2 (1 AmpC)**	1					1 [€] (1 AmpC [#])
Aeromonas jandaei	**1**	1					
Burkholderia pseudomallei	**7**	5	2				
Haemophilus influenzae	**1**		1				
Moraxella asloensis	**1**	1					
Moraxella spp	**1**	1					
Morganella morganii	**1**			1			
Pseudomonas aeruginosa	**2**	1	1				
Pseudomonas mendocina	**1**	1					
Pseudomonas putida	**1**		1				
Sphingomonas paucimobilis	**1**	1					
Stenotrophomonas maltophilia	**1**		1				
Vibrio albensis	**2**	1			1		
Vibrio vulnificus	**3**	2				1	

¥ Bacterial meningitis, € Bacterial pleurisy, * All were CAIs, # All were HCAIs, Ø Of which, 10 were CAIs and 9 were HCAIs, @ Of which, 8 were CAIs and 2 were HCAIs, $ Of which, 1 was CAIs and 2 were HCAIs, ∞ Of which, 1 was CAIs and 1 was HCAIs

3.3.3 Management

Initial management in the first 48 hours of ICU admission was evaluated in all 838 study patients (Table 3.5). 98 patients (11.7%) had a central venous catheter, 82 (9.8%) had an arterial catheter, 128 (15.3%) had a urinary catheter, 138 (16.5%) had a nasogastric tube and 12 (1.4%) underwent lumbar puncture. Abdominal paracentesis was performed in 59 patients (7%) for diagnosis and in 2 (0.2%) for treatment of respiratory distress. Pleural paracentesis was undertaken in 16 patients (1.9%) for diagnosis and in 7 (0.8%) for management of respiratory failure. 352 patients (42%) were given supplementary oxygen through nasal cannula or face mask, 78 (9.3%) were intubated orally and 53 (6.3%) received tracheostomy. 11 patients (1.3%), 115 (13.7%) and 32 (3.8%) were on non-invasive ventilation, invasive ventilation and renal replacement therapy, respectively during the first 48 hours of ICU admission. Vasopressors were used in 95 patients (11.3%). Packed red blood cells, fresh frozen plasma, cryoprecipitate and platelets were transfused in 37 patients (4.4%), 22 patients (2.6%), 12 patients (1.4%) and 15 patients (1.8%), respectively.

Table 3.5 Management in the first 48 hours of ICU admission

Interventions	n (%)
Antimicrobial therapy	605 (72.2)
Oxygen therapy (nasal cannula or mask)	352 (42)
Intubation	78 (9.3)
Tracheostomy	53 (6.3)
Non-invasive ventilation	11 (1.3)
Invasive ventilation	115 (13.7)
Hemofiltration	32 (3.8)
Vasopressors	95 (11.3)
Transfusion of red blood cells	37 (4.4)
Transfusion of fresh frozen plasma	22 (2.6)
Transfusion of cryoprecipitate	12 (1.4)
Transfusion of platelets	15 (1.8)
Diagnostic abdominal paracentesis	59 (7)
Therapeutic abdominal paracentesis	2 (0.2)
Diagnostic pleural paracentesis	16 (1.9)
Therapeutic pleural paracentesis	7 (0.8)
Central venous catheter	98 (11.7)
Arterial catheter	82 (9.8)
Urinary catheter	128 (15.3)
Nasogastric tube	138 (16.5)
Lumbar puncture	12 (1.4)

The percentage of patients being treated with antimicrobials in the first 48 hours of ICU hospitalization was 72.2% (605 patients) (Table 3.5). Detailed antimicrobial regimens for 334 patients being clinically suspected of bacterial infection (228 patients with CAIs and 106 patients with HCAIs) are summarized in Table 3.6. Ceftriaxone was the most common prescribed antimicrobial which could be monotherapy or combination therapy and ranged from 30.6% to 66.7% per origin of infection (not including SSTI). Levofloxacin (11.7%) or azithromycin (17.5%) was given to cover atypical bacteria causing pneumonia while cotrimoxazole (sulfamethoxazole/trimethoprim) (5.1%) was combined in case of suspected *Pneumocystis jirovecii* pneumonia in HIV infected patients. Doxycycline (4.4%) was the main antimicrobial for suspected scrub typhus. For UTI, the second most frequently used antimicrobial was levofloxacin which accounted for 21.7% of infection cases. For SSTI, oxacillin (33.3%) and vancomycin in combination with ceftriaxone (20%) or imipenem (20%) were a common practice. In total, ceftriaxone (44.6%) was the most frequently prescribed antimicrobial agent for treatment of infection in Adult ICU of the HTD, followed by imipenem (13.5%).

Microbiological information was not available in the first 48 hours of ICU admission, so all of the above antimicrobial choices were empirical therapy. Inappropriate initial antimicrobial use was evaluated once the microbiological results became available (Table 3.7). 23/89 CAIs (25.8%) received inappropriate antimicrobial therapy, with a similar pattern for HCAIs (13/54, 24.1% inappropriate). Taking into account of infection types, the percentage of inappropriate antimicrobial indication in sepsis and septic shock was 25.3% (21/83). Those of pneumonia, UTI, SBP, SSTI were 25% (2/8), 43.5% (10/23), 11.1% (2/18) and 14.3% (1/7), respectively.

Table 3.6 Antimicrobial use on admission to ICU

Antimicrobial therapy n (%)	Pneumonia (n = 137)	Urinary tract infection (n = 46)	Spontaneous bacterial peritonitis (n = 33)	Skin and soft tissue infections (n = 15)	Other infections (n = 36)	Unknown (n = 67)	Total (n = 334)
Ceftriaxone	42 (30.6)	20 (43.5)	20 (60.6)		24 (66.7)	43 (64.1)	149 (44.6)
Ceftriaxone + Amikacin		4 (8.7)			1 (2.8)	1 (1.5)	6
Ceftriaxone + Levofloxacin	16 (11.7)			1 (6.7)	1 (2.8)	3 (4.5)	21 (6.3)
Ceftriaxone + Azithromycin	24 (17.5)						24 (7.2)
Ceftriaxone + Cotrim *	7 (5.1)						7
Ceftriaxone + Doxycycline #	6 (4.4)						6
Ceftriaxone + Vancomycin				3 (20)	1 (2.8)	1 (1.5)	5
Imipenem	13 (9.5)	7 (15.2)	9 (27.3)		2 (5.5)	14 (20.9)	45 (13.5)
Imipenem + Azithromycin or Levofloxacin	3 (2.2)						3
Imipenem + Vancomycin	9 (6.6)	1 (2.2)	1 (3)	3 (20)	2 (5.5)	1 (1.5)	17 (5.1)
Meropenem	3 (2.2)					3 (4.5)	6 (1.8)
Meropenem + Vancomycin	2 (1.5)	1 (2.2)			1 (2.8)	1 (1.5)	5
Ertapenem		1 (2.2)	3 (9.1)		1 (2.8)		5
Levofloxacin	1 (0.7)	10 (21.7)					11 (3.3)
Oxacillin				5 (33.3)	1 (2.8)		6
Vancomycin					2 (5.5)		2
Other regimes	11 (8) [@]	2 (4.3) [$]	NA	3 (20) [∞]	NA	NA	16

* HIV infection, # Scrub typhus, @ Azithromycin (2), doxycyline (4), cotrim (5); $ Piperacillin/tazobactam (1), amoxcillin-clavulanic acid (1)
∞ Piperacillin/tazobactam (1), piperacillin/tazobactam + vancomycin (2)

Table 3.7 Inappropriate antimicrobial use for microbiologically proven infections

Types of infection (n = 143)	CAIs (n = 89)		HCAIs (n = 54)	
	Appropriate	Inappropriate *	Appropriate	Inappropriate *
Sepsis	19 (65.5)	10 (34.5)	15 (75)	5 (25)
Septic shock	19 (86.4)	3 (13.6)	9 (75)	3 (25)
Pneumonia	4 (66.7)	2 (33.3)	2 (100)	0
Urinary tract infection	8 (57.1)	6 (42.9)	5 (55.6)	4 (44.4)
Spontaneous bacterial peritonitis	9 (90)	1 (10)	7 (87.5)	1 (12.5)
Skin and soft tissue infection	5 (83.3)	1 (16.7)	1 (100)	0
Other infections	2 (100)	0	2 (100)	0

* Inappropriate antimicrobial treatment was defined as use of antimicrobials to which bacterial isolates were detected to be resistant in vitro.

3.3.4 Admission colonisation status

A total of 1,829 swabs were taken within 48 hours of ICU admission, of which 838 were nasal swabs, 838 were rectal swabs and 153 were ETA samples. The culture results are presented in Table 3.8.

3.3.4.1 Nasal colonisation

The nasal nares of 148 (17.7%) patients were colonised with at least one of the five study bacteria. 110 patients (13.1%) had nasal *S. aureus* colonisation: 72 (65.5%) MRSA, and 38 (34.5%) MSSA. The number of nasal *K. pneumoniae* colonised patients was 23 (2.7%), of whom 2 (8.7%) had ESBL-producing *K. pneumoniae*. 21 (2.5%) patients had nasal *E. coli* colonisation (15 with ESBL-producing *E. coli* and 2 with AmpC-producing *E. coli*).

3.3.4.2 Rectal colonisation

762 (90.9%) patients had rectal colonisation with one or more of the bacteria of interest. 740 (88.3%) had rectal *E. coli* colonisation. Of them, 437 (59.1%) patients were colonised with ESBL-producing *E. coli*, 45 (6.1%) with AmpC-producing *E. coli*, and 8 (1.1%) with carbapenemase-producing *E. coli*. 268 (32%) patients had rectal *K. pneumoniae* colonisation. The number of colonised patients with ESBL, AmpC and carbapenemase-producing *K. pneumoniae* were 41 (15.3%), 34 (12.7%) and 9 (3.4%), respectively. The number of rectal *P. aeruginosa* colonised patients were 22 (2.6%), of whom 4 had carbapenemase-producing *P. aeruginosa*. The number of rectal *S. aureus* colonised patients were 15 (11 MRSA).

3.3.4.3 Endotracheal colonisation

ETA samples were taken from 153 out of 838 study subjects in the first 48 hours of ICU admission. They were taken when tracheostomy was performed due to severe tetanus (78 patients, 50.9%) or intubation due to sepsis (30, 19.6%), septic shock (30, 19.6%), severe Dengue infection (9, 5.9%), hepatic encephalopathy (4, 2.6%), severe malaria (1, 0.7%) and myocarditis with cardiogenic shock (1, 0.7%). Of these 153 subjects, 37 (24.2%) had tracheal colonisation with one of the five study bacteria. 18 (11.8%) were colonised with *K. pneumoniae*. 14 (9.2%) were colonised with *S. aureus* (8 MRSA and 6 MSSA) and 6 of them also had nasal *S. aureus* colonisation (5 MRSA and 1 MSSA).

Overall, the colonisation rate at all sites of *S. aureus*, *E. coli*, *Klebsiella* spp., *Acinetobacter* spp. and *Pseudomonas* spp. was 15.3% (128 patients), 89.1% (747), 35.3% (296), 1.1% (9) and 3.7% (31), respectively. The colonisation rate at all sites with one of the five study bacteria on ICU admission was 93.7% (785/838). Colonisation also varied in different subgroups of patients: 95.8% (319/333) for subjects originated from the community, 77.6% (392/505) for those being transferred to ICU from other hospitals ($p \leq 0.001$, OR = 6.57, 95% CI (3.7 - 11.67)).

For AROs, 87 (10.4%) patients were colonised with MRSA at any of the sites. The rate of ESBL-producing *E. coli* colonisation was 51.3% (430/838) and that of ESBL-producing *K. pneumoniae* was 3.9% (33/838). 8 (1%) patients colonised with carbapenemase-producing *E. coli* whereas 9 (1.1%) with carbapenemase-producing *K. pneumoniae*. 5 (0.6%) patients were found to be carbapenemase-producing *Acinetobacter* spp. and that of carbapenemase-producing *Pseudomonas* spp. was 7 (0.8%). In this study, colonised patients with at least one of AROs in the nasal cavity, rectal cavity and endotracheal tract were 88 (10.5% of 838), 491 (58.6% of 838), 15 (9.8% of 153) respectively. The overall rate of AROs colonisation on ICU admission was 63.1% (529/838). 62.2% (207/333) patients admitted from the community were colonised with AROs and that of patients transferred from other hospitals was 63.8% (322/505) ($p = 0.64$).

3.3.4.4 Antimicrobial susceptibility of colonizing bacteria on ICU admission

A total of 1,905 bacterial isolates were cultured within the first 48 hours in ICU, of which 143 (7.5%) were *S. aureus*, 1370 (71.9%) were *E. coli*, 345 (18.1%) were *Klebsiella* spp., 15 (0.8%) were *Acinetobacter* spp., and 32 (1.7%) were *Pseudomonas* spp.. Their antimicrobial susceptibility is summarized in Table 3.9 according to resistance in absolute number and percentage. MRSA accounted for 63.6% of all *S. aureus* isolates. The proportion of resistance to ciprofloxacin, levofloxacin and clindamycin was 49%, 49% and 62.2%, respectively. Vancomycin-resistant *S. aureus* was not detected. 3rd-generation cephalosporin and ciprofloxacin resistance in *E. coli* were about 50% for each. 3rd-generation cephalosporin and ciprofloxacin resistance in *Klebsiella* spp. were about 20% and 12.5%, respectively. Ceftazidime-resistant *Pseudomonas* spp. was nearly 10%. Carbapenem resistance was also reported for all Gram-negative bacteria cultured in this study. Noticeably, 60% of *Acinetobacter* spp. were resistant to imipenem or meropenem. Colistin, the antimicrobial of last resort for

carbapenemase-producing organisms, was also reported to be resistant among *E. coli* and *Klebsiella* spp. (3.6% and 6.4%, respectively).

Table 3.8 Admission colonisation status of 838 enrolled patients (pts)

Samples (Total pts Total swabs)	No. isolates No.(%) positive pts	*E. coli* No. isolates No.(%) colonised pts (No. colonised pts with AROs)	*K. pneumonia* No. isolates No.(%) colonised pts (No. colonised pts with AROs)	*A. baumannii* No. isolates No.(%) colonised pts (No. colonised pts with AROs)	*P. aeruginosa* No. isolates No.(%) colonised pts (No. colonised pts with AROs)	*S. aureus* No. isolates No.(%) colonised pts (No. colonised pts with AROs)
Nasal swabs (838 pts 838 swabs)	**173 isolates** **148 (17.7)**	**26** **21 (2.5)** **(15 ESBL, 2 AmpC)**	**23** **23 (2.7)** **(2 ESBL)**	**5** **4 (0.5)** **(4 ESBL+CPO)** *Acinetobacter* **spp 1** **1 (0.1)** **(1 ESBL)**	**4** **3 (0.4)**	**114** **110 (13.1)** **(72 MRSA)**
Rectal swabs (838 pts 838 swabs)	**1,689 isolates** **762 (90.9)**	**1339** **740 (88.3)** **(437 ESBL, 45 AmpC, 8 CPO)**	**300** **268 (32)** **(41 ESBL, 34AmpC, 9 CPO)** *Klebsiella* **spp 4** **4 (0.5)** **(1 AmpC)**	**5** **5 (0.6)** **(1 ESBL, 2 ESBL+CPO)** *Acinetobacter* **spp 2** **(0.2)** **(1 ESBL)**	**22** **22 (2.6)** **(4 CPO)** *Pseudomonas* **spp** **2 (0.2)** **(1 CPO)**	**15** **15 (1.8)** **(11 MRSA)**
Endotracheal aspirate (153 pts 153 swabs)	**43 isolates** **37 (24.2)**	**5** **5 (3.3)** **(3 ESBL)**	**18** **18 (11.8)** **(2 ESBL)**	**1** **1 (0.7)** **(1 ESBL+CPO)** *Acinetobacter* **spp 1** **1 (0.7)**	**2** **2 (1.3)** *Pseudomonas* **spp 2** **2 (1.3)** **(1 ESBL+CPO, 1 CPO)**	**14** **14 (9.2)** **(8 MRSA)**

Table 3.9 Antimicrobial resistance of colonizing bacteria on ICU admission

Antimicrobials n (%)	***S. aureus* (n=143)**	***E. coli* (n=1370)**	***Klebsiella* spp. (n=345)**	***Acinetobacter* spp. (n=15)**	***Pseudomonas* spp. (n=32)**
Amoxcillin-clavulanic acid		746 (54.5)	82 (23.8)		
Ceftazidime		**702 (51.2)**	**74 (21.4)**	13 (86.7)	**3 (9.4)**
Ceftriaxone		**703 (51.3)**	**76 (22)**	13 (86.7)	
Cefepime		639 (46.4)	56 (16.2)	12 (80)	3 (9.4)
Ticarcillin-clavulanate		828 (60.4)	87 (25.2)	12 (80)	21 (65.6)
Piperacillin-tazobactam		651 (47.5)	51 (14.8)	12 (80)	3 (9.4)
Ofloxacin		632 (46.1)	31 (9)		
Ciprofloxacin	**70 (49)**	646 (47.2)	43 (12.5)		
Levofloxacin	**70 (49)**			10 (66.7)	3 (9.4)
Sulfamethoxazole-trimethoprim	5 (3.5)	953 (69.6)	106 (30.7)	7 (46.7)	29 (90.6)
Amikacin		26 (1.9)	8 (2.3)	6 (40)	2 (6.3)
Gentamycin					2 (6.3)
Ertapenem		**29 (2.1)**	**10 (2.9)**		
Imipenem		**13 (0.9)**	**10 (2.9)**	**9 (60)**	**6 (18.8)**
Meropenem		**15 (1.1)**	**10 (2.9)**	**9 (60)**	**7 (21.9)**
Colistin		**50 (3.6)**	**22 (6.4)**	0	0
Penicillin	137 (95.8)				
Oxacillin	**91 (63.6)**				
Vancomycin	0				
Erythromycin	95 (66.4)				
Rifampicin	3 (2.1)				
Clindamycin	**89 (62.2)**				

3.3.5 Characteristics of colonised and non-colonised patients on ICU admission

The goal of this study was also to characterize colonised and non-colonised patients on ICU admission in order to determine the risk factors of colonisation for future intervention projects. However, the colonisation on admission with *Acinetobacter* spp. and *Pseudomonas* spp. seemed to be too small for reliable analysis. Moreover, most patients had *E. coli* in their stool. Therefore, I just focused on some clinically important bacteria: *S. aureus*, MRSA, antimicrobial-resistant *E. coli* (including 3rd-generation cephalosporin-resistant *E. coli* and carbapenem-resistant *E. coli*), and antimicrobial-resistant *Klebsiella* spp. (including 3rd-generation cephalosporin-resistant *Klebsiella* spp. and carbapenem-resistant *Klebsiella* spp.). In univariate analysis, no significant risk factors were associated with *S. aureus* nasal colonisation (Table 3.10) or MRSA nasal carriage (Table 3.11). In terms of rectal antimicrobial-resistant *E. coli* or *Klebsiella* spp. colonisation, I did not find any clinical characteristics that remained significantly associated with colonisation (Table 3.12 and Table 3.13).

Table 3.10 Clinical characteristics of *S. aureus* nasal colonisation (univariate analysis)

Characteristics	*S. aureus* (n = 110)	Non-*S. aureus* (n = 728)	p	OR (95% CI)
Age (mean ± SD) (yrs)	42.5 ± 17.3	45 ± 19	0.16	
Male sex (n, %)	65 (59.1)	432 (59.3)	0.96	
Charlson Comorbidity Index score (mean ± SD)	1 ± 1.7	1 ± 1.7	0.82	
APACHE II score (mean ± SD)	9.3 ± 6.4	9.8 ± 7.1	0.39	
Hospital stay ≥ 48hrs prior to ICU (n, %)	26 (23.6)	132 (18.1)	0.17	
ICU stay prior to ICU (n, %)	7 (6.4)	67 (9.2)	0.33	
Invasive procedures prior to ICU (n, %) *	9 (8.2)	71 (9.8)	0.6	
Antimicrobial use within 24hrs prior to ICU (n, %)	16 (14.5)	165 (22.7)	0.05	
History of previous hospitalization within 90 days prior to ICU (n, %)	16 (14.5)	92 (12.6)	0.58	
History of previous ICU hospitalization within 90 days prior to ICU (n, %)	2 (1.8)	16 (2.2)	0.8	
History of previous antimicrobial use within 90 days prior to ICU (, n%)	9 (8.2)	49 (6.7)	0.58	

* Invasive procedures prior to ICU admission: intubation, tracheostomy, mechanical ventilation, central venous catheter, arterial catheter, urinary catheter, nasogastric tube.

Table 3.11 Clinical characteristics of MRSA/MSSA nasal colonisation (univariate analysis)

Characteristics	MRSA (n = 72)	MSSA (n = 766)	p	OR (95% CI)
Age (mean ± SD) (yrs)	42.4 ± 18.2	44.9 ± 18.9	0.28	
Male sex (n, %)	40 (55.6)	457 (59.7)	0.5	
Charlson Comorbidity Index score (mean ± SD)	1 ± 1.8	1.1 ± 1.7	0.66	
APACHE II score (mean ± SD)	9.7 ± 6.5	9.8 ± 7	0.89	
Hospital stay ≥ 48hrs prior to ICU (n, %)	16 (22.2)	142 (18.5)	0.44	
ICU stay prior to ICU (n, %)	5 (6.9)	69 (9.0)	0.56	
Invasive procedures prior to ICU (n, %) *	7 (9.7)	73 (9.5)	0.96	
Antimicrobial use within 24hrs prior to ICU (n, %)	10 (13.9)	171 (22.3)	0.1	
History of previous hospitalization within 90 days prior to ICU (n, %)	12 (16.7)	96 (12.5)	0.32	
History of previous ICU hospitalization within 90 days prior to ICU (n, %)	2 (2.8)	16 (2.1)	0.7	
History of previous antimicrobial use within 90 days prior to ICU (n, %)	7 (9.7)	51 (6.7)	0.33	

* Invasive procedures prior to ICU admission: intubation, tracheostomy, mechanical ventilation, central venous catheter, arterial catheter, urinary catheter, nasogastric tube.

Table 3.12 Clinical characteristics of antimicrobial-resistant *E. coli* rectal colonisation (univariate analysis)

Characteristics	Resistant *E. coli* (n = 460)	Non-resistant *E. coli* (n = 378)	p	OR (95%CI)
Age (mean ± SD) (yrs)	43.8 ± 19	45.7 ± 18.6	0.16	
Male sex (n, %)	266 (57.8)	231 (61.1)	0.34	
Charlson Comorbidity Index score (mean ± SD)	1 ± 1.7	1.1 ± 1.8	0.24	
APACHE II score (mean ± SD)	9.7 ± 6.8	9.9 ± 7.2	0.77	
Hospital stay ≥ 48hrs prior to ICU (n, %)	83 (18)	75 (19.8)	0.51	
ICU stay prior to ICU (n, %)	45 (9.8)	29 (7.7)	0.28	
Invasive procedures prior to ICU (n, %) *	52 (11.3)	28 (7.4)	0.06	
Antimicrobial use within 24hrs prior to ICU (n, %)	97 (21.1)	84 (22.2)	0.69	
History of previous hospitalization within 90 days prior to ICU (n, %)	55 (12)	53 (14)	0.37	
History of previous ICU hospitalization within 90 days prior to ICU (n, %)	8 (1.7)	10 (2.6)	0.37	
History of previous antimicrobial use within 90 days prior to ICU (n, %)	28 (6.1)	30 (7.9)	0.29	

* Invasive procedures prior to ICU admission: intubation, tracheostomy, mechanical ventilation, central venous catheter, arterial catheter, urinary catheter, nasogastric tube.

Table 3.13 Clinical characteristics of antimicrobial-resistant *Klebsiella* spp. rectal colonisation (univariate analysis)

Characteristics	Resistant *Klebsiella* spp. (n=64)	Non-resistant *Klebsiella* spp. (n=774)	p	OR (95% CI)
Age (mean ± SD) (yrs)	44.5 ± 17.6	44.7 ± 18.9	0.92	
Male sex (n, %)	39 (60.9)	458 (59.2)	0.78	
Charlson Comorbidity Index score (mean ± SD)	1.2 ± 1.8	1 ± 1.7	0.56	
APACHE II score (mean ± SD)	9.2 ± 6.8	9.8 ± 7	0.49	
Hospital stay ≥ 48hrs prior to ICU (n, %)	8 (12.5)	150 (19.4)	0.18	
ICU stay prior to ICU (n, %)	7 (10.9)	67 (8.7)	0.54	
Invasive procedures prior to ICU (n, %) *	6 (9.4)	74 (9.6)	0.96	
Antimicrobial use within 24hrs prior to ICU (n, %)	18 (28.1)	163 (21.1)	0.19	
History of previous hospitalization within 90 days prior to ICU (n, %)	7 (10.9)	101 (13)	0.63	
History of previous ICU hospitalization within 90 days prior to ICU (n, %)	0 (0)	18 (2.3)	0.22	
History of previous antimicrobial use within 90 days prior to ICU (n, %)	3 (4.7)	55 (7.1)	0.46	

* Invasive procedures prior to ICU admission: intubation, tracheostomy, mechanical ventilation, central venous catheter, arterial catheter, urinary catheter, nasogastric tube.

3.4 DISCUSSION

This study showed several interesting features of the infection, colonisation and resistance dynamic of *S. aureus*, *E. coli, Klebsiella* spp., *Acinetobacter* spp. and *Pseudomonas* spp. isolated among 838 enrolled ICU patients. Sepsis and septic shock represented a high percentage of 14.7% (123/838) and 10% (84/838), respectively among study patients on ICU admission (Table 3.2). The most frequent sites of infection leading to sepsis or septic shock were pneumonia, UTI, SBP, SSTI, and gastrointestinal tract infection (Table 3.3). This is not much different from the other epidemiological studies. Of 1,078 adult patients admitted to the Emergency Department at an urban referral hospital in Haiti in 2012, 224 (20.8%) had sepsis and 99 (9.2%) had severe sepsis [238], while rates of 8% for severe sepsis and 16.5% for septic shock were reported in a Portuguese mixed ICU in 2004 - 2008 [239]. Respiratory, intra-abdominal, urinary tract, and bone/soft tissue infections were identified as the most common sites of infection leading to sepsis and severe sepsis in most ICUs in mainland China, Japan, Portugal and Germany [240-243].

Microbiologically-proven infections were reported to be 30.6% for severe sepsis and septic shock in mainland China in 2009 [240], and 56% for community-acquired severe sepsis in Portugal from 2004 to 2008 [239] compared to 40.1% (83/207) for sepsis and septic shock in my study. The reason may be variable microbiology laboratory facilities and quality control procedures [243]. Moreover, prompt antimicrobial treatment or previous antimicrobial use before taking cultures, the microbiologically positive culture rates may be influenced. Similar to other studies conducted in Portugal, mainland China and Japan [240-242], Gram-negative bacteria were found to be more involved in infections than Gram-positive strains for both CAIs and HCAIs in my study. However, there are no comparable studies about microbiological etiology of infections among ICU patients in Vietnam. Factors significantly associated with antimicrobial-resistant infections

included polymicrobial infection, the presence of a feeding tube, the presence of an urinary catheter, receipt of antimicrobials within 14 days before admission, previous hospitalization within 30 days before admission and the presence of decubitus ulcers [244]. However, I could not assess some of these risk factors because antimicrobial-resistant infections were confirmed in only 35 study patients (19 CAIs and 16 HCAIs) which was too small for reliable analysis. Therefore, more research on AROs from different levels of healthcare services from central to commune is needed for the development and implementation of standard antimicrobial guidelines in Vietnam.

Bacterial colonisation in ICU patients has been studied in some areas around the world. In Turkey, general colonisation with either MRSA, VRE, ESBL-producing *E. coli*, ESBL-producing *Klebsiella* spp., *P. aeruginosa*, *Acinetobacter* spp. or *Candida* spp. on ICU admission was observed in 56.8% (54/95) of patients [2]. The rate of colonisation in patients from community was 60% (9/15), from other services and hospitals was 56.3% (45/80) [2]. It was lower than my study: 95.8% for from community, 77.6% from other clinical settings. This could be explained by a vast difference in the size of study population (95 vs 838) and study swabs taken (mouth/throat, rectal and skin swabs compared to nasal, rectal swabs, and ETA) or microbiological methods. A 5-year study performed in Shanghai in 2006 - 2010 showed that the most common colonizing and pathogenic ICU-on-admission isolates were *P. aeruginosa* (19.6%), *A. baumannii* (15.6%), *K. pneumoniae* (13.3%), *S. aureus* (10.6%) and *E. coli* (7.6%) [245]. However, these strains were cultured from a wide range of specimens (sputum, ETA, oral swab, urine, blood, catheters, and drainage samples). So, it is striking the higher isolates of *P. aeruginosa* and *A. baumannii* on ICU admission compared to my study. It is, therefore, necessary to have a consensus on how to monitor bacterial colonisation among ICU patients for global perspectives and research. In my study, *S. aureus* was representing the most common microorganism colonizing in the nares with the colonisation rate of

13.1% on ICU admission, which was higher than nasal *S. aureus* colonisation among Turkish adult outpatients (8.1%, 23/283) [246]. However, it was lower than other countries, for example, nasal *S. aureus* colonisation rate among healthy adult people in northern China was 16.5% (403/2,448) [247], 27 - 30.7% in the USA [248,249], and 28.2% (102/362) in the UK [250]. *S. aureus* nasopharyngeal colonisation rate among adults in urban and rural northern Vietnam was found to be 21.1% (140/662) [180]. In addition, nasal *S. aureus* colonisation rate among patients, relatives and healthcare workers in Malaysia was 27.1% (146/538) [251]. In terms of nasal MRSA colonisation on ICU admission, the prevalence of 8.6% (72/838) in my study was much higher than that in a 2-year study conducted in a tetanus ICU of the HTD from 2004 to 2006 (2.9%, 5/174) [179]. This suggests that Vietnam is becoming a new MRSA hotspot in the world. Other studies also showed a considerable variety of MRSA colonisation rates between countries: nasal colonisation rate among healthy adult people in northern China (0.3%, 8/2,448) [247], 0.9% in the USA [38] and 1.1% (4/362) in the UK [250]. Nasopharyngeal MRSA colonisation rate among adults in urban and rural northern Vietnam was reported 4.2% (28/662) [180]. Admission rate of nasal colonisation with MRSA among inpatients in southern India was 2.3% (16/683) [252], and that of outpatients in Turkey was 0 - 6.2% [246,253].

The studies of incidence and pattern of Gram-negative bacteria, especially AROs colonisation in ICU patients are limited with rates being reported differently according to the regional areas and patient populations studied. A surveillance and prevention program of multidrug-resistant organisms in Chinese ICUs reported the prevalence of 32.7% on admission for ESBL-producing *Enterobacteriaceae* [254]. A study conducted in a tetanus ICU in the HTD from 2004 to 2006 also confirmed a rate of 12.6% (22/174) for ESBL-producing *Enterobacteriaceae* (excluding *K. pneumoniae*) on admission [179]. Another study in North India revealed that ICU-admission colonisation with ESBL- and

MBL-producing Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *Enterobacter* species, *Citrobacter* species, *A.baumannnii* and *P. aeruginosa*) was detected in 53.1% (51/96) of patients [61]. They were all lower than my study, 55.2% for ESBL-producing *Enterobacteriaceae*. This again suggests that Vietnam is a new hotspot for AROs. An observational cohort study conducted in two Greek general ICUs from 2008 to 2011 found that 3.6% (36/1,007) of patients were colonised with carbapenemase-producing *Enterobacteriaceae* on admission [255] (2.1% for both of carbapenemase-producing *E. coli* and *K. pneumoniae*). Moreover, upon admission to a general ICU of a referral hospital in the Netherlands (2000 - 2003), 14% (25/183) of patients were colonised with at least one *E. coli* isolate that was resistant to one or more antibiotics [60], while I found that 55.4% of patients had 3rd-generation cephalosporin and carbapenem-resistant *E. coli*. In fact, a high rate of ESBL-producing *E. coli* (around 50%) was reported in healthy Vietnamese individuals living in a rural area of each sampling every 6 months from June 2013 to June 2014 [181]. In addition, a prospective study being conducted in a French ICU indicated that 15% of patients were detected to be colonised with ESBL-producing *Enterobacteriaceae* on admission, mostly of *E. coli* or *K. pneumoniae* [62], which was much lower than my study (51.3% for ESBL-producing *E. coli* and 3.9% for ESBL-producing *K. pneumoniae*). It can be explained that just only rectal swabs were collected from French patients for screening ESBL-producing *Enterobacteriaceae* while my study samples came from nasal swabs, rectal swabs and ETA. All things confirmed again that AROs are circulating at a high level not only in hospitals but also in the community, representing a serious threat to public health in Vietnam.

The risk factors of bacterial colonisation on ICU admission have been well studied in some countries [58,63,110,137,268-270]. For example, a study being conducted in North India revealed that co-morbidities, a hospital stay ≥48 hours, use of ≥3 groups of antimicrobials, and mechanical ventilation ≥48 hours before transfer to ICU was

associated with risk of colonisation with ESBL- and MBL-producing Gram-negative bacteria in patients at ICU admission [61]. Prior hospitalization ≥48 hours was also identified as a risk factor for colonisation with *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter* spp. and MRSA on admission to a Spanish ICU [59]. The use of antimicrobials and pre-hospital length of stay were defined as significant risk factors for colonisation with VRE, ESBL-producing *E. coli* and *Klebsiella* spp., *Acinetobacter* spp., *P. aeruginosa*, and *Candida* spp. among Turkish patients within 48 hours of ICU admission [2]. Prior history of antimicrobial treatment was independently associated with carriage of multidrug-resistant organisms on ICU admission [254]. Transfer from another ICU, previous hospital admission in another country, surgery within the past year, prior neurologic disease, and prior administration of 3rd-generation cephalosporins (within 3 - 12 months before ICU admission) remained associated with colonisation by ESBL-producing *Enterobacteriaceae* upon ICU admission [62]. However, no significant risk factors were associated with nasal *S. aureus* or MRSA colonisation, rectal antimicrobial-resistant *E. coli* or *Klebsiella* spp. colonisation. This could be explained that in my study, just some clinically important bacteria at certain special colonisation sites were studies, while previous studies focused on colonisation at all sites (oral, nasal, pharyngeal, rectal and skin swabs).

There are several limitations of this study that warrant further discussion. Firstly, it was a single-center study, so, the findings may not be representative in other institutions. However, to our knowledge, due to a lack of data at the country level, my study is the first to analyze colonisation, infection and risk factors for bacterial colonisation, especially with AROs on ICU admission in Vietnam. Secondly, nearly one quarter of study patients were treated with antimicrobials within 24 hours before ICU admission which may have had a negative impact on microbiological culture of all samples.

Therefore, more sensitive organisms may not have been detected, biasing results in favor of more resistant ones.

3.5 CONCLUSION

The results show that Vietnamese ICU patients have a high rate of colonisation and infection with AROs. These findings are useful to guide empirical antimicrobial therapy for severe infections among patients admitted to ICU. My study is considered as a baseline study to be repeated after implementing infection control measures, strengthening laboratory diagnosis for bacterial resistance and setting regional surveillance networks.

Chapter 4. HOSPITAL-ACQUIRED COLONISATION AND INFECTIONS DURING ICU STAY

4.1 INTRODUCTION

HAIs are a serious health problem, with WHO estimating that millions of patients are affected each year [11]. Critically-ill patients are particularly vulnerable because of decreased immunity, increased variety of diagnostic and treatment procedures, antimicrobial exposure, and the transmission of AROs among crowded hospital populations [9,14]. HAIs occur worldwide and affect both HICs and LMICs with a significant increase in mortality, morbidity, length of hospital and ICU stay, and resource utilization [19,259]. Although data from resource-limited settings are sparse, infection rates are likely higher than in high-income settings [42,178,260].

Prior bacterial colonisation may increase the risk of subsequent HAIs in ICU, which has been well studied all over the world, especially in developed countries. Nasal colonisation with *S. aureus* has been shown to be a significant risk factor for ICU-acquired *S. aureus* infections, including SSI, BSI, pneumonia and UTI [126-129]. Molecular genetics studies revealed that most MRSA isolates were hospital-acquired clones and that nasal and clinical isolates exhibited up to 75% shared identity [129]. Similarly, rectal carriage of multidrug-resistant *Enterobacteriaceae* was a major reservoir of subsequent bloodstream, lung, urinary tract, and central venous catheter infection in ICUs [137,138]. For *K. pneumoniae*, the association between gastrointestinal colonisation and HAIs was established and strong [139,261]. Moreover, genome data indicated matching colonisation and infection isolates in 80% of isolate pairs [139].

Many studies about the associations between colonisation and infection with non-*Enterobacteriaceae* bacteria, for example *P. aeruginosa* and *A. baumannii* have been conducted. A study undertaken in Spain found that among 77 ICU patients who developed *P. aeruginosa* pneumonia, 69 (89.6%) had prior *P. aeruginosa* rectal

colonisation, and 60 (87%) of these paired rectal and clinical isolates exhibited genotyping concordance [141]. Another study of 189 consecutive ICU patients in Spain found that 20 (10.6%) patients were colonised with multidrug-resistant *A. baumannii* upon ICU admission, and 57 (30.2%) additional patients acquired colonisation, mostly during the first week of ICU admission. Rectal colonisation was associated with increased ICU-acquired infections with multidrug-resistant *A. baumannii*, such as respiratory tract infections, BSI, SSI, peritonitis, osteoarthritis and meningitis [142].

Understanding colonisation and its relationship to HAIs is central to providing the necessary data to ensure safe and high-quality healthcare. In LMICs, such as Vietnam, there is a large amount of antimicrobial use in the community [262,263]. I have previously shown high rates of colonisation with patients on ICU admission [264] which, in addition to a paucity of antimicrobial stewardship programs, means that the relationship between colonisation and resistance may be different. To conserve scarce resources and tailor infection control programs appropriately, this relationship needs to be better understood.

4.2 MATERIALS AND METHODS

The detailed methods of this chapter are reported in Chapter 2 “Materials and methods”.

4.3 RESULTS

4.3.1 Patient characteristics

838 adults were enrolled in the study, of whom 364 were admitted for more than 48 hours and were screened for hospital-acquired colonisation and infection in ICU (Figure 4.1). The patient characteristics are described in Table 4.1. The median age was 46 years (IQR 33 - 60). Male sex accounted for 66.5%. 32.2% of patients had chronic pre-existing disease with Charlson Comorbidity Index ≥1. The median APACHE II score was 8 (IQR 3 - 14). The percentage of patients with mild (<5), moderate (5 - 12) and severe (>12) APACHE II scores were 32.4%, 36.8% and 30.8%, respectively. The most common diagnosis among patients on admission to ICU was tetanus (56%) followed by sepsis and septic shock (20.6%), local infections (12.4%) and severe Dengue infection (4.7%). Of the 364 patients admitting to the Adult ICU for more than 48 hours, 40 died (11%). The median ICU and hospital length of stay were 10 (IQR 5 - 18) and 22 (IQR 13 - 32), respectively.

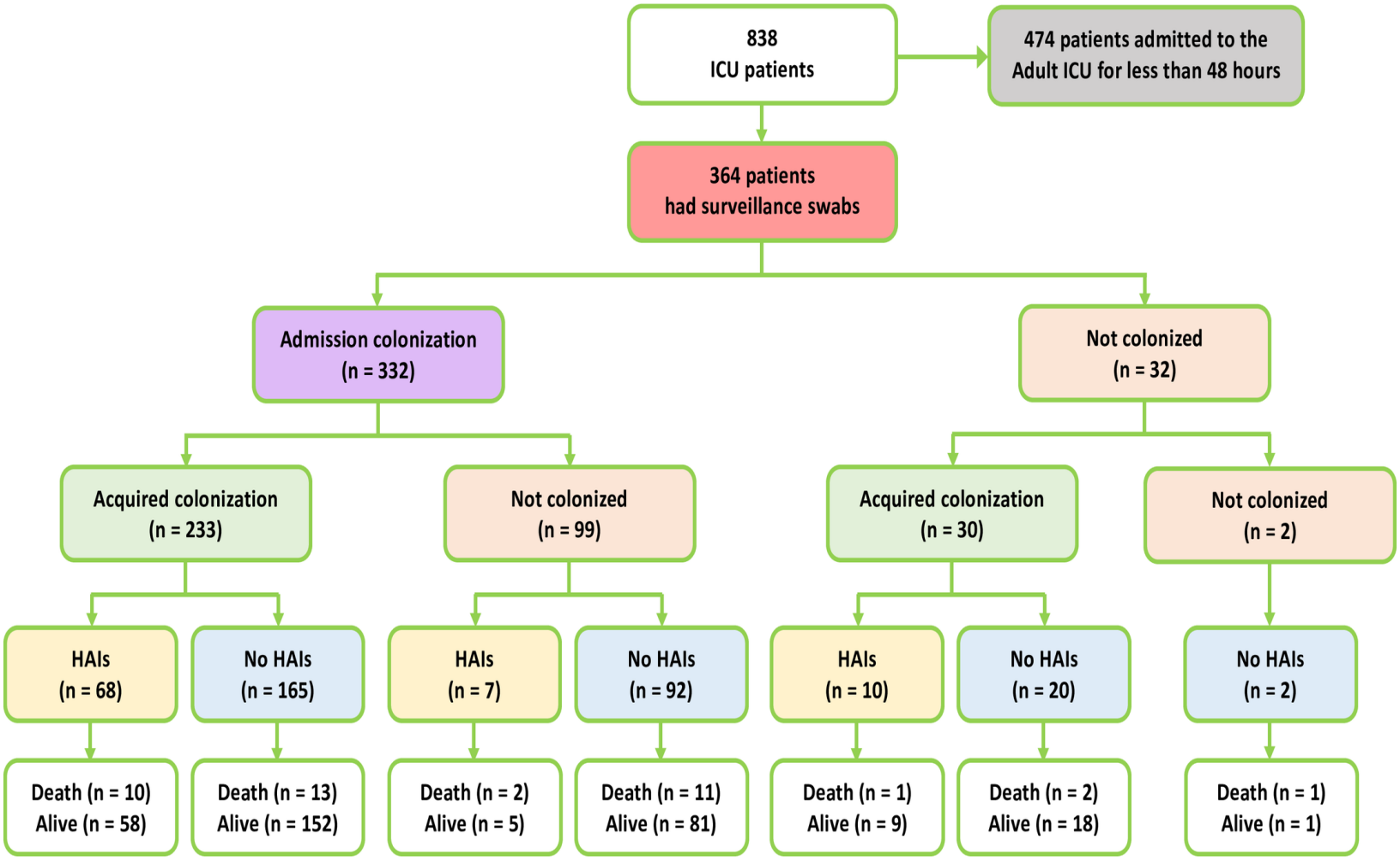
Table 4.1 Patient characteristics during ICU stay

Age (yr) - median (IQR)	46 (33 - 60)
<60 - n (%)	265 (72.8)
≥60 - n (%)	99 (27.2)
Sex - n (%)	
Male	242 (66.5)
Female	122 (33.5)
Charlson Comorbidity Index score - median (IQR)	0 (0 -1)
No comorbidity (0) - n (%)	247 (67.8)
Mild (1 - 2) - n (%)	73 (20.1)
Moderate (3 - 4) - n (%)	24 (6.6)
Severe (≥5) - n (%)	20 (5.5)
APACHE II score - median (IQR)	8 (3 - 14)
Mild (<5) - n (%)	118 (32.4)
Moderate (5 - 12) - n (%)	134 (36.8)
Severe (>12) - n (%)	112 (30.8)
Admitting diagnosis - n (%)	
Tetanus	204 (56)
Sepsis and septic shock	75 (20.6)
Local infections *	45 (12.4)
Severe Dengue infection	17 (4.7)
Internal medicine diseases #	23 (6.3)
Death - n (%)	40 (11)
ICU stay (days) - median (IQR)	10 (5 - 18)
Hospital stay (days) - median (IQR)	22 (13 - 32)

* Local infections included pneumonia (25 cases), cellulitis (4 cases), UTI (15 cases), and SBP (1 cases)

Internal medicine diseases included kidney failure (6 cases), myocarditis (3 cases), myocardial infarction (6 cases), atrial fibrillation (3 cases), malignant *hypertension* (2 cases), diabetic ketoacidosis (2 cases) and epilepsy (2 cases).

Figure 4.1 Inclusion of patients, taking surveillance swabs and outcomes



4.3.2 Acquired colonisation status

In this study, a total of 3,162 surveillance swabs were taken during ICU stay: 1,276 nasal swabs, 1,276 rectal swabs and 610 ETA samples. The culture results are presented in Table 4.2.

4.3.2.1 Nasal colonisation

148 (40.7%) patients acquired colonisation with at least one of the five study bacteria. 75 patients (20.6%) acquired nasal *S. aureus* colonisation: 52 (69.3%) MRSA, and 23 (30.7%) MSSA. 96 (26.4%) patients acquired nasal *Klebsiella* spp. colonisation, of whom 15 (15.6%) had ESBL-producing *Klebsiella* spp., 10 (10.4%) had ESBL and AmpC-producing *Klebsiella* spp., 5 (5.2%) had AmpC-producing *Klebsiella* spp., and 2 (2.1%) had ESBL and carbapenemas-producing *Klebsiella* spp.. There were 82 (22.5%) patients who acquired nasal *Acinetobacter* spp. colonisation: 23 (28%) with ESBL-producing *Acinetobacter* spp., and 57 (69.5%) with ESBL and carbapenemase-producing *Acinetobacter* spp..

4.3.2.2 Rectal colonisation

238 (65.4%) patients acquired rectal colonisation with one or more of the bacteria of interest. 229 (62.9%) patients had rectal *E. coli* colonisation: 124 (54.1%) with ESBL-producing *E. coli*, 18 (7.9%) with ESBL and AmpC-producing *E. coli*, 12 (5.2%) with AmpC-producing *E. coli*, and 11 (4.8%) with ESBL and carbapenemase-producing *E. coli*. 162 (44.5%) patients acquired *Klebsiella* spp. colonisation in the rectum. The number of colonised patients with ESBL-producing *Klebsiella* spp. were 27 (16.7%), 24 (14.8%) with ESBL and AmpC-producing *Klebsiella* spp., 12 (7.4%) with AmpC-producing *Klebsiella* spp., and 9 (5.6%) with ESBL and carbapenemase-producing *Klebsiella* spp..

4.3.2.3 Endotracheal colonisation

ETA samples were collected from 130 patients with endotracheal tubes in place. Of these 130 subjects, 96 (73.8%) acquired tracheal colonisation with one of the five study bacteria. 48 (36.9%) patients colonised with *Klebsiella* spp.. Among 61 (46.9%) patients with *Acinetobacter* spp., 21 (34.4%) has ESBL-producing *Acinetobacter* spp. and 40 (65.6%) with ESBL and carbapenemase-producing *Acinetobacter* spp. *Pseudomonas* spp. endotracheal colonised patients were 37 (28.5%) and 13 (35.1%) of them were found to be colonised with carbapenemase-producing *Pseudomonas* spp.. 48 (36.9%) patients acquired endotracheal *S. aureus* colonisation: 35 (72.9%) MRSA, and 13 (27.1%) MSSA.

Overall, the acquired colonisation rate at all sites of *S. aureus*, *E. coli*, *Klebsiella* spp., *Acinetobacter* spp., and *Pseudomonas* spp. was 24.2% (88/364 patients), 63.5% (231), 51.4% (187), 30.2% (110) and 21.2% (77), respectively. The acquired colonisation rate with one of the five bacteria of interest at all sites was 72.3% (263/364). The proportion of patients who acquired AROs at any sampling site was 61.3% (223/364). The 364 patients included in the study represented a total of 2,276 patient days at risk of AROs acquisition in ICU. For each individual separately, patient days at risk was the follow-up time until the event of AROs acquisition. If the patient did not have the event of interest, patient days at risk was the follow-up time until death or discharge from ICU. Therefore, the incidence rate of ICU patients becoming colonised with AROs was 9.8 (223/2,276) per 100 patient days. The acquired AROs colonisation rate of the tracheal tract was the highest (61.5%, 80/130), followed by the rectal colonisation (54.7%, 199/364) and the nasal colonisation (33.8%, 123/364) (Figure 4.2). The overall acquisition rate at any sampling site with an ESBL-producing *E. coli* was 39.8% (145/364), carbapenemase-producing *Acinetobacter* spp. 22.0% (80/364), MRSA 16.2% (59/364), ESBL-producing *Klebsiella* spp. 13.7% (50/364), carbapenemase-producing

Pseudomonas spp. 11.8% (43/364), carbapenemase-producing *E. coli* 3.3% (12/364) and carbapenemase-producing *Klebsiella* spp. 3.3% (12/364). Carbapenem-resistant *Acinetobacter* spp. and MRSA were the most commonly isolated organisms from nasal swabs, cultured from 15.7% (57/364) and 14.3% (52/364) of patients, respectively. These two organisms were also the most commonly isolated organisms from endotracheal aspirates 30.8% (40/130) and 26.9%, (35/130), respectively. The rate of rectal colonisation with 3rd cephalosporin-resistant *E. coli* among patients was 42.3%, (154/364), followed by 3rd cephalosporin-resistant *Klebsiella* spp. (17.3%, 63/364). 124 (34.1%) patients were rectal colonised with ESBL-producing *E*. coli, 51 (14%) with ESBL-producing *Klebsiella* spp., and the total acquisition rate of rectal ESBL-producing *Enterobacteriaceae* was 44.2% (161/364).

The exact moment of acquired colonisation was unknown, as cultures were taken on schedule (twice a week, on Monday and Thursday). Time zero was the date of ICU admission, and the date of acquired colonisation was assumed to be at the midway point between the latest negative culture and the first positive surveillance culture, and summarized in mean ± SD. Therefore, the mean time to acquired *S. aureus*, *E. coli*, *Klebsiella* spp., *Acinetobacter* spp. and *Pseudomonas* spp. colonisation were 7.4 ± 6.9, 5.5 ± 4.8, 6.0 ± 4.9, 9.2 ± 5.6, and 9.0 ± 5.9 days, respectively. Moreover, the mean time to acquired MRSA, antimicrobial-resistant *E. coli*, antimicrobial-resistant *Klebsiella* spp., antimicrobial-resistant *Acinetobacter* spp. and antimicrobial-resistant *Pseudomonas* spp. colonisation was 7.9 ± 6.6, 6.3 ± 5.7, 8.4 ± 7.8, 9.2 ± 5.6, and 12.4 ± 7.6 (days), respectively. Antimicrobial-resistant *E. coli* was defined as resistance to either 3rd-generation cephalosporins or carbapenems, and it was the same for *Klebsiella* spp., *Acinetobacter* spp. and *Pseudomonas* spp.. The rate of early acquired colonisation (within 5 days of ICU stay) was 82.1% (216/263) and that of late acquired colonisation (after 5 days of ICU stay) was 17.9% (47/263). The proportion of patients acquiring

colonisation increased with length of ICU stay: 66.2% (241/364) in the 1st week, 68.4% (259/364) in the 2nd week and 100% in the 3rd week.

4.3.2.4 Antimicrobial susceptibility of acquired colonizing bacteria during ICU stay

A total of 3,836 bacterial isolates were cultured from 3,162 surveillance swabs during ICU stay, of which 378 (9.9%) were *S. aureus*, 1,550 (40.4%) were *E. coli*, 895 (23.3%) were *Klebsiella* spp., 555 (14.5%) were *Acinetobacter* spp., and 458 (11.9%) were *Pseudomonas* spp.. Their antimicrobial susceptibility is summarized in Table 4.3 according to resistance in absolute number and percentage.

MRSA accounted for 72.8% of all *S. aureus* isolates. The proportion of resistance to ciprofloxacin, levofloxacin and clindamycin was 60.1%, 58.5% and 71.1%, respectively. Vancomycin-resistant *S. aureus* was not detected. 3rd-generation cephalosporin resistance in *E. coli* and *Klebsiella* spp. were about 60% and 30%, respectively. Ceftazidime-resistant *Pseudomonas* spp. was over 20%. Carbapenem resistance was reported with the highest percentage for *Acinetobacter* spp. (nearly 70%). Colistin, the antimicrobial of last resort for carbapenemase-producing organisms, was also detected to be resistant among *E. coli*, *Klebsiella* spp. and *Acinetobacter* spp. (1.7%, 4.9% and 1.1%, respectively).

Table 4.2 Acquired colonisation status of 364 enrolled patients (pts) during ICU stay

Samples (Total pts Total swabs)	No. isolates No.(%) positive pts	*E.coli* No. isolates No.(%) colonised pts (No. colonised pts with AROs)	*K. pneumonia* No. isolates No.(%) colonised pts (No. colonised pts with AROs)	*A.baumannii* No. isolates No.(%) colonised pts (No. colonised pts with AROs)	*P.aeruginosa* No. isolates No.(%) colonised pts (No. colonised pts with AROs)	*S.aureus* No. isolates No.(%) colonised pts (No. colonised pts with AROs)
Nasal swabs (364 pts 1,276 swabs)	**1,051 isolates** 148 (40.7)	**67** 29 (8) (14 ESBL, 1 ESBL+AmpC, 3 ESBL+CPO)	**324** 95 (26.1) (15 ESBL, 5 AmpC, 10 ESBL+AmpC, 2 ESBL+CPO) *Klebsiella* spp. 1 1 (0.3)	**255** 79 (21.7) (21 ESBL, 56 ESBL+CPO) *Acinetobacter* spp. 7 3 (0.8) (2 ESBL, 1 ESBL+CPO)	**201** 49 (13.5) (27 CPO, 1 ESBL+CPO) *Pseudomonas* spp. 5 4 (1.1) (1 CPO)	**191** 75 (20.6) (52 MRSA)
Rectal swabs (364 pts 1,276 swabs)	**2,165 isolates** 238 (65.4)	**1,461** 229 (62.9) (124 ESBL, 12 AmpC, 18 ESBL+AmpC, 11 ESBL+CPO)	**434** 156 (42.9) (26 ESBL, 12 AmpC, 2 ESBL+AmpC+CPO 24 ESBL+AmpC, 9 ESBL+CPO) *Klebsiella* spp. 6 6 (1.6) (1 ESBL)	**125** 64 (17.6) (19 ESBL, 44 ESBL+CPO) *Acinetobacter* spp. 10 6 (1.6) (6 ESBL)	**105** 48 (13.2) (23 CPO) *Pseudomonas* spp. 3 2 (0.5) (2 CPO)	**21** 15 (4.1) (8 MRSA)
Endotracheal aspirate (130 pts 610 swabs)	**620 isolates** 96 (73.8)	**22** 13 (10) (7 ESBL)	**130** 48 (36.9) (6 ESBL, 3 AmpC, 2 ESBL+AmpC 2 ESBL+CPO)	**142** 57 (43.8) (19 ESBL, 38 ESBL+CPO) *Acinetobacter* spp. 16 4 (3.1) (2 ESBL, 2 ESBL+CPO)	**144** 37 (28.5) (13 CPO, 1 ESBL+CPO)	**166** 48 (36.9) (35 MRSA)

Figure 4.2 Acquired colonisation with AROs during ICU stay

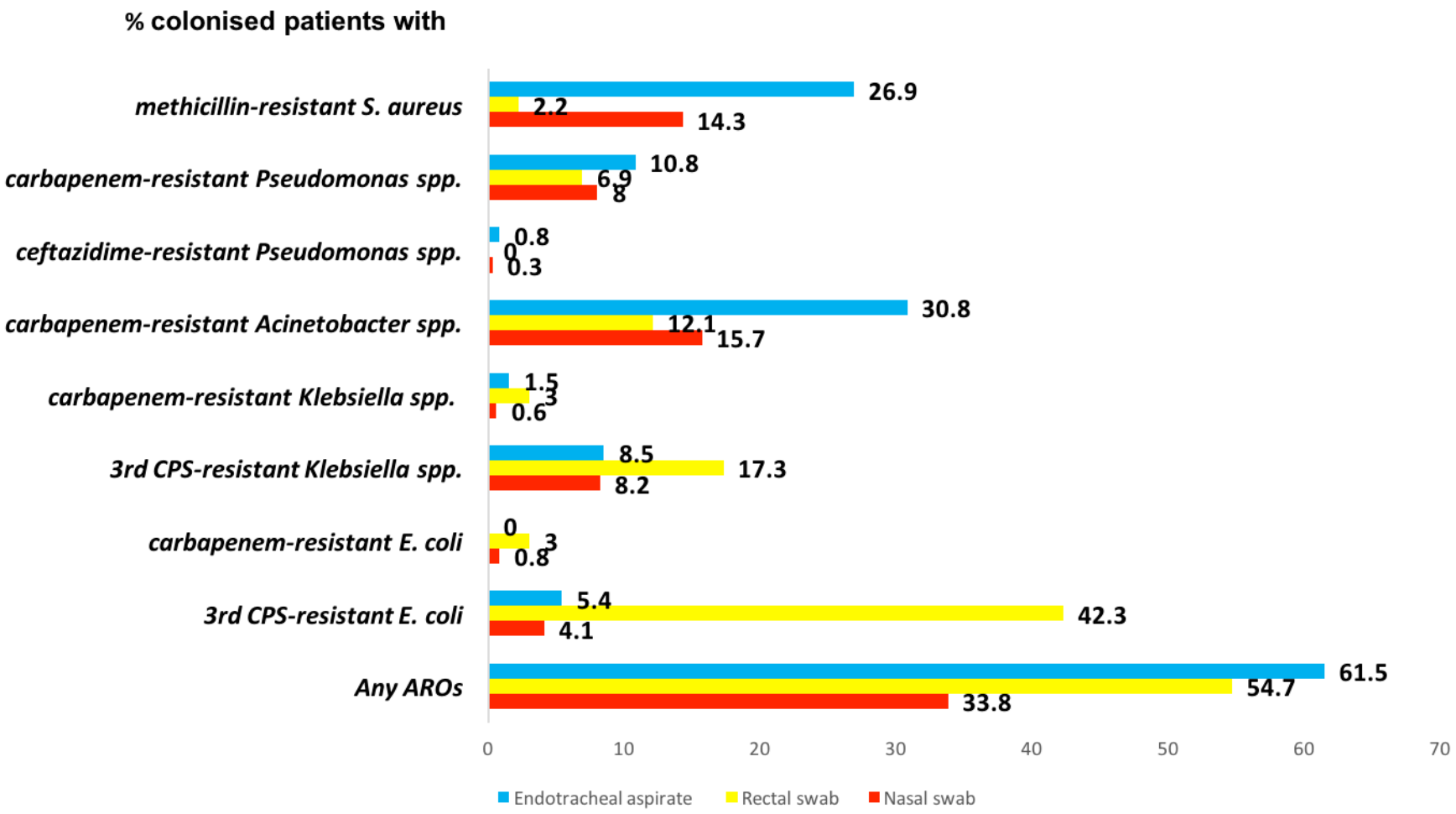


Table 4.3 Antimicrobial resistance of acquired colonizing bacteria during ICU stay

Antimicrobials n (%)	*S.aureus* (n=378)	*E.coli* (n=1,550)	*Klebsiella* spp. (n=895)	*Acinetobacter* spp. (n=555)	*Pseudomonas* spp. (n=458)
Amoxcillin-clavulanic acid		924 (59.6)	260 (29.1)		
Ceftazidime		881 (56.8)	256 (28.6)	539 (97.1)	101 (22.1)
Ceftriaxone		881 (56.8)	256 (28.6)	539 (97.1)	
Cefepime		811 (52.3)	213 (23.8)	539 (97.1)	87 (19)
Ticarcillin-clavulanate		984 (63.5)	277 (30.9)	537 (96.8)	330 (72.1)
Piperacillin-tazobactam		815 (52.6)	219 (24.5)	537 (96.8)	81 (17.7)
Ofloxacin		767 (49.5)	131 (14.6)		
Ciprofloxacin	227 (60.1)	780 (50.3)	183 (20.4)		
Levofloxacin	221 (58.5)			352 (63.4)	56 (12.2)
Sulfamethoxazole-trimethoprim	19 (5)	1,102 (71.1)	335 (37.4)	302 (54.4)	438 (95.6)
Amikacin		25 (1.6)	27 (3)	215 (38.7)	23 (5)
Gentamycin					23 (5)
Ertapenem		42 (2.7)	32 (3.6)		
Imipenem		33 (2.1)	32 (3.6)	371 (66.8)	202 (44.1)
Meropenem		26 (1.7)	32 (3.6)	371 (66.8)	183 (40)
Colistin		27 (1.7)	44 (4.9)	6 (1.1)	0
Penicillin	368 (97.4)				
Oxacillin	275 (72.8)				
Vancomycin	0				
Erythromycin	265 (70.1)				
Rifampicin	30 (7.9)				
Clindamycin	269 (71.1)				

4.3.2.5 Risk factors of acquired colonisation with AROs during ICU stay

In our univariate analysis (Table 4.4), I found Charlson Comorbidity Index score as a significant risk factor for acquired colonisation with AROs, regardless of anatomical sites (all p-values ≤0.01), while receipt of antimicrobial treatment on ICU admission was a significant risk factor for nasal and rectal colonisation with AROs (both p-values ≤0.001). Admission for tetanus disease reduced the risk of AROs acquisition in nasal and rectal cavity (both p-values ≤0.001), and nasogastric tube was associated with reduced risk of rectal AROs colonisation (p = 0.01).

In multivariate analysis (Table 4.5), hazard ratios did not change much, except for antimicrobial treatment, which was reduced by at least 50% and became non-significant, and Charlson Comorbidity Index score, which was reduced and was no longer a significant risk factor for nasal colonisation with AROs. Still, admission for tetanus disease had reduced the risk of nasal and rectal acquisition by AROs, while nasogastric tube was associated with reduced incidence of rectal AROs colonisation in ICU.

Table 4.4 Univariate hazard ratios for risk factors of acquired colonisation with AROs, according to Cox regression analysis

Variables	Nasal colonisation (N = 123)		Rectal colonisation (N = 199)		Endotracheal colonisation (N = 80)	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Admission for tetanus disease	0.40 (0.27 - 0.60)	<0.001	0.37 (0.27 - 0.51)	<0.001	0.63 (0.35 - 1.13)	0.12
Charlson Comorbidity Index score	1.25 (1.08 - 1.44)	0.003	1.24 (1.12 - 1.37)	<0.001	1.45 (1.09 - 1.93)	0.01
Admission colonisation status						
Admission nasal colonisation	0.65 (0.37 - 1.12)	0.12	-	-	-	-
Admission rectal colonisation	-	-	1.12 (0.76 - 1.64)	0.57	-	-
Admission endotracheal colonisation	-	-	-	-	0.86 (0.47 - 1.57)	0.63
Receipt of antimicrobial treatment on admission	1.96 (1.31 - 2.95)	0.001	1.89 (1.38 - 2.59)	<0.001	1.44 (0.83 - 2.51)	0.19
Intensive care procedures on admission						
Nasogastric tube	1.14 (0.79 - 1.66)	0.48	0.69 (0.51 - 0.92)	0.01	0.83 (0.52 - 1.32)	0.42
Respiratory support	1.34 (0.93 - 1.92)	0.11	-	-	0.91 (0.58 - 1.43)	0.69

Table 4.5 Multivariate hazard ratios for risk factors of acquired colonisation with AROs, according to Cox regression analysis

Variables	Nasal colonisation (N = 123)		Rectal colonisation (N = 199)		Endotracheal colonisation (N = 80)	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Admission for tetanus disease	0.46 (0.29 - 0.74)	0.001	0.39 (0.27 - 0.56)	<0.001	0.67 (0.33 - 1.36)	0.27
Charlson Comorbidity Index score	1.14 (0.97 - 1.35)	0.12	1.13 (1.01 - 1.25)	0.04	1.42 (1.03 - 1.94)	0.03
Admission colonisation status						
Admission nasal colonisation	0.57 (0.32 - 1.01)	0.05	-	-	-	-
Admission rectal colonisation	-	-	1.19 (0.80 - 1.76)	0.40	-	-
Admission endotracheal colonisation	-	-	-	-	0.76 (0.40 - 1.45)	0.41
Receipt of antimicrobial treatment on admission	1.28 (0.80 - 2.04)	0.31	1.19 (0.84 - 1.71)	0.33	1.20 (0.66 - 2.18)	0.55
Intensive care procedures on admission						
Nasogastric tube	1.05 (0.68 - 1.61)	0.83	0.62 (0.46 - 0.83)	0.002	0.80 (0.48 - 1.33)	0.40
Respiratory support	1.16 (0.77 - 1.75)	0.49	-	-	0.82 (0.50 - 1.35)	0.45

4.3.3 HAIs development in ICU

4.3.3.1 Characteristic of HAIs

During the study period, 106 episodes of HAIs were observed in 85 patients, of whom 66 patients (77.6%) presented only one episode, 18 patients (21.2%) with two episodes, and 1 patient (1.2%) with four episodes. Moreover, there were 2 patients who developed 2 different types of HAIs with different pathogens at the same time: one with VAP and BSI, and the other one with BSI and UTI. The 364 patients included in the study represented a total of 3,701 patient days at risk of contracting HAIs in ICU. For each individual separately, patient days at risk was the follow-up time until the event of HAIs. If the patient did not have the event of interest, patient days at risk was the follow-up time until death or discharge from ICU. Therefore, the proportion of ICU patients with HAIs was 23.4% (85/364), and the incidence rate of ICU patients contracting HAIs was 2.3 (85/3,701) per 100 patient days.

The median age of these 85 patients was 50 years (IQR 33 - 67) and 35.3% (30/85) of them were ≥60 years of age. Male sex accounted for 68.2% (58/85). 22.4% (19/85) had underlying diseases with Charlson Comorbidity Index score ≥1, and the median APACHE II score was 8 (IQR 4 - 11). Tetanus was the most common diagnosis on ICU admission (65/85, 76.5%) among these patients, followed by sepsis (8/85, 9.4%), septic shock (7/85, 8.2%) and severe Dengue infection (5/85, 5.9%). 13 (15.3%) died in the Adult ICU, and 8 of them (61.5%) patients died within 48 hours of the development of HAIs. The median of length of ICU and hospital stay were 26 (IQR 17 - 33) days and 37 (IQR 27 - 46) days, respectively. Detailed information is shown in Table 4.6.

HAIs included pneumonia (52 episodes in 44 patients), UTI (39 episodes in 38 patients), and BSI (15 episodes in 15 patients). Out of 52 episodes of pneumonia, VAP accounted for 69.2% (36 episodes in 33 patients). In other words, the most common type of HAIs in Adult ICU of the HTD was pneumonia (49.1%), followed by UTI

(36.8%) and BSI (14.1%). The mean duration of the first episode of HAIs development was 11.9 ± 6.4 days. Of the 106 episodes of HAIs, 93 (87.7%) were linked to the culture of a single bacterial species and 13 (14% of 93) were associated with more than one organism. Microbiological analyses found that 106 distinct bacteria were responsible for HAIs: 57 bacteria (53.8%) caused pneumonia, 34 (32.1%) caused UTI, and 15 (14.1%) caused BSI (Table 4.7). Taking into account of HAIs type, the most common pathogens of pneumonia were *K. pneumoniae* (15, 26.3% of 57 isolates), *A. baumannii* (12, 21.1%), *S. aureus* (9, 15.8%), and *P. aeruginosa* (8, 14%). For UTI, the most prevalent causative agents were *E. coli* (12, 35.3% of 34 isolates), *A. baumannii* (6, 17.6%), and *Enterococcus faecalis* (5, 14.7%). For BSI, *S. aureus* was the main pathogen (4, 26.7% of 15 isolates). In general, the most common bacterial causes of HAIs were *A. baumannii* (20, 18.9% of 106 bacteria), *K. pneumoniae* (19, 17.9%), *E. coli* (14, 13.2%), *S. aureus* (14, 13.2%), and *P. aeruginosa* (11, 10.4%). In terms of AROs, carbapenem-resistant *A. baumannii* were 14 (70% of 20 *A. baumannii* isolates), 3rd-generation cephalosporin-resistant *K. pneumoniae* were 4 (21.1% of 19 *K. pneumoniae* isolates), 3rd-generation cephalosporin-resistant *E. coli* were 11 (78.6% of 14 *E. coli* isolates), methicillin-resistant *S. aureus* were 9 (64.3% of 14 *S. aureus* isolates), and carbapenem-resistant *P. aeruginosa* were 2 (18.2% of 11 *P. aeruginosa* isolates).

Table 4.8 shows the relationship between prior colonisation and subsequent HAIs by the same organism. Of patients colonised on admission to ICU, 4.2% (14/332) subsequently developed a HAI with a phenotypically similar bacterium. Similarly, of those who acquired colonisation during ICU stay, 11.4% (30/263) later developed a HAI. In general, more than half (44/77, 57.1%) patients with HAIs had prior colonisation with phenotypically matching organism.

Table 4.6 Characteristics of 85 patients with HAIs during ICU stay

Age (yr) - median (IQR)	50 (33 - 67)
<60 - n (%)	55 (64.7)
≥60 - n (%)	30 (35.3)
Sex - n (%)	
Male	58 (68.2)
Female	27 (31.8)
Charlson Comorbidity Index - median (IQR)	**0 (0 - 0)**
No comorbidity (0) - n (%)	66 (77.6)
Mild (1 - 2) - n (%)	16 (18.8)
Moderate (3 - 4) - n (%)	1 (1.2)
Severe (≥5) - n (%)	2 (2.4)
APACHE II score - median (IQR)	**8 (4 - 11)**
Mild (<5) - n (%)	24 (28.2)
Moderate (5 - 12) - n (%)	43 (50.6)
Severe (>12) - n (%)	18 (21.2)
Admitting diagnosis - n (%)	
Tetanus	65 (76.5)
Sepsis	8 (9.4)
Septic shock	7 (8.2)
Severe Dengue infection	5 (5.9)
Death - n (%)	13 (15.3)
ICU stay (days) - median (IQR)	26 (17 - 33)
Hospital stay (days) - median (IQR)	37 (27 - 46)

Table 4.7 Pathogens causing HAIs in ICU

Microorganisms n (%)	All pathogens isolated (n = 106)	Pneumonia (n = 52)	Urinary tract infection (n = 39)	Bloodstream infection (n = 15)
Gram-positive cocci	**26**			
Staphylococcus aureus	**14 (9 MRSA)**	**9 (6 MRSA)**	**1**	**4 (3 MRSA)**
Coagulase-negative staphylococci	**2**			**2**
Streptococcus constellatus	**1**			**1**
Streptococcus pneumoniae	**2**	**2**		
Enterococcus faecalis	**5**		**5**	
Enterococcus faecium	**1**			**1**
***Enterococcus* spp**	**1**		**1**	
Enterobacteriaceae	**38**			
Escherichia coli	**14 (8 ESBL, 3 AmpC)**		**12 (7 ESBL, 2 AmpC)**	**2 (1 ESBL, 1 AmpC)**
Klebsiella pneumoniae	**19 (2 ESBL, 1 AmpC, 1 ESBL+CPO)**	**15 (1 ESBL, 1 AmpC)**	**2 (1 ESBL)**	**2 (1 ESBL+CPO)**
Proteus mirabilis	**4**	**1**	**3**	
Proteus vulgaris	**1**		**1**	

Nonenterobacterial gram-negative bacteria	**42**			
Acinetobacter baumannii	**20 (3 ESBL, 14 ESBL+CPO)**	**12 (3 ESBL, 9 ESBL+CPO)**	**6 (3 ESBL+CPO)**	**2 (2 ESBL+CPO)**
Acinetobacter nosocomialis	**1 (1 ESBL)**	**1 (1 ESBL)**		
Pseudomonas aeruginosa	**11 (2 CPO)**	**8 (2 CPO)**	**3**	
Stenotrophomonas maltophilia	**4 (1 ESBL)**	**3 (1 ESBL)**		**1**
Haemophilus influenzae	**4**	**4**		
Haemophilus parainfluenzae	**2**	**2**		

Table 4.8 Prior colonisation and subsequent HAIs with the same organisms among ICU patients

Bacteria	Colonisation on ICU admission (subsequent HAIs, n)	Acquired colonisation during ICU stay (subsequent HAIs, n)	Total HAIs (n)	Prior colonisation (n, %)	
				Yes	No
Sensitive *S. aureus*	37 (1)	29 (1)	5	2 (40%)	3 (60%)
Methicillin-resistant *S. aureus*	9 (2)	59 (4)	9	6 (66.7%)	3 (33.3%)
Sensitive *E. coli*	146 (1)	64 (2)	3	3 (100%)	0
ESBL-producing *E. coli*	158 (5)	159 (1)	8	6 (75%)	2 (25%)
AmpC-producing *E. coli*	16 (0)	31 (0)	3	0	3 (100%)
Sensitive *Klebsiella* spp.	117 (4)	96 (11)	15	15 (100%)	0
ESBL-producing *Klebsiella* spp.	6 (0)	72 (1)	1	1 (100%)	0
AmpC-producing *Klebsiella* spp.	7 (0)	51 (1)	1	1 (100%)	0
Carbapenemase-producing *Klebsiella* spp.	3 (0)	12 (0)	1	0	1 (100%)
Sensitive *Acinetobacter* spp.	3 (1)	1 (0)	2	1 (50%)	1 (50%)
ESBL-producing *Acinetobacter* spp.	0	47 (1)	4	1 (25%)	3 (75%)
Carbapenemase-producing *Acinetobacter* spp.	0	75 (7)	14	7 (50%)	7 (50%)
Sensitive *Pseudomonas* spp.	13 (0)	34 (1)	9	1 (11.1%)	8 (88.9%)
Carbapenemase-producing *Pseudomonas* spp.	0	43 (0)	2	0	2 (100%)
Total	14/332 (4.2%)	30/263 (11.4%)	77	44/77 (57.1%)	33/77 (42.9%)

4.3.3.2 Risk factors of HAIs

Because the numbers of patients contracting HAIs by the specified organisms were limited, HAIs were categorized into infection types (pneumonia, UTI and BSI). Similarly, prior colonisation status was tested by including admission colonisation and acquired colonisation regardless of colonizing bacteria. In univariate analysis (Table 4.9), vascular catheters including central venous, arterial and hemofiltration catheter were found to be a significant risk factor for hospital-acquired BSI ($p = 0.01$). Both univariate and multivariate Cox regression analysis (Tables 4.9 and 4.10) demonstrated that admission for tetanus disease was a protective factor against the development of hospital-acquired pneumonia in ICU, whereas none of the study factors was significantly associated with HAIs.

Table 4.9 Univariate hazard ratios for risk factors of HAIs, according to Cox regression analysis

Variables	Pneumonia (N = 44)		Urinary tract infection (N = 38)		Bloodstream infection (N = 15)	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Admission for tetanus disease	0.31 (0.16 - 0.62)	0.001	1.38 (0.52 - 5.13)	0.55	0.34 (0.12 - 1.08)	0.07
Charlson Comorbidity Index score	1.28 (0.92 - 1.62)	0.13	0.87 (0.37 - 1.41)	0.66	1.31 (0.86 - 1.74)	0.17
Prior colonisation status						
Prior nasal colonisation	0.51 (0.22 - 1.33)	0.16	-	-	-	-
Prior rectal colonisation	-	-	0.49 (0.06 - 63.59)	0.66	0.52 (0.06 - 68.40)	0.69
Prior endotracheal colonisation	0.77 (0.40 - 1.55)	0.45	-	-	-	-
Intensive care procedures on admission						
Urinary catheter	-	-	0.83 (0.41 - 1.64)	0.60	-	-
Respiratory support	1.30 (0.71 - 2.38)	0.39	-	-	-	-
Vascular catheters	-	-	-	-	5.06 (1.45 - 15.22)	0.01

Table 4.10 Multivariate hazard ratios for risk factors of HAIs, according to Cox regression analysis

Variables	Pneumonia (N = 44)		Urinary tract infection (N = 38)	
	HR (95% CI)	p	HR (95% CI)	p
Admission for tetanus disease	0.33 (0.16 - 0.67)	0.002	1.25 (0.41 - 4.88)	0.72
Charlson Comorbidity Index score	1.04 (0.71 - 1.53)	0.84	0.93 (0.39 - 1.47)	0.82
Prior colonisation status				
Prior nasal colonisation	0.47 (0.16 - 1.37)	0.17	-	-
Prior rectal colonisation	-	-	0.50 (0.06 - 64.94)	0.67
Prior endotracheal colonisation	0.84 (0.37 - 1.90)	0.69	-	-
Intensive care procedures on admission				
Urinary catheter	-	-	0.85 (0.41 - 1.68)	0.64
Respiratory support	1.28 (0.65 - 2.55)	0.48	-	-
Vascular catheters	-	-	-	-

4.4 DISCUSSION

Reports on ICU-acquired bacterial colonisation are well-described in high-income settings. To our knowledge, my study is the first prospective longitudinal study to investigate ICU-acquired colonisation in Vietnam and one of the few investigating this in LMICs settings. I found that 72.3% of Vietnamese ICU patients acquired colonisation during their ICU stay, and the distribution of colonizing organisms was *E. coli* (40.4%), *Klebsiella* spp. (23.3%), *Acinetobacter* spp. (14.5%), *Pseudomonas* spp. (11.9%), and *S. aureus* (9.9%). A study performed in Shanghai (2006 - 2010) found the most common ICU-acquired strains were *A. baumannii* (19.5%), *P. aeruginosa* (15.6%), *S. maltophilia* (11.5%), *S. aureus* (10.7%), *Enterococcus* spp. (10.6%), and *K. pneumoniae* (9.7%) [245]. Their findings were a little different from ours because in Shanghai, they collected a wide range of specimen types (oral swab, ETA, sputum, urine, blood, catheters, and drainage samples) on admission to ICU and then once weekly during ICU stay. In comparison with my study, I took surveillance cultures including nasal swab, rectal swab and ETA within 48 hour of ICU admission and then twice weekly until ICU discharge; and, I just focused on screening *S. aureus, E. coli, Klebsiella* spp.*, Acinetobacter* spp. and *Pseudomonas* spp..

Our data also show that Vietnam has a high rate of ICU-acquired colonisation with AROs (61.3%). This was much higher than the rates reported from China and France where acquired AROs colonisation ranged from 15.2% to 34.4% [212,254]. The rate of nasal MRSA acquisition in our ICU (14.3%) was also higher than those reported in other ICUs: 7% in Scotland [49], and <1.5% in Ireland [265]. Moreover, the rate of rectal ESBL-producing *Enterobacteriaceae* acquisition in our ICU (44.2%) was also significantly higher than those reported in other geographic areas in the world (eg. 3% in the USA, 4% in Europe, and 21% in the Western Pacific region [213]. The reason behind our high rate of AROs acquisition is multifactorial, but maybe associated with a

high proportion of study patients (38.2%) were treated with broad-spectrum antimicrobials within 48 hours of ICU admission which had a negative impact on surveillance culture of all samples. Therefore, more sensitive organisms may not have been detected, resulting in favor of more resistant ones. Moreover, contact isolation or geographic separation of colonised or infected patients with AROs is rarely applied in our unit, so this can contribute to the spread of AROs among ICU patients. All things mentioned above have shown that Vietnam, a LMIC with unregulated antimicrobial use, limited infection control practices, and lack of routine screening, is more likely to be considered a new endemic area for AROs.

Focusing on early identification of risk factors of acquired AROs colonisation may help to reduce the spread of AROs and subsequent infections in ICU settings. Here, Charlson Comorbidity Index score was a significant risk factor for rectal and endotracheal colonisation of AROs, and it also seems to increase the risk of nasal AROs colonisation in ICU (HR = 1.14, Table 4.5). This is in agreement with previous studies conducted in other regions of the world [207,208,210,213,266]. The higher Charlson Comorbidity Index score, the more severe comorbid diseases are existed in human [202]. Indeed, chronic diseases are associated with physical and chemical changes, marked changes occur in the intestinal and respiratory tract which may enhance the risk of bacterial colonisation [267,268]. Of note is that admission for tetanus disease reduced significantly the risk of nasal and rectal acquisition by AROs, and probably decreased the risk of endotracheal colonisation with AROs (HR = 0.67, Table 4.5). In my study, all tetanus patients were generally healthy with 83.3% of them having a Charlson Comorbidity Index score of 0. They admitted for primarily control of muscular spasm and therefore may have less risk factors for AROs acquisition than others. This may be a reason why admission for tetanus disease was also a protective factor against the development of hospital-acquired pneumonia in ICU. The other explanation may be related to the tracheostomy procedure

being performed in tetanus patients, not intubation technique due to lock-jaw. Notably, 62.4% of tracheostomy were performed in tetanus patients. The tracheostomy tube is placed in the lower airway (below the vocal cords), therefore it is protective against colonisation with pathologic organisms from the upper to lower airway [129]. Furthermore, tracheostomy showed significantly less nosocomial pneumonia because of better hygiene and oral care compared to intubation [269]. Additionally, nasogastric tube significantly decreased the risk of acquiring rectal AROs colonisation, because all ICU patients with nasogastric tube in my study were fed with enteral nutrition formula produced by the Department of Nutritional of the hospital which can affect the human intestinal tract in ways that might change its hospitality to colonizing or pathogenic bacteria [268]. Unsurprisingly, the association between antimicrobial therapy and nasal/rectal acquisition of AROs is strong in my study using univariate analysis (Table 4.4). It is well-known that antimicrobial therapy may promote proliferation of AROs by exerting selective pressure in individual patients, (eg. inhibition of competing microflora but not of resistant organisms) [270]. Once AROs have emerged, antimicrobials may play a crucial role in their subsequent spread from patient to patient [271]. In France, colonised patients with MRSA or ESBL-producing *Enterobacteriaceae* were more likely than non-colonised to be receiving antimicrobial treatment on ICU admission (64% vs 41%, p = 0.003), but there were no significant differences between the 2 groups with respect to age, sex, patient origin, reason for hospitalization, and immune status [137]. In China, patients acquiring MRSA, ESBL-producing *Enterobacteriaceae* as well as *A. baumannii* or ceftazidime-resistant *P. aeruginosa* were more likely to have received antimicrobials in the last 3 months (55.8% vs 30.7%, p <0.001), and to receive >2 antimicrobials in ICU (36.6% vs 9.9%, p = 0.04) than those did not acquire any ARO during ICU [254]. In addition, prior colonisation with multidrug resistant bacteria was also identified as a risk factor for AROs acquisition [212,272]. However, in my study,

we did not find an association between prior AROs colonisation and acquisition of AROs in ICUs. To our knowledge, it may exist a competing activity between different bacterial species, the presence of one organism may prevent the colonisation of another.

In terms of HAIs, the number of patients with HAIs was 23.4%, and pneumonia was the most common type of HAIs (49.1%) in our ICU, followed by UTI (36.8%) and BSI (14.1%). The leading pathogens of HAIs in our ICU were *A. baumannii* (18.9%), *K. pneumoniae* (17.9%), *E. coli* (13.2%), *S. aureus* (13.2%), and *P. aeruginosa* (10.4%). AROs accounted for 41.5% (44/106 isolates), including carbapenem-resistant *Acinetobacter* spp. (14, 31.8% of 44 isolates), 3rd generation-resistant *E. coli* (11, 25%), MRSA (9, 20.5%), 3rd generation-resistant *K. pneumoniae* (4, 9.1%), and carbapenem-resistant *P. aeruginosa* (2, 4.5%). This is consistent with a recent study conducted by Phu et al. in 15 adult ICUs across Vietnam (2012 - 2013) showing that the incidence and prevalence of infection caused by resistant pathogens is high. The study reported the prevalence of HAIs acquired in ICUs was 29.5% (965/3,266 patients), and the top three HAIs were pneumonia, BSI, and SSI [178]. The most frequently isolated pathogens were *A. baumannii* (24.4%), *P. aeruginosa* (13.8%) and *K. pneumoniae* (11.6%), with carbapenem resistance rates of 89.2%, 55.7% and 14.9%, respectively [178]. In my study, we found that vascular catheters including central venous, arterial and hemofiltration catheter were the main risk factor for hospital-acquired BSI. Phu et al. also identified central vascular catheter as one of eight risk factors independently associated with ICU-acquired infections: intubation, urinary catheter, no involvement of a family member in patient care, surgery after admission, admission to ICU from the same hospital, peripheral vascular catheter, and every one day longer of ICU stay [178]. Indeed, many modern diagnostic and therapeutic procedures, such as biopsies, endoscopic examinations, catheterization, intubation/ventilation and suction and surgical procedures increase the risk of infection because of damaging human natural barriers against

infection like skin or mucous membranes. Moreover, contaminated objects or substances may be introduced directly into tissues or normally sterile sites such as the urinary tract and the lower respiratory tract [14].

Despite progress in public health and hospital care, HAIs continue to develop and are among the major causes of death and increased morbidity in both HICs and LMICs. The proportion of patients developing HAIs in my study (23,4%) was much higher than those previously found in other countries: 7.1% in Europe, 4.5% in the USA, 5.7 - 19.1% in other LMICs [11,34]. In HICs, the lungs were the most common site of infection, accounting for 64% of infections, followed by the abdomen (20%), the bloodstream (15%), and the renal tract/genitourinary system (14%) with predominant organisms being *S. aureus*, *Pseudomonas* spp., *Enterobacteriaceae* (mainly *E. coli*), and fungi [33]. In other LMICs, the most frequent type of HAIs was SSI (29.1%), followed by UTI (23.9%), BSI (19.1%), pneumonia (14.8%) with Gram-negative bacteria representing the most common nosocomial isolates [34]. Those things confirm again the disparity in distribution of HAIs types and pathogens between different ICU settings. Therefore, the management of ICU-acquired infections requires knowledge about patients' characteristics, the epidemiology of infections, as well as the AMR patterns of the local microbiologic flora because some of these factors are unique to individual ICU.

In my study, among the 77 patients who developed HAIs with any of the specified bacteria, 44 (57.1%) had prior colonisation with the same organism (Table 4.8), suggesting that prior colonisation was an initial stage in the development of HAIs. The causal relationship between nasal colonisation with *S. aureus* and ICU-acquired *S. aureus* infections is already well-established [126-129]. Moreover, the association between gastrointestinal colonisation and HAIs caused by *K. pneumoniae*, *P. aeruginosa* or *A. baumannii* is also determined strongly [139,141,142,261]. However, our analyses did not demonstrate that prior colonisation was a significant risk factor for HAIs. The

explanation may be due to a low number of observed HAIs with specified bacteria in my study. Furthermore, in this study, I used a combination of bacterial identification on the MALDI-TOF and antimicrobial susceptibility test by disc diffusion method for matching colonizing and infecting isolates. They are all phenotypic tests, not genotypic methods. Therefore, it is possible that the proportion of matching colonisation/infection pairs underestimates the contribution of colonisation to infection. Further understanding of this relationship can be gained through WGS, an approach which would also enable us to better understand transmission of AROs between patients and over time.

My study is limited by being conducted in a single tertiary center, which limits generalization of its results to other centers. Many environmental factors, like workload, hand hygiene compliance, room cleaning protocols, and patient-related factors were not evaluated for the risk of acquired colonisation and infections.

4.5 CONCLUSION

ICU patients are at high risk for acquiring colonisation and contracting HAIs, especially with AROs during ICU stay. More than 50% of patients developed infection with phenotypically similar bacteria to those they were previously colonised with. Future research should focus on monitoring colonisation, and the development of preventive measures that may halt spread of AROs and development of HAI in ICU settings.

Chapter 5. MOLECULAR EPIDEMIOLOGY OF *STAPHYLOCOCCUS AUREUS* COLONISATION AND INFECTIONS IN ICU

5.1 INTRODUCTION

S. aureus is a well-known organism of normal human flora, frequently colonizing the nose, pharynx, and the skin. *S. aureus* colonisation in itself is not harmful but is a significant risk factor for subsequent infections from mild to life-threatening diseases, such as superficial skin or wound infections, cellulitis, abscesses, arthritis, endocarditis, pneumonia and bacteremia [126-129]. The risk of invasive *S. aureus* infection is greatest immediately after acquisition of a strain other than the resident strain [273,274]. It is also known that *S. aureus* can be transmitted between people in a normal population ("community-acquired") via the hands [275], and household contacts [276,277] or contracted in healthcare settings ("hospital-acquired") from other patients, medical staff, and the hospital environment [278,279]. The rate of nasal *S. aureus* colonisation in healthy people is high in HICs: the USA (27 - 30.7%) [248,249], the UK (28.2%) [250], and Netherlands (35%) [280] compared to LMICs: Nigeria (14%), Malaysia (26%), India (16%), Indonesia (<10%) [280] and China (16.5%) [247]. In Vietnam, *S. aureus* nasopharyngeal carriage is present in about one-third of the northern Vietnamese population [180], but there is no data from the southern areas. Most of *S. aureus* strains are sensitive to currently available antimicrobials, and infections can be effectively treated. However, the epidemiology of *S. aureus* has changed, with MRSA strains being a major pathogen that is associated with serious community and hospital-acquired diseases [281]. Different studies showed a considerable variety of nasal MRSA colonisation rates in healthy subjects between countries: the USA (0.9%) [249], the UK (1.1%) [250], and Turkey (0 - 6.2%) [246,253]. Asian countries have shown very high rates (>50%) of MRSA infections, which accounted for 25.5% of community-acquired *S. aureus* infections and 67.4% of hospital-acquired ones [186]. MRSA strains are resistant to many classes of antimicrobials and susceptible only to

vancomycin and new investigational drugs. However, recent reports of MRSA with decreased susceptibility to vancomycin have been a major concern for our ability to treat *S. aureus* infections [282,283]. In Vietnam, MRSA accounts for a significant proportion of *S. aureus* infections, estimated as high as 74.1% of all hospital-acquired and 30.1% of all community-acquired *S.aureus* infections [172]. Therefore, efforts to reduce MRSA infection largely revolve around the identification of risk factors for MRSA infection, investigation and prevention of transmission events, and control of outbreaks. This has led to human interest in understanding the molecular epidemiology of *S. aureus* clones, especially MRSA clones and their relatedness across different geographic regions and healthcare settings [296-298]. However, there are few data about the genetic characterization of *S. aureus* in Vietnam, especialy in ICU settings, including whether particular strains are prevalent, whether there is evidence of patient-to-patient transmission, and whether prior *S. aureus* colonisation leads to staphylococcal infections later.

In recent years, numerous molecular typing methods have been developed for the study of local and global epidemiology of *S. aureus*. The most widely used molecular typing methods has traditionally been pulsed-field gel electrophoresis (PFGE) and *spa* typing [287,288]. PFGE is a technique based on the digestion of bacterial DNA with *Sma*I (a restriction enzyme found in *Serratia marcescens*), thereby generating large fragments of DNA that are separated by pulsed-field electric fields. *Spa* typing is a single-locus typing based on sequencing of the polymorphic X region of the protein A gene (*spa*) of *S. aureus* bacteria. Isolates are assigned to particular *spa* types, then *spa*-clonal lineages by using BURP algorithm. However, these conventional methods lack the resolution necessary to differentiate closely related strains when used to investigate transmission events in the absence of additional epidemiological information (patient-stay data) [130]. A major disadvantage of PFGE is with difficulty in comparing the PFGE results

between different laboratories because the interpretation of PFGE results is often subjective and there is a lack of interlaboratory reproducibility [289]. This has complicated the exchange of strain typing information and the creation of a *S. aureus* and MRSA typing database. *Spa* typing has a high degree of typeability and reproducibility, increased ease of interpretation, and the exchangeability of the typing results to international networks. However, sequencing of a single locus specific for *S. aureus* has made *spa* tying more tedious, expensive and time-consuming. Additionally, the implementation of BURP analysis to group various *spa*-types into *spa*-clonal lineages must be interpreted with caution because some of misclassifications are due to related *spa* repeat successions in isolates of different clonal lineages [290].

Recently, WGS-based typing has been made possible through next-generation sequencing platforms with advantages in speed, costs, unambiguous data interpretation, and simplicity of large-scale database creation. This powerful approach allows *S. aureus* isolates to be compared with each other and with reference sequences across time and space, down to a resolution of a single nucleotide difference [152]. This enhances our knowledge of the population structure of *S. aureus*, the evolutionary history of lineages, and the potential for an outbreak investigator by determining unambiguously the relatedness of isolates. Combined with epidemiological data (patient-stay data), it is then possible to draw inferences about the probability that a transmission event occurred or not to direct better targeting of infection control resources [130,154,155]. These properties give WGS the potential to replace conventional typing methods, and to enhance infection control practice on local, national and international scales.

If we can better understand the relationship of prior colonisation with *S. aureus* and subsequent infections by the same organism, or the genetic relatedness of colonizing and infecting *S. aureus* isolates obtained from the same ICU patients, we are able to manage this pathogen more effectively. The findings also contribute to the assessment

of the efficacy of infection control measures in Adult ICU at the HTD, as well as other ICU settings with similar infection control practices in place.

5.2 MATERIALS AND METHODS

The detailed methods of this chapter are reported in Chapter 2 “Materials and methods”.

5.3 RESULTS

5.3.1 Patient characteristics

During the study period, 19 patients among the 838 enrolled ICU patients with either community or hospital-acquired *S. aureus* infection were included for analysis (Table 5.1). Of them, 68.4% (13/19) were male. The median age was 46 years (IQR 32.5 - 62). 31.6% (6/19) had chronic pre-existing disease with Charlson Comorbidity Index ≥1. The median APACHE II score was 11 (IQR 4 - 19). The most common reasons for ICU admission were tetanus (10 cases, 52.5%) followed by sepsis (5 cases, 26.3%), severe Dengue infection with multi-organ dysfunction (1 case, 5.3%), severe pneumonia with respiratory distress (1 case, 5.3%), hepatic encephalopathy in patient with Hepatitis B virus-related decompensated liver cirrhosis (1 case, 5.3%), and status epilepticus (1 case, 5.3%). Out of the 19 patients, 3 died (15.8%) due to severe MRSA pneumonia with acute respiratory distress syndrome (ARDS): 1 case of secondary pneumonia due to staphylococcal bacteremia in a patient with intravenous drug use and AIDS, and the other two were hospital-acquired pneumonia in one patient with severe tetanus and another patient with end-stage cirrhosis. For deceased patients, the median ICU length of stay was 7 days (IQR 4 - 43). For survivors, the median ICU and hospital length of stay were 18 (IQR 9.8 - 27.3) and 28 (IQR 21.5 - 43.3) days respectively.

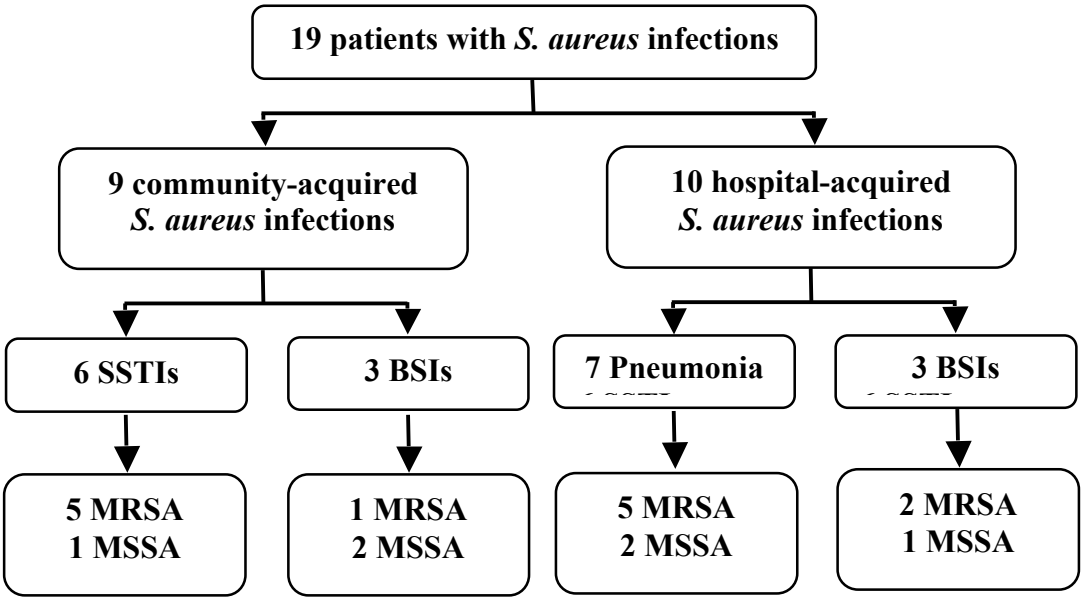
Table 5.1 Characteristics of 19 patients with *S. aureus* infections

Age (yr) - median (IQR)	46 (32.5 - 62)
<60 - n (%)	14 (73.7)
≥60 - n (%)	5 (26.3)
Sex - n (%)	
Male	13 (68.4)
Female	6 (31.6)
Charlson Comorbidity Index score - median (IQR)	0 (0 -1)
No comorbidity (0) - n (%)	13 (68.4)
Mild (1 - 2) - n (%)	4 (21.1)
Moderate (3 - 4) - n (%)	0
Severe (≥5) - n (%)	2 (10.5)
APACHE II score - median (IQR)	11 (4 - 19)
Mild (<5) - n (%)	7 (36.8)
Moderate (5 - 12) - n (%)	4 (21.1)
Severe (>12) - n (%)	8 (42.1)
Admitting diagnosis - n (%)	
Tetanus	10 (52.5)
Sepsis	5 (26.3)
Severe Dengue infection	1 (5.3)
Severe pneumonia	1 (5.3)
Hepatic encephalopathy	1 (5.3)
Status epilepticus	1 (5.3)
***S. aureus* infections**	
Pneumonia	7 (36.8)
Bloodstream infection	6 (31.6)
Skin and soft tissue infection	6 (31.6)
Death - n (%)	3 (15.8)
ICU stay (days) for death patients - median (IQR)	7 (4 - 43)
ICU stay (days) for survivors - median (IQR)	18 (9.8 - 27.3)
Hospital stay (days) for survivors - median (IQR)	28 (21.5 - 43.3)

5.3.2 Characteristics of *S. aureus* infections

The common types of *S. aureus* infection were pneumonia (7 cases), BSI (6 cases), and SSTI (6 cases) (Figure 5.1). I found 9 episodes of community-acquired *S. aureus* infection, including SSTI (6 cases) and BSI (3 cases); and 10 episodes of hospital-acquired *S. aureus* infection, consisting of pneumonia (7 cases) and BSI (3 cases). For CAIs, 6 were MRSA infections, including 5 cases of SSTI and 1 case of BSI; and 3 MSSA infections: 1 case of SSTI and 2 cases of BSI. For HAIs, 7 were MRSA infections, including 5 cases of pneumonia and 2 cases of BSI; and 3 MSSA infections: 2 cases of pneumonia and 1 case of BSI. The mean time from ICU admission to development of hospital-acquired *S. aureus* pneumonia was 15.1 ± 17 days, and that of hospital-acquired *S. aureus* BSI was 18 ± 8.5 days.

Figure 5.1 *S. aureus* infections



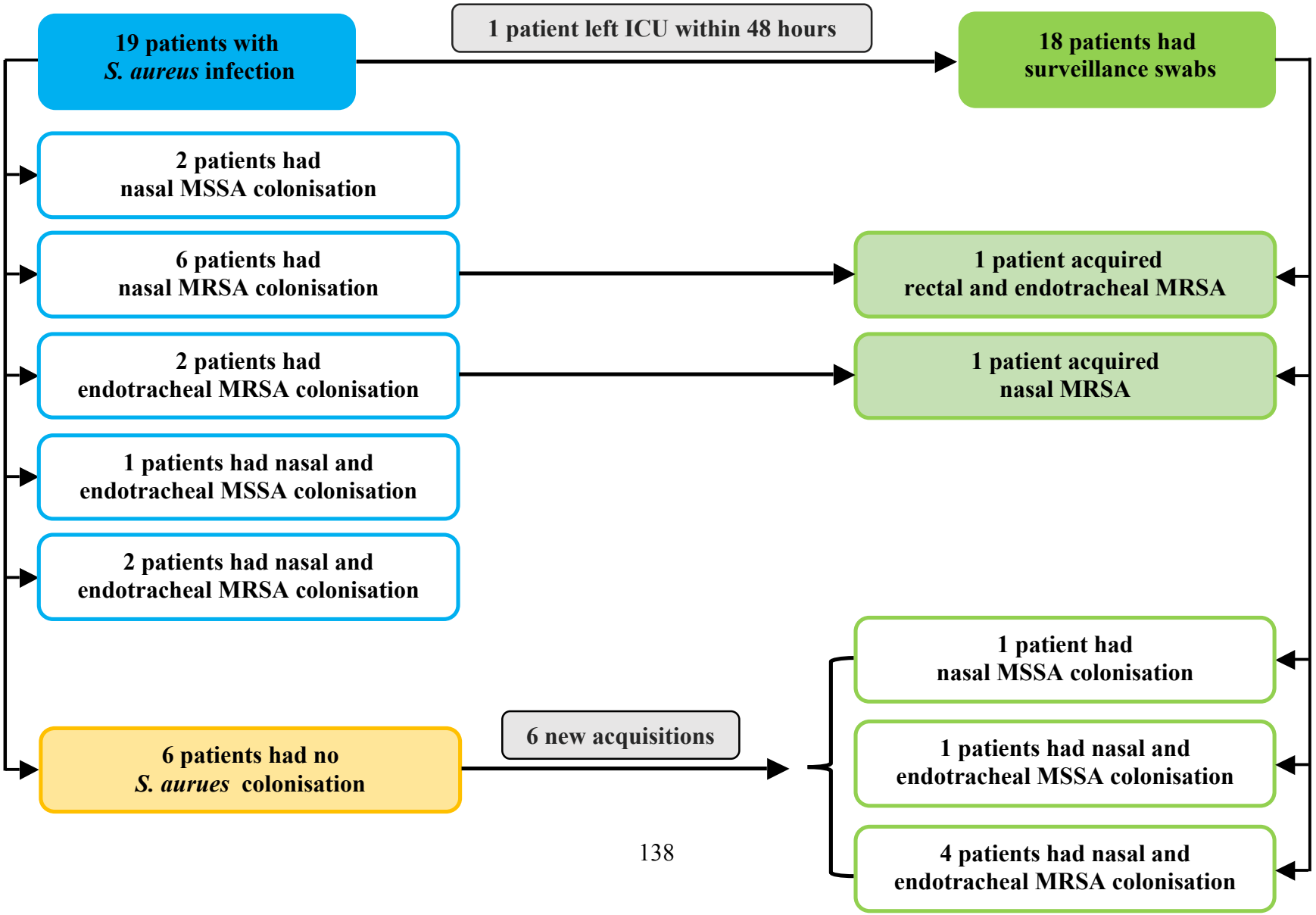
5.3.3 *S. aureus* colonisation on ICU admission assessed by microbiology identification method

All 19 patients had both nasal and rectal admission swabs, and 7 of them (36.8%) had additional ETA taken within 48 hours of ICU admission. 11 patients (57.9%) had nasal *S. aureus* colonisation: 8 MRSA and 3 MSSA. 5 patients had endotracheal *S. aureus* colonisation (4 MRSA and 1 MSSA). 3 patients were colonised with *S. aureus* in both nasal cavity and endotracheal tract. Overall, a total of 13 patients (68.4%) yielded *S. aureus* from either nasal swab or ETA (Figure 5.2).

5.3.4 Acquired *S. aureus* colonisation during ICU stay assessed by microbiology identification method

One patient was discharged from ICU within 2 days of admission. So, 18 patients (94.7%) had surveillance cultures taken to assess acquired *S. aureus* colonisation during their ICU stay. 6 new acquisitions (4 MRSA) were identified in 6 patients who were negative for *S. aureus* colonisation on ICU admission. I also noted another patient became nasally colonised with MRSA, who was positive for endotracheal MRSA colonisation on admission. One more patient became colonised with MRSA in rectal and endotracheal samples although they already had nasal MRSA colonisation on admission.

Figure 5.2 *S. aureus* colonisation on ICU admission and during ICU stay assessed by conventional microbiology method



5.3.5 Phenotypic and genotypic detection of AMR

5.3.5.1 Phenotypic detection of AMR

I collected all colonizing and infecting *S. aureus* isolates (phenotypically bacterial identification) from all 19 patients with community and hospital-acquired *S. aureus* infections for assessment of antimicrobial resistance. However, 5 *S. aureus* isolates could not be retrieved from storage leaving only 67 *S. aureus* available for further analysis. Of the 67 *S. aureus* isolates, 19 (28.4%) were community-acquired isolates and 48 (71.6%) were hospital-acquired strains. 54 (80.6%) were methicillin resistant (39 of them originated from the hospital), and 13 (19.4%) were methicillin susceptible (9 of them from the hospital). Hospital-acquired MRSA isolates were resistant to clindamycin, ciprofloxacin and levofloxacin at a higher rate than community-acquired MRSA isolates. 66.7% of hospital-acquired MSSA isolates and 50% of community-acquired MSSA isolates were resistant to penicillin. 7.4% of MRSA isolates were resistant to rifampin. All MRSA isolates were susceptible to vancomycin. All 6 *S. aureus* isolates causing BSI were susceptible to linezolid and teicoplanin. The AMR profile of *S. aureus* isolates is summarized in Table 5.2. Overall, the resistance rate of *S. aureus* to penicillin was 92.5%, 80.6% for methicillin, 76.1% for erythromycin, 74.6% for clindamycin, 65.7% for ciprofloxacin and 61.2% for levofloxacin.

5.3.5.2 Genotypic detection of AMR

In this study, only 6 *S. aureus* isolates (9% of 67) did not carry any resistance genes against methicillin, penicillin, erythromycin, clindamycin, and fluoroquinolones. 82.1% (55/67) of *S. aureus* isolates had *mecA* gene which is responsible for resistance to methicillin (Table 5.3). In detail, the *mecA* gene was found in 98.2% (53/54) of phenotypically methicillin resistant isolates or MRSA, whereas 15.4% (2/13) of phenotypically methicillin susceptible isolates or MSSA carried *mecA* gene. With regard to the *blaZ* gene (a gene encoding resistance to penicillin), the majority of

staphylococcal strains (82.1%, 55/67) possessed the *blaZ* gene, of which 54 (98.2%) isolates expressed the phenotypic resistance to penicillin and only 1 (1.8%) strain was susceptible to penicillin. In contrast, there were 8 (11.9% of 67) *S. aureus* organisms phenotypically penicillin resistant without carrying the *blaZ* gene. A total of 50 (74.6%) *S. aureus* isolates contained at least one of the erythromycin resistance genes (*ermA, ermB, ermC* and *msrA*), and 98% (49/50) strains were resistant phenotypically to erythromycin. The *lnuA* gene was detected in only 4 isolates, but clindamycin resistance by disk diffusion method was detected in 74.6% (50/67) of *S. aureus* isolates. The fluoroquinolones resistance genes were present in 46 (68.7%) *S. aureus* isolates: 41 of them had *gyrA* and all were phenotypically resistant to fluoroquinolones, while other 5 isolates carried *norA* without phenotypic resistance to fluoroquinolones. Of the 46 *S. aureus* isolates harboring the fluoroquinolones resistance genes, 45 (97.8%) were MRSA strains. The vanA gene was not observed in this study.

In general, the presence of some known resistance genes, such as *mecA*, *blaZ*, *ermB* and *gyrA* highly correlated with phenotypic resistance because of high sensitivity (>85%). Moreover, the absence of some known resistance genes (*ermA, ermC*, *msrA* and *lnuA*) and phenotypic sensitivity were highly linked with specificity of 100%. Detailed information about the correlation between resistant phenotype and genotype is shown in Table 5.4.

Table 5.2 Antimicrobial resistance of 67 *S. aureus* isolates

Antimicrobial agent	Community-acquired isolates		Hospital-acquired isolates		MRSA	MSSA	Total
	MRSA n = 15 (%)	MSSA n = 4 (%)	MRSA n = 39 (%)	MSSA n = 9 (%)	N = 54 (%)	N = 13 (%)	N = 67 (%)
Penicillin	15 (100)	2 (50)	39 (100)	6 (66.7)	54 (100)	8 (61.5)	62 (92.5)
Oxacillin	15 (100)	0	39 (100)	0	54 (100)	0	54 (80.6)
Vancomycin	0	0	0	0	0	0	0
Erythromycin	14 (93.3)	1 (25)	36 (92.3)	0	50 (92.6)	1 (7.7)	51 (76.1)
Rifampicin	1 (6.7)	0	3 (7.7)	0	4 (7.4)	0	4 (6.0)
Clindamycin	13 (86.7)	1 (25)	36 (92.3)	0	49 (90.7)	1 (7.7)	50 (74.6)
Trimethoprim/sulfamethoxazole	3 (20)	1 (25)	1 (2.6)	0	4 (7.4)	1 (7.7)	5 (7.5)
Ciprofloxacin	8 (53.3)	1 (25)	35 (89.7)	0	43 (79.6)	1 (7.7)	44 (65.7)
Levofloxacin	8 (53.3)	1 (25)	32 (82.1)	0	40 (74.1)	1 (7.7)	41 (61.2)
Linezolid *	0	0	0	0	0	0	0
Teicoplanin *	0	0	0	0	0	0	0

* Just applicable for 6 *S. aureus* isolates causing BSI

Table 5.3 Antimicrobial resistance genes among MRSA and MSSA isolates

Gene	Gene name	N (n = 67)	MRSA (n = 54)	MSSA (n = 13)
mecA	Alternate penicillin binding protein 2, defining MRSA	55	42	13
blaZ	Beta-lactamase operon	55	43	12
ermA	Methyltransferases, erythromycin, clindamycin resistance	1	1	0
ermB	Methyltransferases, erythromycin, clindamycin resistance	45	34	11
ermC	Methyltransferases, erythromycin, clindamycin resistance	4	3	1
msrA	Energy dependent efflux of erythromycin	4	3	1
lnuA	Lincosamid - Nucleotidyltransferase	4	3	1
gyrA	DNA gyrase subunit A of fluoroquinolone	41	31	10
norA	Efflux pump gene of fluoroquinolones	5	4	1
vanA	Vancomycin resistance gene	0	0	0

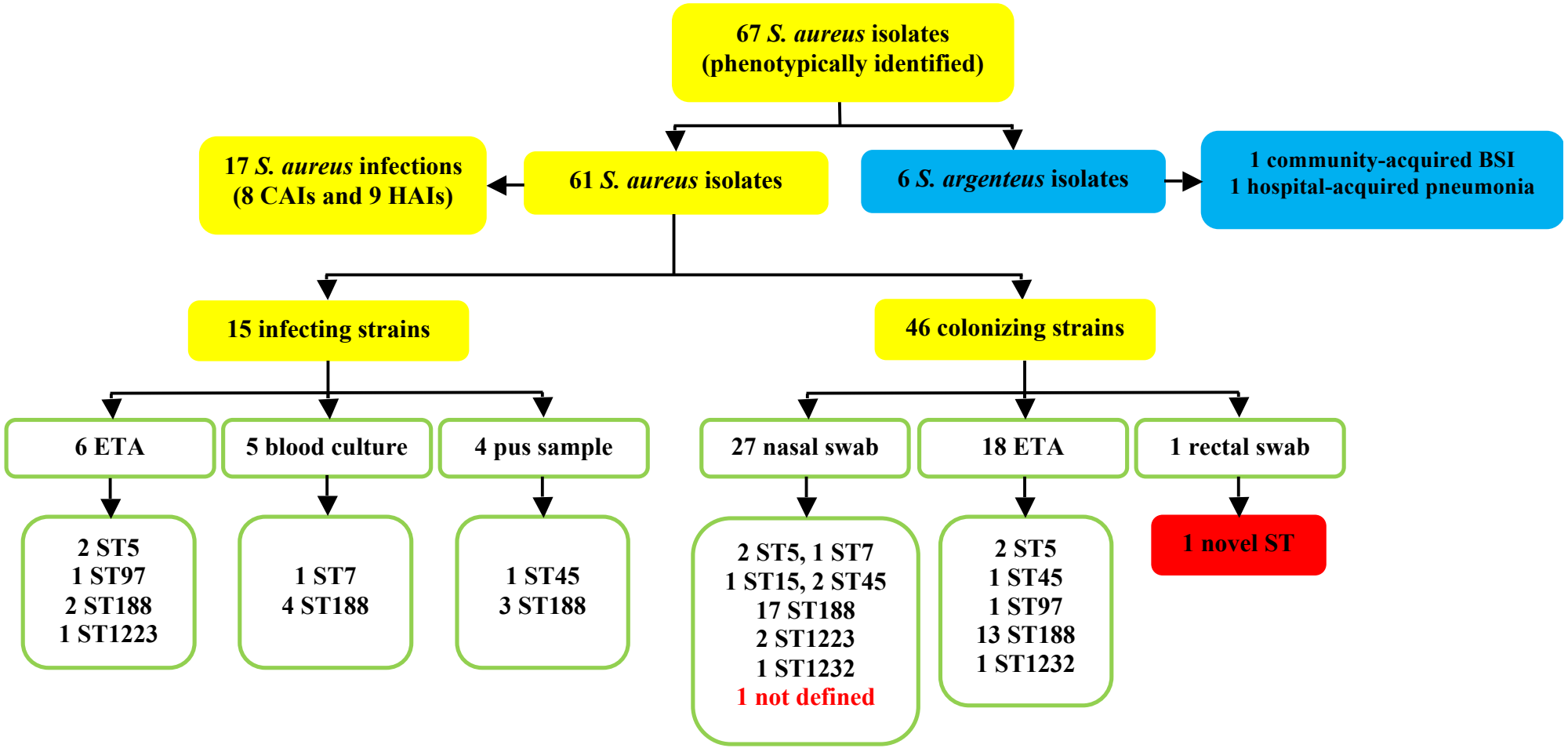
Table 5.4 Correlation of resistant phenotype and genotype

Antimicrobials	AMR genes		Phenotype		Sensitivity (%)	Specificity (%)
			Resistant	Susceptible		
Oxacillin	*mecA*	Yes	53	2	98.2	84.6
		No	1	11		
Penicillin	*blaZ*	Yes	54	1	87.1	80
		No	8	4		
Erythromycin	*ermA*	Yes	1	0	2.0	100
		No	50	16		
	ermB	Yes	44	1	86.3	93.8
		No	7	15		
	ermC	Yes	4	0	7.8	100
		No	47	16		
	msrA	Yes	4	0	7.8	100
		No	47	16		
	ermA/ermB/ ermC/msrA	Yes	49	1	96.1	93.8
		No	2	15		
Clindamycin	*lnuA*	Yes	4	0	8.0	100
		No	46	17		
Ciprofloxacin	*gyrA*	Yes	41	0	93.2	100
		No	3	23		
	norA	Yes	0	5	-	78.3
		No	44	18		
	gyrA/norA	Yes	41	5	93.2	78.3
		No	3	18		
Levofloxacin	*gyrA*	Yes	41	0	100	100
		No	0	26		
	norA	Yes	0	5	-	80.8
		No	41	21		
	gyrA/norA	Yes	41	5	100	80.8
		No	0	21		

5.3.6 Genomic investigation

A total of 67 *S. aureus* isolates were collected for molecular typing analysis (Figure 5.3). Although identified phenotypically as *S. aureus* by using a combination of microscopic examination and production of coagulase, then re-checking on the MALDI-TOF, molecular typing showed that 6 (9%) isolates were actually *Staphylococcus argenteus (S. argenteus)*. These 6 *S. argenteus* isolates were cultured in 2 different patients: one with hospital-acquired pneumonia (3 isolates recovered from ETA and 1 from nasal swab) and one with community-acquired BSI (1 isolate cultured from blood and 1 from nasal swab). Therefore, only 61 *S. aureus* isolates were confirmed by sequencing method in 17 patients with *S. aureus* infection. Out of them, 15 (24.6%) isolates were infecting *S. aureus* isolates (7 of them were community-acquired *S. aureus*) and 46 (75.4%) were colonizing *S. aureus* strains (12 of them were community-acquired *S. aureus*). For infecting *S. aureus* isolates, 6 isolates were recovered from ETA, 5 from blood and 4 from pus samples. For colonizing *S. aureus* strains, 27 strains were cultured from nasal swabs, 18 from ETA and 1 from rectal swab. In terms of methicillin resistance, 54 (88.5% of 61 *S. aureus* isolates) were MRSA strains, 39 (72.2% of 54) originated from the hospital. The frequency of MRSA strains originated from the community (15/17, 88.2%) was comparable to those acquired from hospital sources (39/44, 88.6%).

Figure 5.3 *S. aureus* identification by molecular typing method



5.3.6.1 *S. aureus* diversity assessed by MLST analysis

The MLST results indicated that the 6 *S. argenteus* isolates belong to a single sequence ST, ST2250. Among the 61 *S. aureus* isolates subjected to MLST analysis, two loci were not found in a nasal isolate (a community-acquired MRSA strain) leaving only 60 *S. aureus* strains for further analysis. By using the *S. aureus* MLST database, there were 9 different allelic profiles which were then assigned to 8 STs and a novel ST (Figure 5.4). The most prevalent ST was ST188, represented by 39 isolates (65% of 60); the next prevalent clones were ST5 (6 isolates, 10%), ST45 (4 isolates, 6.7%) and ST1223 (3 isolates, 5%). The other genotypes, ST7, ST97 and ST1232 were found in 2 isolates (3.2%) per each, whereas ST15 contained only one strain. The major ST188 and ST5 corresponded to both MRSA and MSSA strains. Four other STs (ST45, ST1223, ST1232 and novel ST) belonged to only MRSA isolates, whereas ST7, ST15 and ST97 were presented by MSSA strains. There were differences between colonizing and infecting strains within specific STs. Novel and unknown ST were present only in colonizing strains.

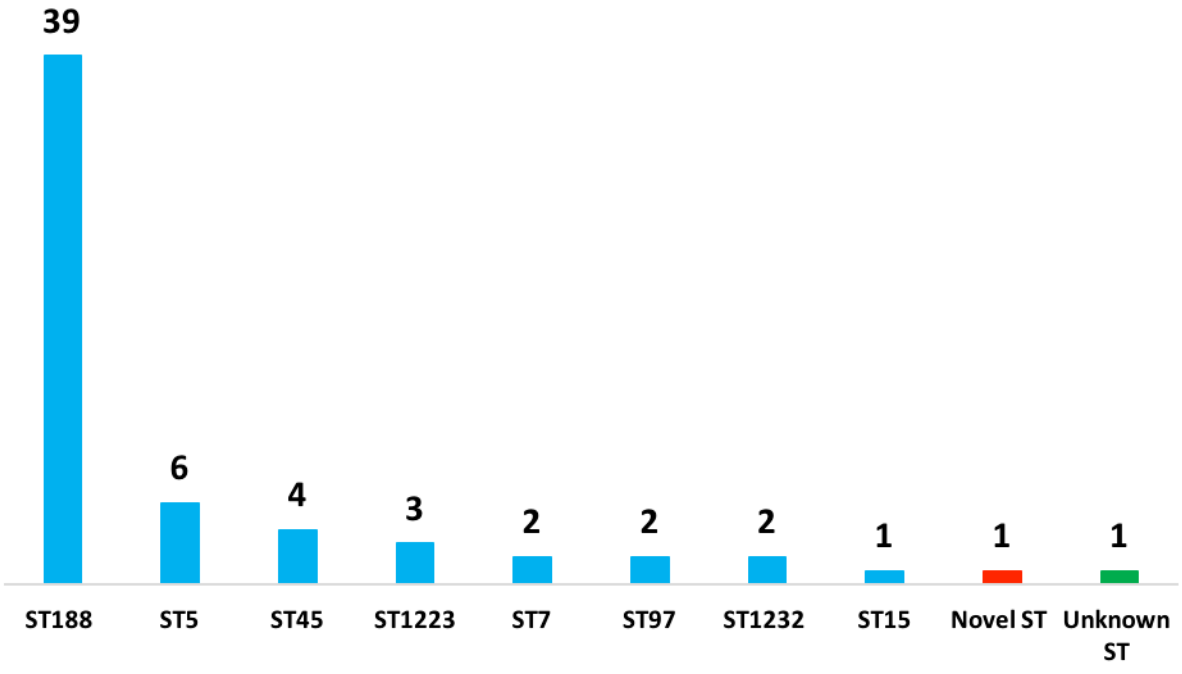


Figure 5.4 Distribution of STs among *S.aureus*

All STs were divided into 4 clonal complexes (CC1, CC5, CC75 and CC97) by BURST and 9 STs (2 ST7, 4 ST45, 2 ST1232 and 1 ST15) were singletons (Table 5.5). ST188 and a novel ST were grouped into CC1 which was also the predominant CC in the study. ST5, ST97 and ST1223 corresponded with CC5, CC97 and CC75, respectively. The 9 singletons did not belong to any CC, and differed from other STs in the study by four or more MLST loci. Out of the 14 community-acquired MRSA isolates, 8 were grouped into CC1 whereas the other 6 did not belong to any CC. Among the 39 hospital-acquired MRSA isolates, the most common CC was CC1 (31 isolates) followed by CC5 (5 isolates) and CC75 (3 isolates). There were no MRSA isolates in CC97. For community-acquired MSSA, one isolate was CC1 and the other one did not belong to any CC. For hospital-acquired MSSA, one isolate was CC5, 2 were CC97, and the other 2 did not belong to any CC.

Table 5.5 Properties of the 61 STs

ST	No. of isolates (No. of MRSA)	No. of CA : HA *S. aureus* isolates	No. of *S. aureus* infections	Allelic profile (allele no.)							CC
				arcC	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*	
188	39 (38)	9 : 30	CAIs: 2 BSI, 1 SSTI HAIs: 2 BSI, 2 pneumonia	3	1	1	8	1	1	1	1
5	6 (5)	0 : 6	HAIs: 1 pneumonia	1	4	1	4	12	1	10	5
45	4 (4)	4 : 0	CAIs: 1 SSTI	10	14	8	6	10	3	2	not defined
1223	3 (3)	0 : 3	HAIs: 1 pneumonia	151	187	20	101	145	150	131	75
7	2 (0)	0 : 2	HAIs: 1 BSI	5	4	1	4	4	6	3	not defined
97	2 (0)	0 : 2	HAIs: 1 pneumonia	3	1	1	1	1	5	3	97
1232	2 (2)	2 : 0		3	35	167	2	20	26	39	not defined
15	1 (0)	1 : 0		13	13	1	1	12	11	13	not defined
Novel	1 (1)	0 : 1		3	1	1	18	1	443	1	1

5.3.6.2 *S. aureus* colonisation on ICU admission assessed by MLST analysis

MLST analysis revealed 2 patients were colonised with *S. argenteus* although microbiology method identified phenotypically as *S. aureus* (Patients 108 and 453, Table 5.6). Therefore, only 11 patients (57.9% of 19) had *S. aureus* colonisation on ICU admission. 3 patients had nasal *S. aureus* ST188; 1 patient with endotracheal *S. aureus* ST188; and 1 patient with both nasal and endotracheal *S. aureus* ST188. 1 patient had endotracheal *S. aureus* ST5. *S. aureus* ST45 strains were recovered from nasal swab of a patient, and from nasal swab and ETA concomitantly in another patient. *S. aureus* ST15 and ST1223 strains were found in nasal cavity of 2 different patients (1 patient per each ST). We could not identify ST in one patient with nasal *S. aureus* colonisation because two loci were not found in this isolate. In general, *S. aureus* ST188 was the predominant clone colonizing ICU patients on admission.

5.3.6.3 Acquired *S. aureus* colonisation during ICU stay assessed by MLST analysis

Out of 6 *S. aureus* acquisitions in 6 patients who were negative for *S. aureus* colonisation on ICU admission (Table 5.6), 2 patients acquired nasal and then endotracheal *S. aureus* ST188 during ICU stay. One patient had endotracheal and then nasal *S. aureus* ST5. One patient became colonised concomitantly with nasal and endotracheal *S. aureus* ST1232. One patient was colonised with endotracheal *S. aureus* ST97 and then nasal *S. aureus* ST5 perhaps due to multiple independent transmissions. Nasal *S. aureus* ST7 acquisition was detected in one patient. Moreover, I also noted one patient became nasal colonised with *S. aureus* ST188 although this patient already had endotracheal *S. aureus* ST188 on admission. Another patient became rectally and endotracheally colonised with *S. aureus* ST188 although this patient already had nasal *S. aureus* ST188 on admission. Therefore, MLST alone could not differentiate whether or not these two patients actually acquired a genetically distinct *S. aureus* strain during

ICU stay or the same *S. aureus* strain was allowed to colonise other body sites under some conditions. This will be clarified by using phylogenetic analysis in the following paragraphs.

5.3.6.4 Transmission of *S. aureus* assessed by MLST analysis

Combined with patient-stay data and AMR profile, it is then possible to draw an inference about the probability that a transmission event occurred between Patient 571 and Patient 575 (Table 5.6). These two patients had shared time in Adult ICU, and their *S. aureus* isolates were well-matched in terms of ST188 and identical antibiogram. Therefore, a total of 7 *S. aureus* acquisitions were detected by MLST analysis compared to 6 *S. aureus* acquisitions by conventional microbiology method.

5.3.6.5 Relationship between *S. aureus* colonisation and infections assessed by MLST analysis

MLST analysis indicated matching STs of the infecting and previously colonizing *S. aureus* isolates in 90% (9/10) ICU patients with hospital-acquired *S. aureus* infections (Table 5.6): ST5 (2 patients), ST7 (1), ST97 (1), ST188 (4) and ST1223 (1). Of them, 4 patients had initial colonisation with *S. aureus* on ICU admission, while the remaining 5 patients acquired *S. aureus* strains during ICU stay. Moreover, 33.3% (3/9) ICU patients suffered CAIs caused by *S. aureus* ST188 isolates which were concordant with the colonizing strains detected on ICU admission.

Table 5.6 *S. aureus* colonisation, acquisition and trasmission in those infected, based on MLST analysis

Patient ID	Length of ICU stay	*S. aureus* infections	*S. aureus* colonisation on ICU admission	*S. aureus* acquisition during ICU stay
19	15th Nov 2014 - 2nd Dec 2014	Pneumonia* (ST97)		ST97 (ETA), ST5 (NS)
36	9th Dec 2014 - 27th Dec 2014	Pneumonia* (ST5)	ST5 (ETA)	
81	10th Jan 2015 - 10th Jan 2015	BSI$ (ST188)	ST188 (NS, ETA)	
108	22nd Jan 2015 - 26th Jan 2015	BSI$ (ST2250)	*S. argenteus*	
129	30th Jan 2015 - 28th Feb 2015	Pneumonia* (ST5)		ST5 (ETA, NS)
170	8th Mar 2015 - 26th May 2015	Pneumonia* (ST188)		ST188 (NS, ETA)
174	10th Mar 2015 - 16th Mar 2015	SSTI$ (ST45)	Not defined (NS)	
282	12nd May 2015 - 22nd May 2015	BSI$ (ST188)	ST188 (NS)	
311	25th May 2015 - 31st May 2015	SSTI$ *(infecting isolates were not retrieved)*	ST15 (NS)	

321	30th May 2015 - 10th Jun 2015	SSTI$ (ST188)	ST45 (NS)	
344	10th Jun 2015 - 19th Jun 2015	SSTI$ (ST188)	ST188 (NS)	
426	16th July 2015 - 21st Aug 2015	BSI* (ST188)	ST188 (ETA)	ST188 (NS)
453	27th July 2015 - 14th Aug 2015	Pneumonia* (ST2250)	*S. argenteus*	
571	8th Sep 2015 - 5th Oct 2015	Pneumonia* (ST188)		ST188 (NS, ETA)
572	8th Sep 2015 - 4th Oct 2015	BSI* (ST7)		ST7 (NS)
575	8th Sep 2015 - 19th Oct 2015	BSI* (ST188)	ST188 (NS)	ST188 (RS, ETA)
623	26th Sep 2015 - 02nd Oct 2015	Pneumonia* (ST1223)	ST1223 (NS)	
689	26th Oct 2015 - 23rd Nov 2015	SSTI$ *(infecting isolates were not retrieved)*		ST1232 (NS + ETA)
807	30th Dec 2015 - 12nd Jan 2016	SSTI$ (ST188)	ST45 (NS, ETA)	

$: CAIs; *: HAIs; Orange color is for ICU patients developing HAIs with their previously colonizing *S. aureus*; Blue color is for ICU patients with CAIs caused by *S. aureus* isolates with the same STs of colonizing *S. aureus* strains; Red rectangle is for a transmission event occurred between Patient 571 and Patient 575.

5.3.6.6 *S. aureus* ST188 assessed by WGS

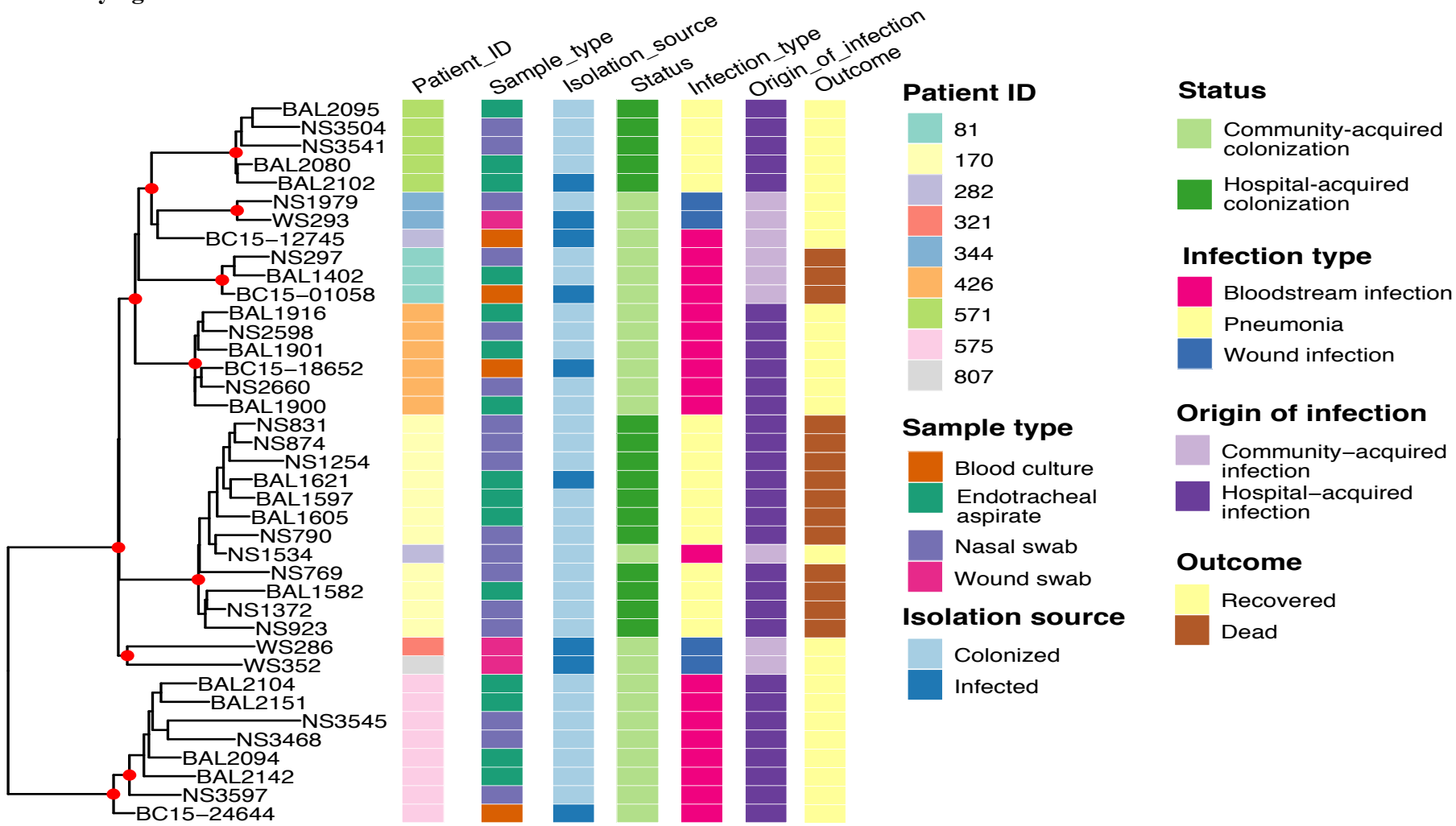
In this study, *S. aureus* ST188 was the predominant clone circulating in Adult ICU. *S. aureus* ST188 isolates were responsible for 5 CAIs (3 SSTI and 2 BSI); and 4 HAIs (2 BSI and 2 pneumonia). However, only infecting ST188 strains were cultured from pus samples, but colonizing ST188 isolates could not be retrieved from storage in 2 patients with SSTI, leaving only 7 patients (1 SSTI, 4 BSI and 2 pneumonia) with full collection of colonizing and infecting ST188 strains available for genetic analysis. Whole-genome SNP typing was used to define the genetic diversity of dominant *S. aureus* ST188 clone in Adult ICU. There was clear phylogenetic clustering of colonizing and infecting ST188 strains within 6 of 7 patients (85.7%) (Figure 5.5): 3 patients with BSI (1 CAI and 2 HAIs, all 3 patients had initial colonisation with *S. aureus* ST188 on ICU admission), 2 patients with hospital-acquired pneumonia (all 2 patients acquired colonisation with *S. aureus* ST188 during ICU stay), and 1 patient with community-acquired SSTI (who also had initial colonisation with *S. aureus* ST188 on ICU admission). Surprisingly, nasal *S. aureus* isolate (NS1534) and bacteremia-associated *S. aureus* isolate (BC15-12745) that were obtained from Patient 282 (another patient with community-acquired BSI) did not cluster phylogenetically. In contrast, NS1534 from Patient 282 clustered with all *S. aureus* isolates from Patient 170 with hospital-acquired pneumonia.

As described above, the diversity of *S. aureus* isolates was also manifested by the median and maximum pairwise SNP differences between isolates within the same patient, and the minimum pairwise SNP differences between isolates obtained between patients (Figure 5.6) with a SNP difference of >40 used to exclude a recent transmission [154,215]. In Patient 426, the median and maximum within-host SNP differences <40 and the minimum between-host SNP differences >40 demonstrated that they did not acquire *S. aureus* during ICU stay, but *S. aureus* isolates from the tracheal

tract disseminated to the nasal cavity. Similarly, the minimum SNP differences >40 between Patient 575 and other patients showed that they did not acquire *S. aureus* during ICU stay, but *S. aureus* isolates from the nasal cavity disseminated to the tracheal and intestinal tract. Furthermore, a possible patient-to-patient transmission between Patient 571 and Patient 575 indicated by MLST analysis plus identical antibiogram and overlapping ICU stay did not occur because of the minimum between-host SNP differences >40. In addition, a nasal *S. aureus* isolate (NS1534) and a bacteremia-associated *S. aureus* strain (BC15-12745) found in Patient 282 differed by 90 SNPs showing that they were originated from different sources. In contrast, NS1534 differed from almost *S. aureus* isolates obtained from Patient 170 by 15 to 40 SNPs (except 43 SNPs for NS769) indicating that they were likely to originate from the same origin. Regard to Patient 282's medical record, NS1534 was recovered from nasal swab taken 1 day after ICU admission, while BC15-12745 was isolated from blood culture performed on the day of ICU admission. It seems that Patient 282 was negative for *S. aureus* colonisation on ICU admission, but acquired *S. aureus* during ICU stay.

To sum up, only 10 patients had *S. aureus* colonisation on ICU admission (neither 11 nor 13 patients as indicated by MLST and conventional microbiology method, respectively), and a total of 7 *S. aureus* acquisitions were detected (6 of them were in patients without *S. aureus* colonisation on ICU admission as indicated by MLST and conventional microbiology method, and one additional acquisition in Patient 282 as described above).

Figure 5.5 Phylogenetic tree for ST188 with full datasets



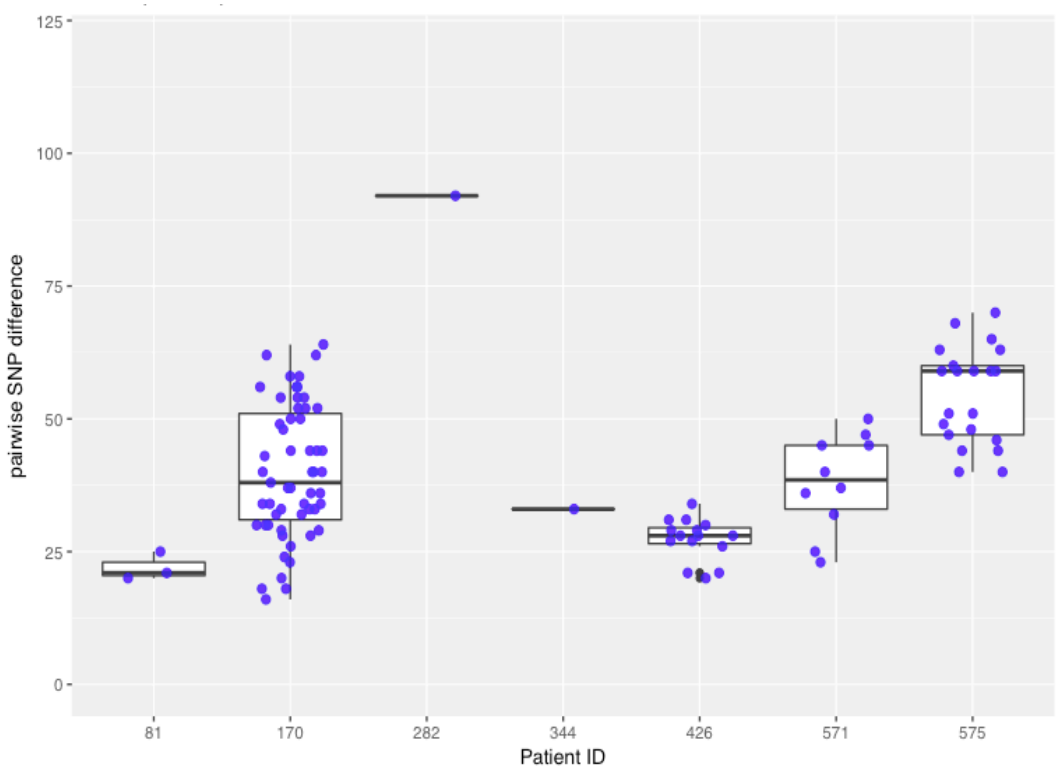


Figure 5.6 SNP differences within individual patients. Each dot represents the SNP difference between two isolates

5.4 DISCUSSION

My study has provided additional molecular data on colonisation, infection and AMR patterns of *S. aureus* among ICU patients in Vietnam. Here, the resistant phenotype and genotype are highly correlated in my study (Table 5.4). However, inconsistency was also found, for example the *mecA* gene was found in 15.4% (2/13) of phenotypically methicillin susceptible isolates (MSSA), or 1 isolate was resistant phenotypically to methicillin (MRSA) without carrying the resistance gene *mecA*. The absence of *mecA* gene within resistant staphylococcal isolates has been reported worldwide [303-305]. The inconsistency was also noted in other antimicrobials (penicillin, erythromycin, clindamycin and fluoroquinolones). A potential explanation for this inconsistency could be the lack of expression of some resistance genes, which can be influenced by numerous genetic and environmental conditions [294]. Isolates which were resistant but

did not have resistant genes, may contain other genes encoding resistance, for example, increased expression of chromosomally encoded efflux pump gene *norA* has been shown to be associated with methicillin resistance [295], or *ermA* and *ermC* genes have also different genetic potentials for selection of clindamycin-resistant mutants [296]. Therefore, more detailed studies of sources and movement of AMR genes are crucial to fight *S. aureus* infection.

Moreover, we also found that microbiological identification methods falsely identified *S. aureus* colonisation and infection. Molecular typing revealed 2 patients were actually colonised and infected with *S. argenteus*, not *S. aureus*. *S. argenteus* can not be distinguished from *S. aureus* using conventional microbiology methods [297,298]. Both strains grow on blood agar with large, round, smooth colonies and colonies demonstrate beta-haemolysis. They are both catalase-positive, coagulase-positive, and Gram-stain-positive cocci in clusters. The biochemical test profile for *S. argenteus* is consistent with *S. aureus*, with mean probability of >95% of identity as *S. aureus* because no test definitively discriminates between both strains. Even using MALDI-TOF MS, it is also difficult to identify *S. argenteus* based on the existing Bruker standard clinical database because identity score of *S. argenteus* is under the manufacturer's recommended threshold for a species level identification. Both strains were then confidently identified into their different groups based on an amended database [299]. However, *S. argenteus* isolates lack the carotenoid pigment staphyloxanthin which is responsible for the characteristic of golden colony color [300]. Staphyloxanthin confers resistance against oxidant-based clearance mechanisms of the host innate immune system and enhanced survival in human neutrophils [301], leading to the hypothesis that *S. argenteus* may be less virulent and less resistant to a range of antimicrobials than *S. aureus* [300]. In fact, all 6 *S. argenteus* in my study grew on blood agar as creamy white colonies, they all had identity scores showing highly probable of *S. aureus* using MALDI-TOF MS.

The name *S. argenteus* was proposed in 2015 [299] but *S. argenteus* was first described in 2009 in northern Australia with isolates belonging to CC75 [302]. Until recently, the use of MLST has made a clear distinction between both strains [298,302,303]. Further isolation of *S. argenteus* has been reported in Trinidad and Tobago where *S. argenteus* belonged to CC1223/1594, ST2250/2277 and ST2596 [297], Thailand (ST2250, ST2854, ST1223 and ST2198) [304,305], Myanmar (ST2250) [306] and Cambodia (ST1223) [303]. This is the first time *S. argenteus* has been detected in Vietnam and sequenced as ST2250. This suggests that international dissemination of ST2250 has taken place. In northern Australia*, S. argenteus* was predominantly associated with community impetigo but rarely with nosocomial bacteremia [307]. In Thailand, *S. argenteus* has been proposed to be an important cause of community-acquired invasive infections with SSTI and bacteremia being the most common clinical features and the mortality rate was about 6.9 - 20% [298,304]. These data have demonstrated that *S. argenteus* may be associated with serious morbidity, death, and nosocomial infection. Only two infected patients with *S. argenteus* were identified in my study, so the clinical picture might be different: one with community-acquired bacteremia and another one with hospital-acquired pneumonia. *S. argenteus* was considered the main pathogen leading to infection in these 2 patients because ICU doctors did not detect any other origin of infection or bacteria from their clinical samples. These two patients were both discharged alive whereas 3 of 17 patients (17.7%) with *S. aureus* infection died of severe MRSA pneumonia. Therefore, further studies to fully elucidate the clinical and molecular epidemiology of *S. argenteus* are warranted, and sequencing methods will be an indispensable tool.

In my study, MLST analysis revealed 8 different STs and a novel ST among the 60 *S. aureus* isolates. The ST diversity suggests that *S. aureus* colonisation and infection in ICU patients were not epidemiologically related [308]. This is in line with similar findings in other studies in Africa [309] and other parts of the world [310]. ST188 was the

predominant *S. aureus* clone circulating in our ICU and this is also the first time ST188 has been detected in southern Vietnam. ST188 was initially reported in northern Vietnam by Bich et al. (2016) who conducted a first study to characterize the population structure of colonizing and invasive *S. aureus* strains in northern Vietnam [311]. In this study, colonizing *S. aureus* isolates were collected from February to May 2012 by collecting nasal and throat swabs of healthy people living in the Dong Da and Ba Vi districts in northern Vietnam (community settings). Clinical invasive *S. aureus* isolates were obtained from positive blood cultures of patients admitted to National Hospital for Tropical Diseases from November 2009 to December 2012. Then, 85 colonizing isolates and 77 invasive ones were randomly selected for MLST analysis. The most dominant STs in this study were ST59 (39 isolates, 24.1%), ST188 (27 isolates, 16.7%) and ST45 (26 isolates, 16.1%) while in my study, ST188 (39 isolates, 65%) was by far the most commonly sequenced. In comparison with our ST188, the clone had the most MRSA isolates (38/39), ST188 in northern Vietnam had the most MSSA isolates (about 24/27). The other STs 5, 7, 15, 97 and 1232 being found in my study were also reported in the study of Bich et al. with different prevalence.

MLST was also used to investigate an outbreak of severe infections due to community-acquired MRSA following routine immunization in southern Vietnam, in May 2006. Nine children presented with adverse events, ranging from fatal toxic shock syndrome, necrotizing soft tissue infection, purulent abscesses, to fever with rash. Eight children had been vaccinated by the same healthcare worker. *S. aureus* recovered from skin infections of 4 children and *S. aureus* isolated from nasal and throat swabs of healthcare worker were sequenced for analysis. MLST analysis found that strains from children and healthcare worker were indistinguishable, and were ST59. Deficiencies in vaccine quality, storage practices, or preparation and delivery were not found. Therefore, the investigation suggests that insufficient attention to hand hygiene may have led to

transmission of community-acquired MRSA in southern Vietnam at that time [312]. Moreover, in an international study to investigate the evolutionary pattern and genotypic characteristics of MRSA strains in the Asian region, 5 MRSA strains isolated from Vietnam were analyzed by MLST to be ST239 (3 isolates) and ST241 (2 isolates) [313]. Therefore, for more comprehensive information about the population structure of *S. aureus* isolates throughout Vietnam, further studies at a national level are essential.

Molecular epidemiology of *S. aureus* has been well-described in the world. ST239 and ST5 have been reported to be dominant STs in some Asian countries, such as Taiwan, Korea, Japan, China and Singapore [326-331]. ST80 is the predominant community-acquired MRSA clone in Europe, USA300 (ST8) and USA400 (ST1) in the USA, ST93 in Australia, and ST30 in Oceania [320]. Interestingly, we did not detect any ST239, ST80, ST8, ST1, ST93 or ST30 isolates in my study. This has demonstrated a unique geographic distribution and evolutionary pattern of *S. aureus*, especially for MRSA clones around the world.

ST188 is a double locus variant of ST1. ST188 with allelic profile of 3-1-1-8-1-1-1 differs at only 2 loci from ST1 with allelic profile of 1-1-1-1-1-1-1. WGS analysis showed that ST188 may have first arisen in livestock around 1960 and were then transmitted to other species. There was no significant genomic or virulence difference between ST188 isolated from livestock and humans. The virulence of ST188 is related to its nasal colonisation and biofilm formation ability [321]. The first draft genome sequence of a MRSA ST188 strain isolated from a patient with hospital-acquired bacteremia at a university teaching hospital in Hong Kong has just been reported in 2014 and designated CUHK_188 [322]. The reference sequence of ST188 has not been published yet on NCBI genome database (http://www.ncbi.nlm.nih.gov/RefSeq/). ST188 has not yet been described as a global pandemic strain, but ST188-associated infections have been increasingly reported particularly in the Asia-Pacific region: China

[321], Hong Kong [323], Malaysia [324] and Taiwan [325]. Most reported infections in China and Taiwan were caused by MSSA ST188 [321,325], but methicillin resistance in ST188 has been increasingly described in Hong Kong and Malaysia [323,324]. In my study, 97.4% ST188 were MRSA and ST188 was responsible for both *S. aureus* CAIs and HAIs. Noticeably, 3 patients died of severe MRSA pneumonia with respiratory distress, and 2 of them were identified to have ST188. Our findings suggest that ST188 may be endemic in Vietnam and provide additional evidence to confirm the complex evolutionary process of ST188 in the world, especially in Vietnam.

My study is also the first one to investigate and confirm the role of prior *S. aureus* colonisation as the source of subsequent *S. aureus* infections in Vietnamese ICU patients. 90% (9/10) ICU patients became infected with their previously colonizing *S. aureus* by the use of MLST analysis (Table 5.6). A phylogenetic cluster of colonizing and infecting *S. aureus* ST188 isolates was observed within 4 ICU patients with HAIs (2 BSI and 2 pneumonia) by the use of whole-genome SNP typing (Figure 5.5). For the other 2 ICU patients with CAIs (1 SSTI and 1 BSI), genome data also indicated a genetic cluster of colonisation and infection isolates. The temporal relationship between nasal *S. aureus* colonisation and subsequent *S. aureus* infections has been well-established typically based on clinical and epidemiologic data, as well as conventional microbiology methods [120-129]. Recently, molecular methods have been used successfully to describe the global population structure of *S. aureus*, to investigate the finer details of staphylococcal outbreaks and transmission events, to predict AMR and virulence, to evaluate infection control practice, and to guide preventive measures [130]. For example, WGS was employed to confirm the transmission of MRSA and MSSA among patients during wound care in a healthcare center in eastern Ghana [131], or to "rule in" the transmission of MRSA via deceased donor liver transplantation in the USA when the donor and recipient MRSA isolates are indistinguishable [132]. WGS is an ideal method to

"rule out" a suspected outbreak of MRSA-related SSI in a rural hospital in the USA. In this hospital, the MRSA strain from a colonised surgical team member was identified not to be the source of the SSI cases and no evidence of transmission occurred among the patients with SSI [133]. However, there is a paucity of studies using WGS analysis to investigate the role of prior *S. aureus* colonisation as a source of infections, especially in less-resourced healthcare settings. In a multicenter study of *S. aureus* bacteremia conducted in Germany, the blood isolates were clonally identical to those obtained from nasal swabs in 82.2% of patients (180/219) [134]. In another multicenter study conducted in the USA, colonizing MRSA isolates belonged to the same clonal types as the strains causing SSTI in most cases (73.7%) [135]. In Korea, concordance rates by methicillin susceptibility and ST between colonizing and clinical *S. aureus* isolates obtained from children were 90.3% and 84%, respectively among the 31 pairs of healthcare-associated *S. aureus*, and 100% concordance was observed by methicillin susceptibility and ST for 6 pairs of community-associated *S. aureus* [136].

To sum up, WGS is more reliable than conventional microbiology method with regard to investigation of colonisation with genetically confirmed *S. aureus*. In 13 patients (68.4% of 19 enrolled patients) who were positive with *S. aureus* colonisation on ICU admission by conventional microbiological methods, there were only 10 patients (52.6%) actually colonised with *S. aureus* by genomic typing (not including 2 patients colonised with *S. argenteus,* and one patient (Patient 282) acquired *S. aureus* only 1 day after ICU admission). WGS-SNP-based analysis is also more accurate than MLST in terms of disproving a possible patient-to-patient transmission event indicated by the use of MLST plus antibiogram and overlapping ICU stay, and identifying one additional transmission which was not detected. Therefore, a total of 7 *S. aureus* acquisitions were detected by WGS-SNP-based analysis (41.2% of 17, not including 2 patients with *S. argenteus* infection) compared to also 7 acquisitions by MLST (including 1 patient-to-

patient transmission event falsely identified, and 6 acquisitions by conventional microbiological identification). Our *S. aureus* acquisition rate is higher than the acquisition rate of 14.2% (45/316) reported by Bloemendaal et al. in 6 European ICUs [326].

This study has some limitations. Firstly, some colonizing and infecting *S. aureus* strains were not retrieved from storage, it is possible that the proportion of matching colonisation/infection pairs underestimates the contribution of colonisation to infection and misses some instances of transmission events. Secondly, it was performed in a single Vietnamese center, so the findings may not be representative. However, these findings should be generally applicable to ICUs where similar infection control measures are in place. Thirdly, I did not screen *S. aureus* in medical staff and the hospital environment, so it is possible that I missed some transmission sources.

5.5 CONCLUSION

This study shows that prior *S. aureus* colonisation plays a role as a source of infections in ICU patients, and MRSA is an important pathogen in ICU. The predominant clone of *S. aureus* in southern Vietnam is ST188, and perhaps most surprising was the presence of *S. argenteus* strains. These findings were only apparent through the use of WGS. In conclusion, I highlight the potential of WGS in the analysis of *S. aureus* colonisation, acquisition and infection in ICU settings. Wide-scale surveillance of *S. aureus* and establishment of a strategy to prevent its further spread are urgently needed in ICU setting. With advances in technology and reduced costs, I believe that WGS will be widely used.

Chapter 6. MOLECULAR EPIDEMIOLOGY OF *KLEBSIELLA PNEUMONIAE* COLONISATION AND INFECTIONS IN ICU

6.1 INTRODUCTION

K. pneumoniae has been identified as one of the top bacteria of concern in recent AMR reports from WHO [327], the CDC [167] and the UK Department of Health [328]. In humans, *K. pneumoniae* is known to asymptomatically colonise the skin, nares, mouth, pharynx, and gastrointestinal tracts with different carriage rates. In the USA, *K. pneumoniae* was detected in 3.8% of stool samples and 9.5% of nasal specimens collected from 242 healthy participants [329]. In Korea, *K. pneumoniae* was identified in 21.1% of stool samples obtained from 1,174 healthy adults [330]. *K. pneumoniae* nasopharyngeal carriage was found in about 15% of Indonesian adults and 7% of children [331], while the overall carriage rate of *K. pneumoniae* (either nose, throat, or both) was 14.1% among 1,029 Vietnamese adults and children [182].

K. pneumoniae is able to cause a wide variety of infections in humans, including soft tissue infection, cholecystitis, liver abscess, meningitis, UTI, pneumonia and septicemia. In recent years, multidrug-resistant *K. pneumoniae* has emerged as an important nosocomial pathogen likely to be resulting from selection pressures generated by changes in medical practice and antimicrobial use [332], and due to its ability to spread rapidly in the hospital environment [22,327,333]. *K. pneumoniae* is becoming resistant to a number of antimicrobials, mainly extended-spectrum cephalosporins and penicillins, due to the acquisition of plasmids encoding for the ESBL production. Data obtained from all WHO regions reported over 30% resistance in *K. pneumoniae* against 3rd-generation cephalosporins, and exceeding 50% in some countries [327]. Alarming rates of carbapenem resistance - exceeding 50% - have also been reported in *K. pneumoniae* in many settings, and associated with mortality rates of up to 50% because few alternative treatment options are available [327].

At the HTD, according to a recent microbiological report from January 2015 to January 2018 (unpublished data), *K. pneumoniae* was one of the most frequently isolated pathogens in Adult ICU. This pathogen accounted for 12% of pneumonia cases with ESBL rate of 30%, and carbapenemase rate of 16%; 15% of SBP cases (ESBL rate of 4%); 9% of UTI cases (ESBL rate of 30% and carbapenemase rate of 29%) and 9% of BSI cases (ESBL rate of 6%). The association between gastrointestinal colonisation and subsequent HAIs due to *K. pneumoniae* was established and strong [139,261]. Moreover, genome data indicated matching colonisation and infection isolates in 80% of isolate pairs [139].

To efficiently control *K. pneumoniae* requires a detailed understanding of the epidemiology and pathogenesis of these bacteria. Various methods have been developed for *K. pneumoniae* typing to clarify if these is an outbreak or just coincidental, sporadic cases, and to identify the source of infection and the route of transmission. Phenotypic methods include biotyping, capsular serotyping, phage typing and bacteriocin typing. Biotyping is based on an extended panel of biochemical and culture tests with long cultivation times, so it is not a suitable epidemiological tool [334]. Capsular serotyping is performed by countercurrent immuno-electrophoresis to determine capsular K antigen by testing *K. pneumoniae* isolates against the specific antisera. However, this method is time-consuming and its interpretation is subjective because of the large number of serological cross-reactions among the capsule types. Moreover, anti-capsule antisera are not commercially available, and about 10 - 30% of *K. pneumoniae* isolates are serologically non-typeable, either because they express a novel capsule (most commonly for clinical isolates) or are non-capsulated [335,336]. Phage typing is a technique that uses various known bacteriophages to identify *K. pneumoniae* on the basis of their reaction (susceptibility or resistance) to specific phages. Depending on which groups of phages can kill or not kill

a *K. pneumoniae* strain (the reaction pattern), *K. pneumoniae* is given a number, the phage type. The phage-typing procedure is easily interpretable, but the method itself requires a variety of phages and substantial technical expertise to perform. Furthermore, this technique shows a relatively poor typing rate of 19 - 67%, so this method can generally only be performed at reference laboratories [337,338]. *K. pneumoniae* typing via bacteriocin sensitivity has been recommended to enable more precise epidemiological analysis. Bacteriocin are bactericidal substances, usually proteins, produced by bacteria to inhibit the growth of other bacteria, usually members of the same species. However, this method shows considerable disadvantages: the instability of bacteriocin preparations, the low reproducibility and typability of *K. pneumoniae* strains, so its usefulness for epidemiological studies is doubtful [334,339].

Therefore, molecular typing methods have presented as accurate tools in epidemiological investigations of *K. pneumoniae*. Ribotyping is based on the digestion of genomic DNA with *Ecor*I (a restriction enzyme found in *E. coli*), then the DNA fragments are separated by agarose gel electrophoresis, hybridized with a labelled 16S or 23S rRNA probe, and finally compared with reference organisms in a computer database [340]. Pulsed-field gel electrophoresis (PFGE) involves the digestion of chromosomal DNA with *Xba*I (a restriction enzyme found in *Xanthomonas badrii*), thereby generating an average of fragments that are separated by a contour-clamped homogeneous electric field [341]. Randomly amplified polymorphic DNA (RAPD) is based on the use of three primers which hybridize with sufficient affinity to DNA sequences at low annealing temperatures such that they can be used to initiate amplification of regions of the bacterial genome. The amplification products are separated by agarose gel electrophoresis to generate a bacterial fingerprint and the banding patterns are used to compare the relatedness of bacterial strains [341]. Amplified fragment length polymorphism (AFLP) is to digest whole DNA with several restriction

enzyme combinations and two primers for PCR amplification. The amplified fragments are subjected to high-resolution gel electrophoresis and characteristic separation profiles are generated and compared using an automated DNA sequencer. However, the ribotyping's interpretation of banding pattern variation has both practical and theoretical limitations [342]. PFGE is a technically demanding and time-consuming technique that requires specific equipment, costly reagents, and long DNA preparation and electrophoresis times [341]. RAPD analysis, compared with PFGE, is significantly simpler and faster to perform, but RAPD typing suffers from problems in low inter-run and inter-laboratory reproducibility and from a lack of consensus rules for interpretation of pattern differences [343]. AFLP is a complex procedure involving a great number of steps, the requirement for an automated DNA sequencer in analysis and the consequent costs, which is a crucial factor to warrant reproducibility [344]. Recently, MLST is an adequate nucleotide sequence-based approach for characterizing the genetic relationships among *K. pneumoniae* isolates, with the advantage over traditional molecular typing of manipulation with ease, unambiguous and portable data, and convenient comparison [342]. Additionally, with the rapid development of WGS, the population structure of *K. pneumoniae*, especially MDR pathogens can be dissected at a higher resolution level. Thus, WGS has been applied to efficiently control and prevent the dissemination of MDR *K. pneumoniae* in hospital settings thanks to its ability of early detection and tracking of colonised or infected or patients [345].

If we can better understand the relationship of prior colonisation with *K. pneumoniae* and subsequent infections by the same organism, or the genetic relatedness of colonizing and infecting *K. pneumoniae* isolates obtained from the same ICU patients, we are able to manage this pathogen more effectively. The findings also contribute to the assessment of the efficacy of infection control measures in Adult ICU at the HTD, as well as other ICU settings with similar infection control practices in place.

6.2 MATERIALS AND METHODS

The detailed methods of this chapter are reported in Chapter 2 “Materials and methods”.

6.3 RESULTS

6.3.1 Patient characteristics

During the study period, 28 of the 838 enrolled ICU patients had *K. pneumoniae* infection (Table 6.1). The median age was 59 years (IQR 45 - 66). 71.4% (20/28) were male and 39.3% (11/28) had chronic pre-existing disease with Charlson Comorbidity Index ≥1. The median APACHE II score was 10.5 (IQR 7 - 19). The most common reasons for admission to ICU were tetanus (13 cases, 46.4%) followed by sepsis and septic shock (8 cases, 28.6%), severe pneumonia with respiratory distress (2 case, 7.1%), and hepatic encephalopathy in patients with Hepatitis B virus-related decompensated liver cirrhosis (2 case, 7.1%). Out of the 28 patients, 8 died (28.6%): 5 with prolonged shock and the other 3 cases with acute respiratory distress syndrome. For patients who died, the median ICU length of stay was 5.5 (IQR 2.8 - 26.5). For survivors, the median ICU and hospital length of stay were 25.5 (IQR 6.8 - 32.5) and 36.5 (IQR 16.5 - 42), respectively.

Table 6.1 Characteristics of 28 patients with *K. pneumoniae* infections

Age (yr) - median (IQR)	59 (45 - 66)
<60 - n (%)	16 (57.1)
≥60 - n (%)	12 (42.9)
Sex - n (%)	
Male	20 (71.4)
Female	8 (28.6)
Charlson Comorbidity Index score - median (IQR)	0 (0 -1.3)
No comorbidity (0) - n (%)	17 (60.7)
Mild (1 - 2) - n (%)	5 (17.9)
Moderate (3 - 4) - n (%)	3 (10.7)
Severe (≥5) - n (%)	3 (10.7)
APACHE II score - median (IQR)	10.5 (7 - 19)
Mild (<5) - n (%)	4 (14.2)
Moderate (5 - 12) - n (%)	12 (42.9)
Severe (>12) - n (%)	12 (42.9)
Admitting diagnosis - n (%)	
Tetanus	13 (46.4)
Sepsis and septic shock	8 (28.6)
Severe pneumonia	2 (7.1)
Hepatic encephalopathy	2 (7.1)
Severe Dengue infection	1 (3.6)
Urinary tract infection	1 (3.6)
Status epilepticus	1 (3.6)
***K. pneumoniae* infections**	
Pneumonia	16 (57.2)
Bloodstream infection	6 (21.4)
Urinary tract infection	4 (14.3)
Spontaneous bacterial peritonitis	2 (7.1)
Death - n (%)	8 (28.6)
ICU stay (days) for death patients - median (IQR)	5.5 (2.8 - 26.5)
ICU stay (days) for survivors - median (IQR)	25.5 (6.8 - 32.5)
Hospital stay (days) for survivors - median (IQR)	36.5 (16.5 - 42)

6.3.2 Characteristics of *K. pneumoniae* infections

Of the 28 episodes of *K. pneumoniae* infection included for analysis in this study, pneumonia (16 cases, 57.2%) was the most common type of infection, followed by BSI (6, 21.4%), UTI (4, 14.3%) and SBP (2, 7.1%). Of them, I found 5 episodes of community-acquired *K. pneumoniae* infection, including BSI (2 cases), SBP (2) and pneumonia (1); 5 episodes of healthcare-associated *K. pneumoniae* infection consisting of UTI (3) and BSI (2); and 18 episodes of hospital-acquired *K. pneumoniae* infection: pneumonia (15, 12 of them were ventilator-associated pneumonia), BSI (2) and UTI (1). All CAIs were related to sensitive *K. pneumoniae* strains. For HCAIs, there were 3 cases of UTI (60%) caused by antimicrobial-resistant *K. pneumoniae* (1 with AmpC-producing *K. pneumoniae*, 1 with ESBL-producing *K. pneumoniae*, and 1 with ESBL and carbapenemase-producing *K. pneumoniae*). For HAIs, 2 cases (11.1%) were antimicrobial-resistant *K. pneumoniae* infections: 1 case of pneumonia with AmpC-producing *K. pneumoniae*, and 1 case of BSI with ESBL and carbapenemase-producing *K. pneumoniae*. The mean time from ICU admission to development of hospital-acquired *K. pneumoniae* pneumonia was 14.5 ± 10.1 days, and that of hospital-acquired *K. pneumoniae* BSI was 11 ± 7.1 days. Further information is described in Figure 6.1.

6.3.3 Phenotypic detection of AMR

Of the 28 patients with *K. pneumoniae* infection, a total of 141 infecting and colonizing *K. pneumoniae* isolates (phenotypically bacterial identification) were collected for AMR testing. Of them, 11 (7.8%) were community-acquired isolates (6 infecting and 5 colonizing isolates) and 130 (92.2%) were hospital-acquired strains (21 infecting and 109 colonizing strains). 21 (14.9%) were AmpC-producing *K. pneumoniae* (20 of them originated from the hospital), 12 (8.5%) were ESBL-producing *K. pneumoniae* (all of them from the hospital), and 3 (2.1%) were ESBL and carbapenemase-producing *K. pneumoniae* (all of them from the hospital). Hospital-acquired *K. pneumoniae* isolates were resistant to almost antimicrobials at a higher rate than community-acquired strains for both infecting and colonizing organisms. Over 20% of *K. pneumoniae* isolates were resistant to ceftazidime and ceftriazone, and fewer than 3% of *K. pneumoniae* isolates were resistant to ertapenem, imipenem and meropenem. However, 3.5% of *K. pneumoniae* isolates showed intermediate phenotypes to colistin, which were counted as resistant in this analysis. The AMR profile of *K. pneumoniae* isolates is summarized in Table 6.2.

Figure 6.1 *K. pneumoniae* infections

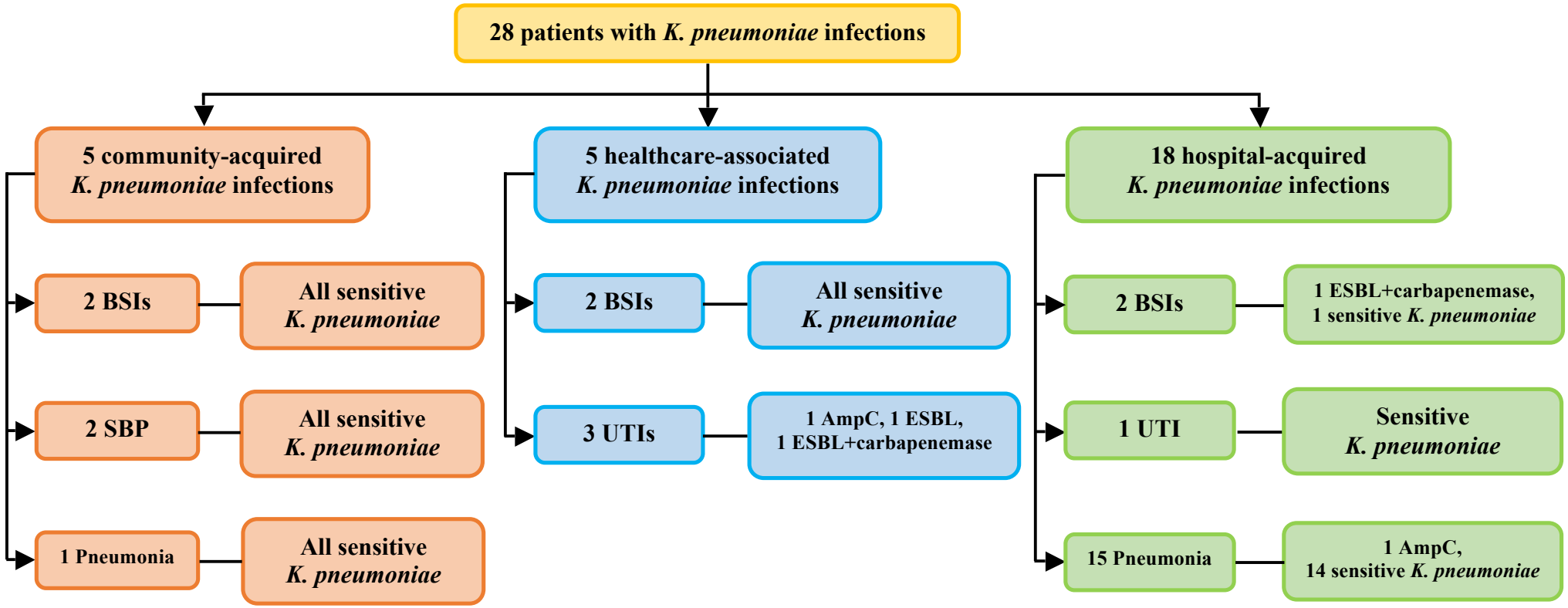


Table 6.2 Antimicrobial resistance of 141 *K. pneumoniae* isolates

Antimicrobial agents / mechanisms	Community-acquired isolates		Hospital-acquired isolates		Infecting n = 27 (%)	Colonizing n = 114 (%)	Total N = 141 (%)
	Infecting n = 6 (%)	Colonizing n = 5 (%)	Infecting n = 21 (%)	Colonizing n = 109 (%)			
Amoxcillin-clavulanic acid	0	1 (20.0)	4 (19.1)	26 (23.9)	4 (14.8)	27 (23.7)	31 (22.0)
Ceftazidime	0	1 (20.0)	4 (19.1)	25 (22.9)	4 (14.8)	26 (22.8)	30 (21.3)
Ceftriaxone	0	1 (20.0)	4 (19.1)	25 (22.9)	4 (14.8)	26 (22.8)	30 (21.3)
Cefepime	0	0	3 (14.3)	13 (11.9)	3 (11.1)	13 (11.4)	16 (11.3)
Ticarcillin-clavulanate	4 (66.7)	1 (20.0)	5 (23.8)	27 (24.8)	9 (33.3)	28 (24.6)	37 (26.2)
Piperacillin-tazobactam	0	0	3 (14.3)	15 (13.8)	3 (11.1)	15 (13.2)	18 (12.8)
Ofloxacin	0	1 (20.0)	4 (19.1)	13 (11.9)	4 (14.8)	14 (12.3)	18 (12.8)
Ciprofloxacin	0	2 (40.0)	5 (23.8)	18 (16.5)	5 (18.5)	20 (17.5)	25 (17.7)
Trimethoprim-sulfamethoxazole	1 (16.7)	2 (40.0)	6 (28.6)	46 (42.2)	7 (25.9)	48 (42.1)	55 (39.0)
Amikacin	0	0	2 (9.5)	2 (1.0)	2 (7.4)	2 (1.8)	4 (2.8)
Ertapenem	0	0	4 (19.1)	0	4 (14.8)	0	4 (2.8)
Imipenem	0	0	4 (19.1)	0	4 (14.8)	0	4 (2.8)
Meropenem	0	0	3 (14.3)	0	3 (11.1)	0	3 (2.1)
Colistin	0	0	3 (14.3)	2 (1.8)	3 (11.1)	2 (1.8)	5 (3.5)
AmpC	0	1 (20.0)	1 (4.8)	19 (17.4)	1 (3.7)	20 (17.5)	21 (14.9)
ESBL	0	0	0	12 (11.0)	0	12 (10.5)	12 (8.5)
ESBL + Carbapenemase	0	0	3 (14.3)	0	3 (11.1)	0	3 (2.1)

6.3.4 *K. pneumoniae* colonisation on ICU admission assessed by microbiology identification method

All 28 infected patients had both nasal and rectal swabs on admission, and 16 of them (57.1%) had additional ETA taken within 48 hours of ICU stay. On ICU admission, only 2 patients (7.1%) had nasal *K. pneumoniae* colonisation, and all with sensitive bacteria whereas 17 patients (60.7%) had rectal *K. pneumoniae* colonisation: 2 with ESBL-producing *K. pneumoniae*, 2 with AmpC-producing *K. pneumoniae* and 2 with ESBL and AmpC-producing *K. pneumoniae*. Moreover, 4 patients (25% of 16) had endotracheal *K. pneumoniae* colonisation, and all with sensitive strains. Overall, a total of 21 patients (75%) showed colonisation with *K. pneumoniae* from either the admission nasal swab, rectal swab or ETA.

6.3.5 Acquired *K. pneumoniae* colonisation during ICU stay assessed by microbiology identification method

Six patients (5 of them initially colonised with *K. pneumoniae*) were discharged from ICU within 2 days of admission and had no further samples taken (Figure 6.2). The remaining 22 patients (12 of them initially colonised with *K. pneumoniae*) had further surveillance cultures taken to assess acquired *K. pneumoniae* colonisation during their ICU stay. 12 patients (54.5%) who were negative for nasal *K. pneumoniae* colonisation on ICU admission became nasal colonised with *K. pneumoniae* during ICU stay (1 with ESBL-producing *K. pneumoniae*, 1 with AmpC-producing *K. pneumoniae* and 1 with ESBL and AmpC-producing *K. pneumoniae*). Similarly, 6 patients (27.3%) who did not show colonisation with *K. pneumoniae* in the rectum on admission became rectally colonised with *K. pneumoniae* during ICU stay (1 with AmpC-producing *K. pneumoniae* and 2 with ESBL and AmpC-producing *K. pneumoniae*), and 12 patients (54.5%) acquired endotracheal colonisation with *K. pneumoniae* (2 with AmpC-producing strain) without *K. pneumoniae* colonisation on admission. In general, 6 new

K. pneumoniae acquisitions were found in 6 patients not having admission *K. pneumoniae* colonisation from either nasal swab, rectal swab or ETA (Patients 225, 227, 334, 341, 349 and 485) (Table 6.3). Moreover, I also detected 2 other patients who had rectal ESBL and AmpC-producing *K. pneumoniae* colonisation on admission and subsequently developed colonisation with fully sensitive *K. pneumoniae* in their rectum during ICU stay (Patients 488 and 648). In addition, five more patients became colonised in different body sites with *K. pneumoniae* isolates sharing the same antibiogram with *K. pneumoniae* strains colonizing on admission (Patients 452, 453, 572, 623 and 644). For these patients, just based on antimicrobial susceptibility testing I did not know if they actually acquired a genetically distinct *K. pneumoniae* isolate or the same *K. pneumoniae* strain was allowed to colonise another body sites. Microbiology identification methods are typically less informative for this situation. Therefore, only 8 new *K. pneumoniae* acquisitions were identified in this study by using traditional culture method (6 new acquisitions in patients without colonisation on admission, and 2 new acquisitions with sensitive *K. pneumoniae* in patients who had admission colonisation with ESBL and AmpC-producing *K. pneumoniae*).

Figure 6.2 Acquired *K. pneumoniae* colonisation during ICU stay assessed by conventional microbiology method

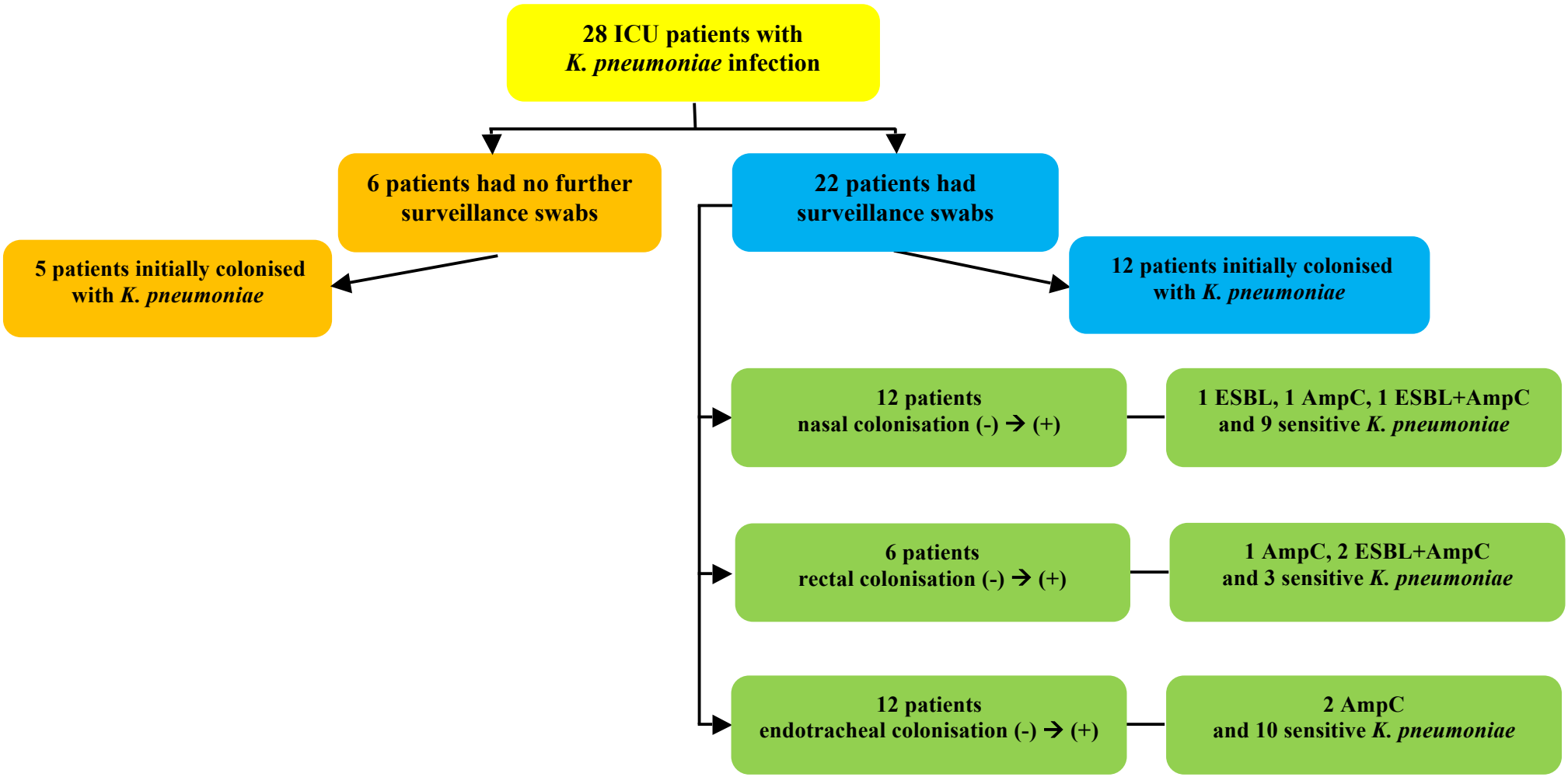


Table 6.3 *K. pneumoniae* colonisation, acquisition and transmission in those infected, based on conventional microbiology method and MLST analysis

Patient ID	Length of ICU stay	Infections	Conventional microbiology identification method		MLST analysis	
			Colonisation on ICU admission	Acquired colonisation during ICU stay	Colonisation on ICU admission	Acquired colonisation during ICU stay
37	10th Dec 2014 - 11th Dec 2014	BSI# (ST23)				
67	5th Jan 2015 - 12th Jan 2015	UTI# (ST1779)	AmpC (RS)		ST1779 (RS)	
96	16th Jan 2015 - 6th Mar 2015	Pneumonia* (ST65)	Sensitive (NS, ETA)		ST65 (NS, ETA)	
121	28th Jan 2015 - 30th Jan 2015	BSI$ (ST375)	AmpC (RS)		ST15 (RS)	
139	3th Feb 2015 - 5th Feb 2015	Pneumonia$ (novel ST)	Sensitive (RS)		ST22 (RS)	
170	8th Mar 2015 - 26th May 2015	Pneumonia* (ST86)	Sensitive (NS, ETA)		ST86 (NS, ETA)	
225	4th Apr 2015 - 11st May 2015	Pneumonia* (ST592)		Sensitive (NS, ETA)		ST592 (NS, ETA)
227	5th Apr 2015 - 13th May 2015	Pneumonia* (ST86)		Sensitive (NS, RS, ETA)		novel ST (RS), ST86 (NS, ETA)
256	18th Apr 2015 - 5th May 2015	BSI* (ST17)	ESBL (RS)		ST17 (RS)	
331	2nd Jun 2015 - 10th Jun 2015	UTI# (not retrieved)	ESBL (RS)		ST15 (RS)	
334	4th Jun 2015 - 1st Jul 2015	Pneumonia* (ST17)		Sensitive (NS, RS, ETA)		ST17 (NS, RS, ETA)
341	8th Jun 2015 - 12nd Jul 2015	Pneumonia* (ST592)		ESBL (NS), AmpC (RS), Sensitive (NS, RS, ETA)		ST101 (NS), ST17 (RS), ST592 (NS, RS, ETA)
349	12nd Jun 2015 - 22nd Jul 2015	Pneumonia* (ST193)		AmpC, AmpC + ESBL (RS), Sensitive (NS, ETA)		ST709 (RS), ST193 (NS, ETA)
410	9th Jul 2015 - 14th Jul 2015	BSI* (ST828)	Sensitive (RS)		ST828 (RS)	
443	23th Jul 2015 - 23th Jul 2015	BSI$ (ST592)	Sensitive (RS)		novel ST (RS)	

452	27th Jul 2015 - 16th Aug 2015	UTI* (not retrieved)	Sensitive (ETA)	Sensitive (NS, RS)	ST420 (ETA)	ST420 (NS, RS)
453	27th Jul 2015 - 14th Aug 2015	Pneumonia* (ST1215)	Sensitive (RS)	Sensitive (NS, ETA)	ST17 + ST3109 (RS)	ST23 (NS), ST1215 (NS, ETA)
472	3rd Aug 2015 - 7th Aug 2015	SBP$ (novel ST)	Sensitive (RS)		novel ST (RS)	
485	9th Aug 2015 - 2nd Oct 2015	Pneumonia* (ST25)		AmpC + ESBL (RS), Sensitive (NS, ETA)		ST1473 (RS), ST25 (NS, ETA)
488	10th Aug 2015 - 10th Sep 2015	Pneumonia* (ST35)	AmpC + ESBL (RS)	Sensitive, AmpC (NS, ETA); Sensitive, AmpC + ESBL (RS)	ST17 (RS)	ST35 + ST2054 (NS, ETA), ST37 + ST1035 + ST2054 (RS)
572	8th Sep 2015 - 4th Oct 2015	Pneumonia* (ST1245)	Sensitive (RS)	Sensitive (NS, ETA)	ST483 + ST1245 (RS)	ST1245 (NS, ETA)
592	15th Sep 2015 - 17th Sep 2015	BSI# (ST23)	Sensitive (RS)		ST719 (RS)	
600	19th Sep 2015 - 20th Sep 2015	UTI# (ST15)	Sensitive (RS)		ST709 (RS)	
623	26th Sep 2015 - 2nd Oct 2015	Pneumonia* (ST35)	Sensitive (RS)	Sensitive (ETA)	ST35 (RS)	ST35 (ETA)
644	8th Oct 2015 - 2nd Nov 2015	Pneumonia* (ST23)	Sensitive (RS)	Sensitive (NS, ETA)	ST36 + ST37 (RS)	ST23 (NS, ETA)
648	9th Oct 2015 - 5th Nov 2015	Pneumonia* (ST86)	AmpC + ESBL (RS)	Sensitive (RS), AmpC + ESBL (NS, RS), AmpC (NS, ETA)	novel ST (RS)	ST363 (RS), ST34 (NS, RS), ST86 (NS, ETA)
732	13th Nov 2015 - 15th Nov 2015	SBP$ (ST420)	Sensitive (RS)		ST420 (RS)	
788	4th Dec 2015 - 2nd Jan 2016	Pneumonia* (ST65)	Sensitive (ETA)		ST65 (ETA)	

$: CAIs, #: HCAIs, *: HAIs; NS: nasal swab, RS: rectal swab, ETA: endotracheal aspirate.
Orange color is for ICU patients developing HAIs with their previously colonizing *K. pneumoniae*; Blue color is for ICU patients with CAIs or HCAIs caused by *K. pneumoniae* isolates with the same STs of colonizing *K. pneumoniae* strains.
Red, green and violet rectangles are for transmission events occurred between Patient 170 and Patient 227, Patient 225 and Patient 341, and Patient 453 and Patient 644, respectively.

6.3.6 Genomic investigation

141 *K. pneumoniae* isolates were collected for molecular typing analysis: 27 (19.1%) infection isolates and 114 (80.9%) colonisation ones (Figure 6.3). The 27 infection isolates were responsible for 25 *K. pneumoniae* infections in 25 different patients; 2 patients with CAIs (1 with bacteremic UTI and another with bacteremic SBP) had *K. pneumoniae* isolated from blood and the infection site. The 27 infection isolates were recovered from ETA (16 isolates), from blood (8 isolates), from peritoneal fluid (2 isolates) and one isolate from urine. The 114 colonisation isolates were found in 27 of 28 patients; 42 cultured from nasal swabs, 39 from rectal swabs and 33 from ETA.

6.3.6.1 *K. pneumoniae* diversity assessed by MLST analysis

The MLST results indicated that the sequenced *K. pneumoniae* collection harbored great diversity, which was composed of 28 distinct STs and 5 novel STs (Figure 6.4). These STs differed among themselves by at least two MLST loci. The most prevalent ST was ST25, represented by 18 isolates (12.8% of 141); the next prevalent clones were ST86 (17 isolates, 12.1%), ST420 (14, 9.9%), ST17 (13, 9.2%), ST23 (12, 8.5%), ST592 (8, 5.7%), ST65 (7, 5.0%), ST2054 (6, 4.3%) and ST35 (5, 3.5%). The other STs were found in less than 5 isolates per ST. The main STs corresponding to ESBL-producing *K. pneumoniae* were ST17 (4 isolates), ST34 (2) and another 6 STs (15, 101, 709, 1035, 1437 and a novel ST, 1 isolate per ST). AmpC-producing *K. pneumoniae* were presented by ST2054 (6 isolates), ST86 (5), ST17 (3), ST709 (2) and other 5 STs (15, 34, 1473, 1779 and a novel ST, 1 isolate per ST), whereas ESBL and carbapenemase-producing *K. pneumoniae* belonged to only ST15 (2 isolates) and ST17 (1 isolate). Four of 5 novel STs were found in rectal isolates.

Taking into account the population structure of infection *K. pneumoniae* isolates, the distribution of *K. pneumoniae* STs for the 16 episodes of pneumonia was also diverse: ST86 (3 episodes), ST35 (2), ST65 (2), ST592 (2), and other STs (1 episode per each

ST) including ST17, ST23, ST25, ST193, ST1215, ST1245 and a novel ST. The 6 episodes of BSI were caused by *K. pneumoniae* ST23 (2 episodes), ST17 (1), ST375 (1), ST592 (1), and ST828 (1). The 4 episodes of UTI were related to *K. pneumoniae* ST15 (1 episode) and ST1779 (1 episode), while *K. pneumoniae* isolates were not retrieved from storage in the other 2 UTI cases for genomic analysis. *K. pneumoniae* ST420 and a novel ST were responsible for 2 cases of SBP.

Figure 6.3 *K. pneumoniae* identification by molecular typing method

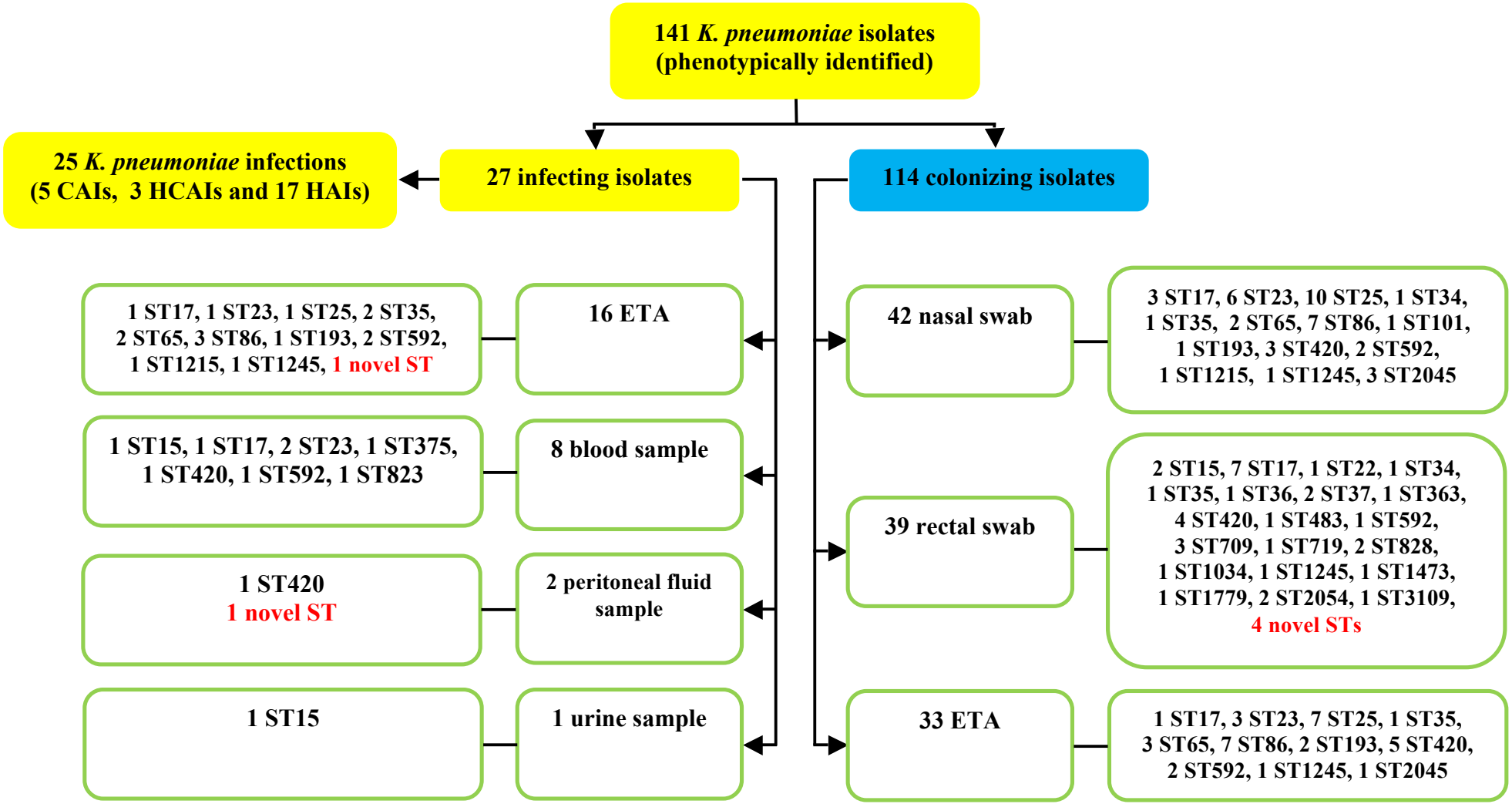


Figure 6.4 Distribution of STs among *K. pneumoniae*

6.3.6.2 *K. pneumoniae* colonisation on ICU admission assessed by MLST analysis

On ICU admission, 2 patients had nasal and endotracheal *K. pneumoniae* colonisation with the same STs (1 with ST65 and 1 with ST86). The other 2 patients colonised with *K. pneumoniae* only in the endotracheal tract with ST65 or ST420. The distribution of STs among 17 patients with admission rectal *K. pneumoniae* colonisation was diverse (Table 6.2): 2 patients with ST15, 2 patients with ST17 and 3 patients with novel STs. 3 other patients concomitantly colonised with 2 different *K. pneumoniae* STs (1 with ST17 and ST3109, 1 with ST483 and ST1245 and 1 with ST36 and ST37). The remaining 7 patients colonised with only one ST22, ST35, ST420, ST709, ST719, ST828 or ST1779.

6.3.6.3 Acquired *K. pneumoniae* colonisation during ICU stay assessed by MLST analysis

As previously described, six new *K. pneumoniae* acquisitions were detected in 6 patients not colonised with *K. pneumoniae* on ICU admission (Patients 225, 227, 334, 341, 349 and 485) (Table 6.3). All these 6 patients acquired nasal and endotracheal *K. pneumoniae* with the same STs that caused their infection, ventilator-associated pneumonia (2 patients with ST592, other 4 patients with ST17, ST25, ST86 or ST193). However, 5 of these 6 patients acquired rectal *K. pneumoniae* with different STs compared to their nasal and endotracheal strains, including a novel ST, ST17, ST592, ST709 and ST1473. We also noted 4 patients became nasal, rectal and/or endotracheal colonised with *K. pneumoniae* of different STs compared to their rectal *K. pneumoniae* isolates on admission (Patient 453, 488, 644 and 648). Furthermore, 3 patients developed nasal, rectal and/or endotracheal colonisation with *K. pneumoniae* of the same STs, which were previously colonised in different body sites on admission (Patient 452, 572 and 623). Therefore, a total of 10 new *K. pneumoniae* acquisitions were identified in this study by using MLST analysis (in comparison of only 8 new *K. pneumoniae* acquisitions suggested by using traditional culture method).

6.3.6.4 Transmission of *K. pneumoniae* assessed by MLST analysis

During the study period, we discovered a possible transmission event occurring from Patient 170 to Patient 227 (Table 6.3). These two patients had overlapping ICU stay (but they were not next to each other), and their *K. pneumoniae* isolates were well-matched in terms of ST86 and identical antibiogram. Moreover, we also detected 2 patients (Patients 225 and 341) acquired *K. pneumoniae* with the same ST592 and identical antibiogram although they did not share time in Adult ICU (the time interval between their admissions was about 2 months). The same situation was experienced in Patients 453 and 644 when they both acquired *K. pneumoniae* ST23 sharing identical

antibiogram (the time interval between their admissions was about 3 months). However, whether or not the *K. pneumoniae* ST86, ST592 and ST23 in 3 pairs of patients (Patients 170 and 227, Patients 225 and 341, Patients 453 and 644, respectively) were identical was explored by a phylogenetic analysis.

6.3.6.5 Relationship between *K. pneumoniae* colonisation and infections assessed by MLST analysis

MLST analysis indicated an agreement in STs of the infecting and previously colonizing *K. pneumoniae* isolates in 94.4% (17/18) ICU patients with hospital-acquired *K. pneumoniae* infections (Table 6.3): ST23, ST25, ST193, ST828, ST1215 and ST1245 (1 patient per each ST); ST17, ST35, ST65, ST592 (2 patients per each ST); and ST86 (3 patients). Of them, 7 patients had initial colonisation with *K. pneumoniae* on ICU admission, and 10 patients acquired colonisation by *K. pneumoniae* during ICU stay. Moreover, 40% (2/5) ICU patients suffered CAIs and 20% (1/5) patients with HCAI caused by *K. pneumoniae* isolates which shared the same STs with their colonizing strains detected on ICU admission (1 ST420 and 1 novel ST for CAIs; 1 ST1779 for HCAIs).

6.3.7 Phylogenetic analysis

The population structure of infecting and colonizing *K. pneumoniae* isolates in this study was diverse. However, only 3 STs were included for further phylogenetic analysis since they were isolated from more than three patients in Adult ICU with a quite high number of isolates obtained per patient compared to the remaining STs. They were *K. pneumoniae* ST17 (13 isolates, 5 patients), ST23 (12 isolates, 4 patients) and ST86 (17 isolates, 3 patients). Moreover, *K. pneumoniae* ST23 and ST86 are also of great clinical importance since they have been considered to be hypervirulent clones associated with hypermucoviscous capsular type K1 and K2 respectively, which can cause severe and highly fatal invasive diseases [153,228,346].

6.3.7.1 *K. pneumoniae* ST17 assessed by phylogenetic analysis

In this study, 13 isolates of *K. pneumoniae* ST17 were identified in 5 patients with hospital-acquired *K. pneumoniae* infections (1 BSI and 4 VAP). However, only colonizing ST17 isolates (not infecting ones) were recovered in 3 patients (Patients 341, 453 and 488 with pneumonia), leaving only 2 patients (Patient 256 with BSI and Patient 334 with VAP) with full collections of colonizing and infecting ST17 strains.

For Patients 256 and 334, a clear phylogenetic clustering was observed among colonizing and infecting ST17 isolates within each patient, and indicated by distinct clades (Figure 6.5). Of note, Patient 256 had rectal colonisation with ST17 isolate on ICU admission, whereas Patient 334 acquired nasal, rectal and endotracheal ST17 strains during ICU stay. Besides, two ST17 isolates colonizing in Patient 488 (E2979 and E3079) clustered tightly and they were separated from other ST17 strains obtained from other patients (E1988 in Patient 341, and E2715 in Patient 453). This suggested that ST17 isolates were derived from different genetic pools and an outbreak of ST17 did not occur in the ICU.

All 13 *K. pneumoniae* ST17 isolates were screened for capsular K antigen, virulence factors, and AMR genes (Tables 6.4 and 6.5). There were 3 different K serotypes found in ST17 strain: K23 serotype in Patient 334 and Patient 453, K25 serotype in Patient 256 and Patient 488, and K122 serotype in Patient 341. Virulence factors identified include siderophore systems yersiniabactin, aerobactin, colibactin, salmochelin; and the "regulators of mucoid phenotype" *rmpA*. The yersiniabactin locus (*ybt*) was detected in 92.3% (12/13) of ST17 isolates, not including a rectal ST17 strain (E1988) obtained from Patient 341 (Table 6.4). They were *ybt*2 lineage in Patient 334 (6 isolates) and Patient 453 (only 1 isolate); and *ybt*15 lineage in Patient 488 (2 isolates), whereas the *ybt* lineage of 3 ST17 strains in Patient 256 did not correspond to any lineage in Kleborate [347]. Patient 256 is the only patient who died among the 5 patients with *K.*

pneumoniae ST17 infections due to refractory shock. *Ybt* locus sequence types (YbSTs) were further explored using MLST analysis: YbST324 (Patient 334 and Patient 453), and YbST230 (Patient 488). All *ybt* loci detected were located within ICEKp (*K. pneumoniae* integrative conjugative element): *ybt*2 in the ICE*Kp1* and *ybt*15 in the ICE*Kp11*. The synthesis of aerobactin and colibactin encoded by *iuc* and *clb* locus, respectively was not found in ST17 isolates. However, salmochelin *iro* synthesis loci was reported in 53.4% (7/13) of ST17 isolates, which belonged to SmST7. All these 6 strains were present in Patient 334 and Patient 453. In addition to siderophores, *rmpA* was found only in 46.2% (6/13) of ST17 isolates obtained from Patient 334 and Patient 453.

In terms of phenotypic detection of antimicrobial resistance, 2 of 13 ST17 isolates were ESBL positive (15.4%), 1 AmpC producer (7.7%), 2 concomitantly ESBL and AmpC producers (15.4%), and 1 ESBL and carbapenemase producer (7.7%). Using AMR genotype, all 2 ESBL-producing ST17 isolates carried a *bla*ESBL gene (bla_{SHV-5}) (Table 6.5). The only one ESBL and carbapenemase-producing ST17 strain isolated in the study had *bla*ESBL gene (bla_{SHV-5}), not *bla*CARB gene (carbapenemase). The AmpC-producing ST17 isolate possessed DHA-1 AmpC enzymes, and all 2 ESBL and AmpC-producing ST17 strains contained only DHA-1 AmpC enzymes, not *bla*ESBL gene. Overall, phenotypic resistance did not correlate with the presence of a known resistance gene in the population of ST17 isolates.

6.3.7.2 *K. pneumoniae* ST23 assessed by phylogenetic analysis

For *K. pneumoniae* ST23, 12 isolates were collected in 4 ICU patients: 2 with healthcare-associated BSI (Patient 37 and 592, one died) and 2 with VAP (Patient 453 and 644). They were sensitive to all antimicrobials tested inluding cefriaxone, ceftazidime, cefepime, amoxcillin/clavulanic acid, gentamycin, ertapenem, imipenem, ciprofloxacin, ofloxacin, amikacin, piperacillin/tazobactam, ticarcillin/clavulanic acid

and colistin. Colonizing and infecting ST23 isolates were recovered fully in Patient 644, whereas only colonizing or infecting ST23 strains were retrieved in the remaining 3 patients. Almost all ST23 isolates in Patient 644 were related phylogenetically except that a nasal one, namely E3979, was represented by a distinct branch (Figure 6.6). In this study, Patient 644 acquired ST23 isolates during ICU stay (not colonisation with ST23 strains on ICU admission). They may have been exposed to different reservoirs of ST23 isolates in ICU, so that the phylogenetic pattern of ST23 strains was different. Phylogeny reconstruction also demonstrated a phylogenetic cluster of ST23 colonizing Patient 453 (E2746-1, E2746-2 and E2817) by a separate clade from other ST23 isolates obtained from other patients. This helped to disprove a transmission event occurring between Patient 453 and Patient 644, contrary to what the MLST analysis suggested. Moreover, the ST23 isolates outside Adult ICU (CM3204 and CM3660 recovered from Patient 37 and 592 with healthcare-associated BSI) formed a different clade compared to the ST23 strains inside ICU belonging to 2 other distinct clusters.

All ST23 isolates had the K1 serotype (Table 6.4). The iron-scavenging siderophore yersiniabactin (ICE*Kp10*/*ybt*1) was detected in 100% (12/12) of ST23 strains. This *ybt* lineage was assigned to 2 different YbSTs: YbST47 belonging to Patient 37, Patient 453, and Patient 592; and YbST46 (Patient 644). The aerobactin, colibactin and salmochelin loci (*iuc*, *clb* and *iro*, respectively) were found also in 100% ST23 isolates. However, we found *rmpA* in 58.3% (7/12) of ST23 isolates obtained from Patient 453 (a nasal strain), Patient 592 (a strain from blood sample), and Patient 644 (3 isolates from ETA and 2 from nasal swab). Consistent the with phenotypic drug susceptibility testing results, *bla*, *bla*ESBL and *bla*CARB genes were not detected in the population of ST23 isolates.

Table 6.4 Virulence factors in *K. pneumoniae* isolates

ID	Sample	K type	Yersiniabactin	YbST	Colibactin	CbST	Aerobactin	AbST	Salmochelin	SmST	Hypermucoidy	ST
256	CM3425	25	*ybt* unknown	-	-	-	-	-	-	-	-	
	E1288	25	*ybt* unknown	-	-	-	-	-	-	-	-	
	E1385	25	*ybt* unknown	-	-	-	-	-	-	-	-	
334	B1762	23	ICE*Kp1*/*ybt*2	324	-	-	-	-	*iro5*	7	*rmpA*	
	B1774	23	ICE*Kp1*/*ybt*2	324	-	-	-	-	*iro5*	7	-	
	E1940	23	ICE*Kp1*/*ybt*2	324	-	-	-	-	*iro5*	7	*rmpA*	
	E1941	23	ICE*Kp1*/*ybt*2	324	-	-	-	-	*iro5*	7	*rmpA*	**17**
	E1983	23	ICE*Kp1*/*ybt*2	324	-	-	-	-	*iro5*	7	*rmpA*	
	E2025	23	ICE*Kp1*/*ybt*2	324	-	-	-	-	*iro5*	7	*rmpA*	
341	E1988	122	-	-	-	-	-	-	-	-	-	
453	E2715	23	ICE*Kp1*/*ybt*2	324-1LV	-	-	-	-	*iro5*	7-1LV	*rmpA*	
488	E2979	25	ICE*Kp11*/*ybt*15	230	-	-	-	-	-	-	-	
	E3079	25	ICE*Kp11*/*ybt*15	230	-	-	-	-	-	-	-	
37	CM3204	1	ICE*Kp10*/*ybt*1	47	*clb2*	29	*iuc2*	1	*iro3*	2	-	
453	E2746-1	1	ICE*Kp10*/*ybt*1	47	*clb2*	29-1LV	*iuc2*	1	*iro3*	2	*rmpA*	
	E2746-2	1	ICE*Kp10*/*ybt*1	47	*clb2*	29-1LV	*iuc2*	1	*iro3*	2	-	
	E2817-2	1	ICE*Kp10*/*ybt*1	47	*clb2*	29-1LV	*iuc2*	1	*iro3*	2	-	
592	CM3660	1	ICE*Kp10*/*ybt*1	47-1LV	*clb2*	29-1LV	*iuc2*	1	*iro3*	2	*rmpA*	**23**
644	B2185	1	ICE*Kp10*/*ybt*1	46	*clb2*	29	*iuc2*	1	*iro3*	2	-	
	B2201	1	ICE*Kp10*/*ybt*1	46	*clb2*	29	*iuc2*	1	*iro3*	2	*rmpA*	
	B2212	1	ICE*Kp10*/*ybt*1	46	*clb2*	29	*iuc2*	1	*iro3*	2	*rmpA*	
	B2217	1	ICE*Kp10*/*ybt*1	46	*clb2*	29	*iuc2*	1	*iro3*	2	*rmpA*	

	E3979	1	ICE*Kp10*/*ybt*1	46	*clb2*	29	*iuc2*	1	*iro3*	2	-	
	E4034	1	ICE*Kp10*/*ybt*1	46-1LV	*clb2*	29	*iuc2*	1	*iro3*	2	*rmpA*	
	E4089	1	ICE*Kp10*/*ybt*1	46	*clb2*	29	*iuc2*	1	*iro3*	2	*rmpA*	
170	B1476	2	ICE*Kp12*/*ybt*0	13-1LV	-	-	*iuc2*	1	*iro3*	1	*rmpA*	**86**
	B1481	2	ICE*Kp12*/*ybt*0	13-1LV	-	-	*iuc2*	1	*iro3*	1	*rmpA*	
	E740	2	ICE*Kp12*/*ybt*0	13-1LV	-	-	*iuc2*	1	*iro3*	1	*rmpA*	
	E769	2	ICE*Kp12*/*ybt*0	13-1LV	-	-	*iuc2*	1-1LV	*iro3*	1	-	
227	B1573	2	-	-	-	-	*iuc2*	1	*iro3*	1	*rmpA*	
	B1609	2	-	-	-	-	*iuc2*	1	*iro3*	1	*rmpA*	
	B1624	2	-	-	-	-	*iuc2*	1	*iro3*	1	-	
	B1631	2	-	-	-	-	*iuc2*	1	*iro3*	1	*rmpA*	
	B1637	2	-	-	-	-	*iuc2*	1	*iro3*	1	*rmpA*	
	E1148	2	-	-	-	-	*iuc2*	16-1LV	*iro3*	1	-	
	E1295	2	-	-	-	-	*iuc2*	1	*iro3*	1	-	
	E1356	2	-	-	-	-	*iuc2*	1	*iro3*	1	-	
648	B2204	2	ICE*Kp3*/*ybt*9	202	-	-	*iuc2*	1	*iro3*	1	-	
	B2214	2	ICE*Kp3*/*ybt*9	202	-	-	*iuc2*	1	*iro3*	1	*rmpA*	
	B2222	2	ICE*Kp3*/*ybt*9	205-1LV	-	-	*iuc2*	1	*iro3*	1	-	
	E3985-1	2	ICE*Kp3*/*ybt*9	202	-	-	*iuc2*	1	*iro3*	1	*rmpA*	
	E4093	2	ICE*Kp3*/*ybt*9	202	-	-	*iuc2*	1	*iro3*	1	-	

Table 6.5 Phenotypic and genotypic antimicrobial resistance in *K. pneumoniae* isolates

ID	Sample	Phenotypic antimicrobial resistance	Genotypic antimicrobial resistance			ST *
			bla	***bla*ESBL**	***bla*CARB**	
256	CM3425	ESBL and carbapenemase production	DHA-1, OXA-1	SHV-5	-	**17**
	E1288	ESBL production	DHA-1, OXA-1	SHV-5	-	
	E1385	ESBL production	DHA-1, OXA-1	SHV-5	-	
334	B1762	-	-	-	-	
	B1774	-	-	-	-	
	E1940	-	-	-	-	
	E1941	-	-	-	-	
	E1983	-	-	-	-	
	E2025	-	-	-	-	
341	E1988	AmpC β-lactamase production	DHA-1	-	-	
453	E2715	-	-	-	-	
488	E2979	ESBL and AmpC β-lactamase production	DHA-1, OXA-1	-	-	
	E3079	ESBL and AmpC β-lactamase production	DHA-1, OXA-1	-	-	
170	B1476	-	-	-	-	**86**
	B1481	-	-	-	-	
	E740	-	-	-	-	
	E769	-	-	-	-	

227	B1573	-	-	-	-	
	B1609	-	-	-	-	
	B1624	-	-	-	-	
	B1631	-	-	-	-	
	B1637	-	-	-	-	
	E1148	-	-	-	-	
	E1295	-	-	-	-	
	E1356	-	-	-	-	
648	B2204	AmpC β-lactamase production	DHA-1	-	-	
	B2214	AmpC β-lactamase production	DHA-1	-	-	
	B2222	AmpC β-lactamase production	DHA-1	-	-	
	E3985-1	AmpC β-lactamase production	DHA-1	-	-	
	E4093	AmpC β-lactamase production	DHA-1	-	-	

* ST23 strains were not mentioned in Table 6.5 because they were sensitive to all antimicrobials tested and did not carry any AMR genes.

6.3.7.3 *K. pneumoniae* ST86 assessed by phylogenetic analysis

For *K. pneumoniae* ST86, 17 isolates were obtained from 3 ICU patients with VAP (Patient 170, 227 and 648). Out of the 3 patients, one died (Patient 170) due to ARDS. Phylogenetic analysis identified 3 different phylogroups corresponding to the discrete clustering of colonizing and infecting ST86 strains within 3 patients (Figure 6.7). These phylogroups were evolving independently, which disproved a transmission event occurring between Patient 170 and Patient 227, contrary to the MLST analysis. Of note, Patient 170 colonised with ST86 on ICU admission, whereas Patients 227 and 648 acquired ST86 strains during ICU stay.

In this study, ST86 is associated with the K2 serotype (Table 6.4). The yersiniabactin synthesis was detected in 52.9% (9/17) ST86 isolates recovered from Patient 170 and Patient 648 (Table 6.4). The ICE*Kp12*/*ybt*0 (YbST13) was present in Patient 170, while the ICE*Kp3*/*ybt*9 (YbST202 and YbST205) was found in Patient 648. The colibactin synthesis was not revealed, but the aerobactin and salmochelin were produced in all ST86 isolates (100%). In addition, 52.9% (9/17) ST86 strains carried *rmpA*. Of them, 4 ST86 isolates came from Patient 227 who did not harbour yersiniabactin-producing bacteria. All five AmpC-producing *K. pneumoniae* ST86 (phenotypic antimicrobial resistance) isolated from Patient 648 carried DHA-1 AmpC enzymes detected by genotypic technique. This finding indicated an agreement between phenotypic and genotypic characterization of AMR in the population of ST86 isolates.

Figure 6.5 Phylogenetic tree for *K. pneumoniae* ST17 with full datasets

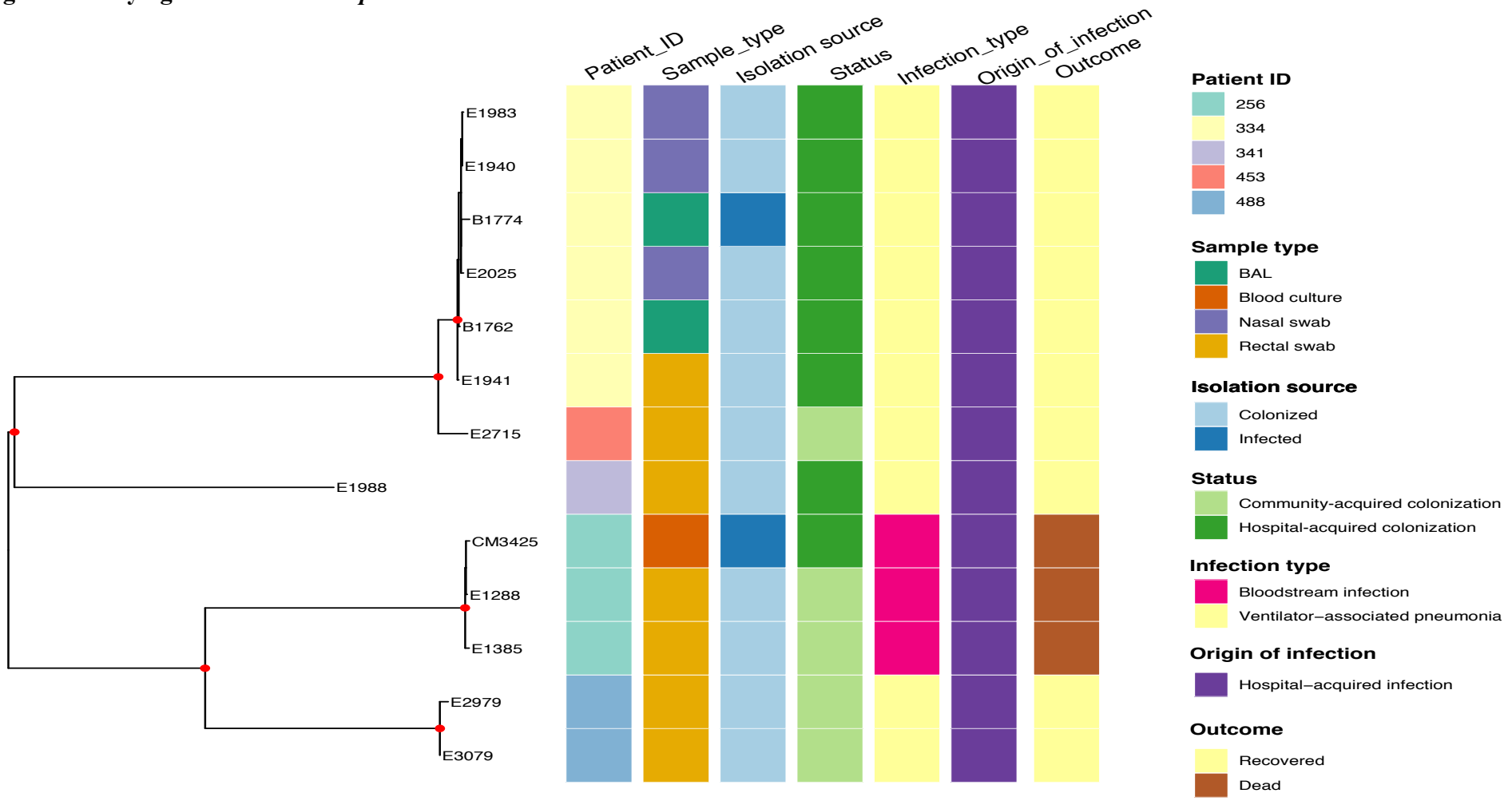


Figure 6.6 Phylogenetic tree for *K. pneumoniae* ST23 with full datasets

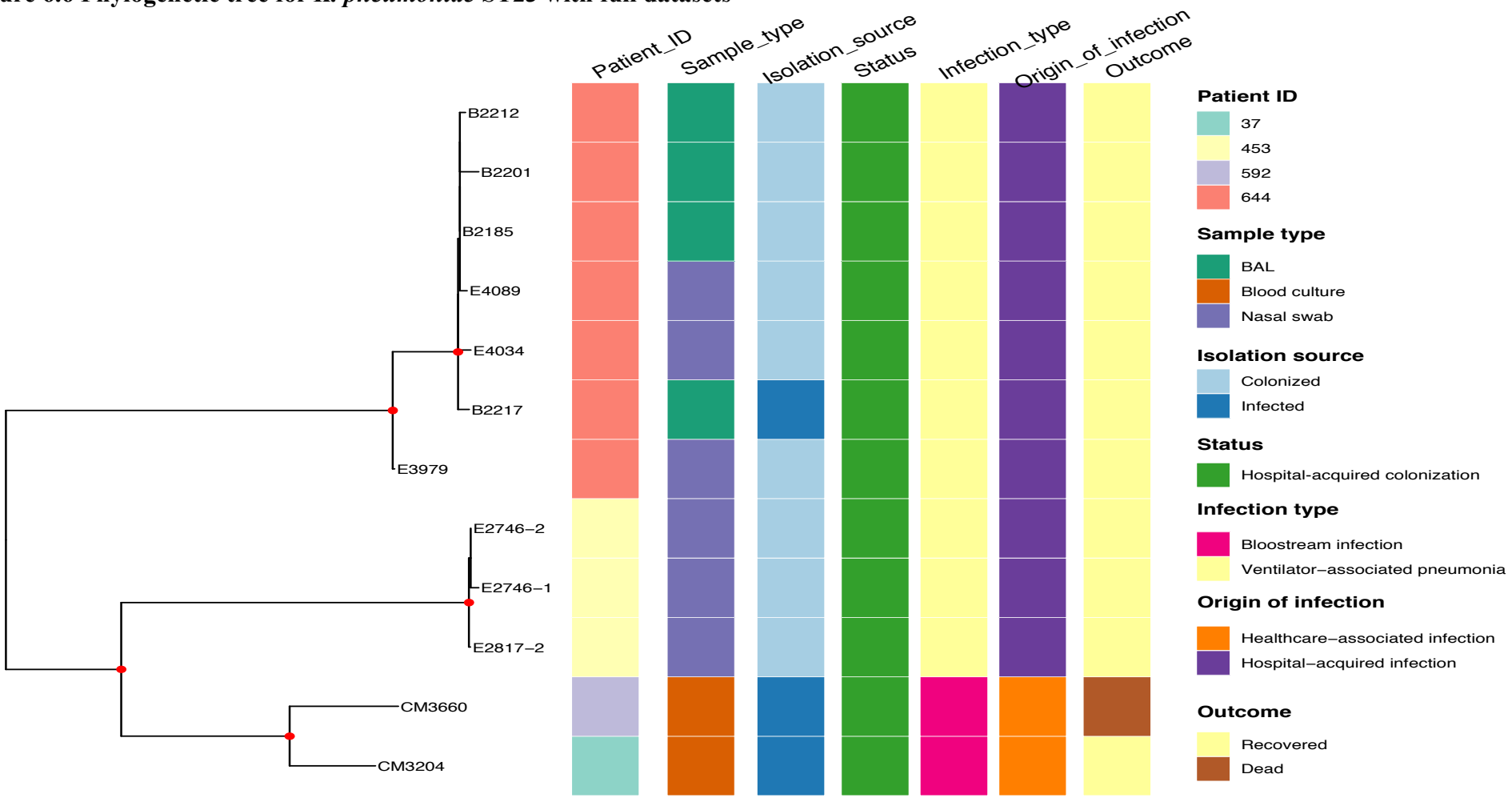
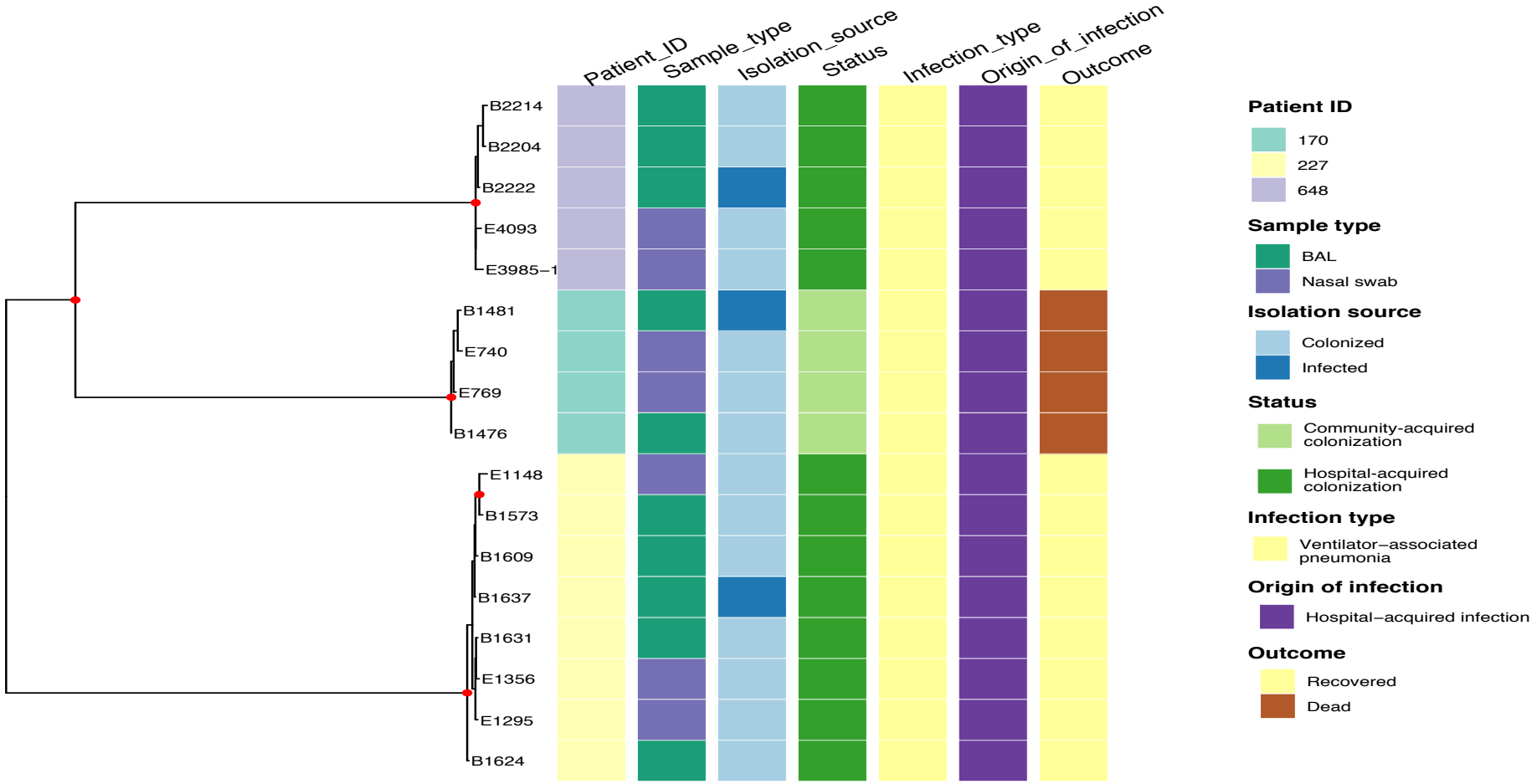


Figure 6.7 Phylogenetic tree for *K. pneumoniae* ST86 with full datasets



6.4 DISCUSSION

This study is, to the best of our knowledge, the first to investigate the relationship between colonisation and infection with *K. pneumoniae* among adult ICU patients at a tertiary hospital in Vietnam. The clinical manifestation of *K. pneumoniae* infection in our ICU is not much different from other healthcare settings, which includes bacteraemia, urinary tract and respiratory tract infections [348,349]. Pneumonia was the most common type of infection (57.2%, 16/28) and 75% (12/16) were VAP. The majority of infected patients had severe tetanus, or sepsis and septic shock on admission to ICU (Table 6.1).

With regard to the genetic resistance determinants in *K. pneumoniae*, the $bla_{\text{CTX-M}}$ was the most common resistance gene of ESBL (61.5 - 100%) previously reported in *K. pneumoniae* isolated in hospitals in Ho Chi Minh City [350,351], while $bla_{\text{SHV-5}}$ was found in 23.1% (3/13) of *K. pneumoniae* ST17 in my study. We screened acquired resistance genes for only three *K. pneumoniae* STs of great clinical importance (ST17, ST23 and ST86), so that the rate of ESBL resistance gene in *K. pneumoniae* in my study was not comparable to previously reported data [350,351]. For carbapenemase-encoding genes, the $bla_{\text{NDM-1}}$ gene was first detected in a urinary *K. pneumoniae* isolate in Vietnam in 2010 from a 62-year-old male patient with UTI [352]. A cross-sectional study conducted later to investigate oropharyngeal *K. pneumoniae* carriage in urban and rural Vietnamese communities (northern Vietnam, 2011) also found two participants colonised with carbapenem-resistant *K. pneumoniae*, which were confirmed to be NDM-1 positive [182]. However, carbapenemase-encoding genes were not detected in my study, and a carbapenem resistance phenotype was only found in *K. pneumoniae* ST15 and ST17. Therefore, along with phenotypic antimicrobial resistance, further investigation on genotypic AMR in *K. pneumoniae* is essential to inform optimal management,

antimicrobial stewardship, prevention and infection control to reduce the burden of antimicrobial-resistant *K. pneumoniae* in ICUs.

Here, we also used WGS in an attempt to investigate the population diversity of *K. pneumoniae*, to screen for the presence of virulence factors (siderophores and *rmpA*), and to identify the genomic relatedness of colonizing and infecting *K. pneumoniae* isolates in adult ICU patients. The population structure of *K. pneumoniae* assessed by MLST in our ICU was diverse with 28 distinct STs and 5 novel STs. Of them, only three *K. pneumoniae* STs (ST17, ST23 and ST86) were of great clinical importance because they were isolated from more than 3 ICU patients with a quite high number of isolates for genomic analysis. Moreover, they are hypervirulent clones because of their ability to cause invasive infections in ICU patients, including BSI and bacteremic pneumonia complicated by septic shock, which appear similar to those reported from other studies [153,224]. The ST17 and ST23 strains have previously been described in Vietnam during 2007 - 2008 (data of the infection type not shown) [353]. Little is known about the ST17 strain in the world. During 2008 - 2009, an outbreak caused by ESBL-producing (type CTX-M-15) *K. pneumoniae* ST17 occurred in the neonatal ICU at Stavanger University Hospital in Norway [354]. Despite the high colonisation rate among infants, the rate of clinical infection was low, and no death was associated with the outbreak, indicating low virulence properties of the outbreak ST17 strain [354]. However, in my study, 3 of 13 ST17 isolates were ESBL producers (type SHV-5) and they were also responsible for hospital-acquired BSI diagnosed in Patient 256 who died due to refractory shock. Therefore, further molecular studies are warranted to clarify the virulence properties of the ST17 strain in Vietnam. The ST23 of the K1 serotype has been documented as a major aetiological agent of liver abscess in Taiwan [355], Hong Kong [356], Singapore [357], and South Korea [358], but this type of invasive infection was not detected in our ICU patients during the study period for unknown reasons. *K.*

pneumoniae ST23 of serotype K1 is considered to be a particular virulent clone, which typically carry all four acquired siderophore systems (yersiniabactin, aerobactin, colibactin, salmochelin) and *rmpA* [153]. Crucially, siderophores are considered the key function of iron acquisition which enhances the ability of bacteria to survive and replicate within the host. Evidence is mounting that *K. pneumoniae* isolates carrying acquired siderophores are highly pathogenic and can cause invasive diseases [153,222-224]. Moreover, the "regulator of mucoid phenotype" *rmpA*, which can up-regulate capsule production, has been identified as a virulence factor in *K. pneumoniae* [225]. Indeed, the molecular characteristics of ST23 K1 strains isolated in my study are concordant with those findings, and it was associated with poor outcome through an ICU patient with BSI who died, despite a fully sensitive bacteria.

K2 strains have not received as much attention as K1 strains, but severe and fatal infections due to ST86 of the K2 serotype were reported recently in France and Korea [228,346]. In my study, ST86 of the K2 serotype was also responsible for a fatal case of severe pneumonia although its antibiogram was fully sensitive. It is of note that all 4 K2 strains recovered from this patient harboured yersiniabactin, aerobactin and salmochelin, while 3 of them possessed *rmpA*, and none of them carried *bla*ESBL or *bla*CARB. Therefore, my study suggests that the acquisition of siderophore and *rmpA* was superior to the antibiogram for the prognostic of *K. pneumoniae* infection in humans. Clinicians should be aware of the virulence of *K. pneumoniae* and efforts should be made to investigate the sources of infection.

The aim of this study was to determine whether or not colonizing and infecting *K. pneumoniae* isolates obtained from the same ICU patients were genetically similar. 94.4% (17/18) ICU patients became infected with their previously colonizing *K. pneumoniae* by the use of MLST analysis (Table 6.3). WGS data indicated a cluster of previously colonizing and infecting *K. pneumoniae* ST17, ST23 and ST86 strains

obtained from 6 ICU patients which confirmed the role of prior *K. pneumoniae* colonisation as the source of subsequent HAIs (Figures 6.5, 6.6 and 6.7). It is of note that one patient with BSI had concordant pairs based on WGS data of blood and rectal swab isolates. ETA and nasal swab isolates from the remaining 5 patients with pneumonia also demonstrated perfect concordance. These findings are consistent with the Michigan study (the USA) and the Alfred project (Australia) [139,140]. A 2016 study at the University of Michigan Health System reported a significant association between rectal *K. pneumoniae* colonisation and subsequent infections (pneumonia, UTI and BSI). Moreover, there was high concordance among colonizing and infecting isolates, particularly for pneumonia and UTI, as measured by genome analyses [140]. The 2013 *Klebsiella* Acquisition Surveillance Project at Alfred Health confirmed that *K. pneumoniae* colonisation is a significant risk factor for infection in ICU, and genome comparison indicated matching carriage and infection isolates in 80% (12/15) of isolate pairs [139]. This strong concordance suggests that characterization of colonizing *K. pneumoniae* isolates to inform infection prevention strategies or treatment decisions is feasible. A recent study in long-term acute care hospitals concluded that a bundled intervention based on screening for carbapenemase-producing *K. pneumoniae* decreased significantly the rates of colonisation and infection [359]. Moreover, understanding the pathogenesis of progression from *K. pneumoniae* colonisation to disease could enable novel diagnostics and therapeutics to prevent and rapidly treat these common nosocomial infections. Our data also demonstrated that 33.3% (2/6) *K. pneumoniae* HAIs were associated with the patients' own *K. pneumoniae* strains which were tested positive from screening swabs on ICU admission, and 66.7% (4/6) occurred in patients who acquired *K. pneumoniae* during ICU stay. For *K. pneumoniae* CAIs and HCAIs, there was an agreement in STs of the colonizing and infecting strains which were all screened on ICU admission. This suggests that not only measures to reduce cross-

trasmission between patients are necessary, but also measures to minimize the risk of *K. pneumoniae* infection with the patients' own microbiome deserves significant attention [360,361].

In this study, 6 *K. pneumoniae* acquisitions were found in 6 patients not having admission colonisation by conventional microbiology identification method. By using MLST analysis, 4 new patients were detected to acquire *K. pneumoniae* of different STs compared to admission isolates, and 3 likely transmission chains were identified which were responsible for 16.7% (3/18) of *K. pneumoniae* infections acquired in ICU. However, WGS data allowed us to disprove 2 of 3 transmission events related to ST23 and ST86 indicated by MLST (WGS analysis were not used for ST592). Therefore, WGS performed better than conventional methods for detection of nosocomial *K. pneumoniae* transmission.

This study has several strengths. Firstly, this is the first study to provide comprehensive information on the population structure of *K. pneumoniae* in a Vietnamese ICU setting. Secondly, we used WGS to screen for concordance between colonizing and infecting *K. pneumoniae* isolates regardless of antimicrobial susceptibility and overlapping patient stay. Lastly, whereas most previous studies of *K. pneumoniae* have focused on ESBL and/or carbapenemase-producing isolates which do not represent the major burden of *K. pneumoniae* infections, we tested all *K. pneumoniae* isolates during a 14-month collection period that minimized potential selection bias.

The main limitations of this study arise from the collection of *K. pneumoniae* isolates for genomic analysis. As some colonizing and infecting *K. pneumoniae* strains were not retrieved from storage, it is possible that the proportion of matching colonisation/infection pairs underestimates the contribution of colonisation to infection and misses some instances of transmission events. Moreover, genomic comparison was applied for only 3 STs of *K. pneumoniae*, which can impede the characterization of the

burden of *K. pneumoniae* colonisation and infections in ICU. Additionally, this study was performed in a single Vietnamese center, so the findings may not be representative. However, our findings should be generally applicable to ICUs where similar infection control measures are in place. Lastly, I did not screen *K. pneumoniae* for medical staff and hospital environment, so it is possible that I missed some transmission sources.

6.5 CONCLUSION

Even though our findings may not be representative of the overall situation of Vietnam, it is noteworthy that ICU patients often become infected with their colonizing *K. pneumoniae* isolates. Moreover, some hypervirulent *K. pneumoniae* clones associated with life-threatening human infectious diseases are circulating in Vietnam. Wide-scale surveillance of *K. pneumoniae* and establishment of a strategy to prevent its further spread are urgently needed in ICU settings.

Chapter 7. GENREAL DISCUSSION AND FUTURE DIRECTIONS

My thesis has explored colonisation and infection with 5 bacteria identified as global health concerns in a single ICU in Vietnam. Through an observational study with the use of conventional microbiology and DNA sequencing, I have endeavoured to characterize colonisation, identify risk factors for colonisation, especially with AROs among adult patients on ICU admission and during their ICU stay, and understand its relationship with infection (chapters 3 and 4). Notably, my study is also one of the few investigating the genetic characterization of *S. aureus* or *K. pneumoniae* colonisation, and its contribution to infections in ICU setting (chapters 5 and 6). Such data is urgently needed to inform optimal management, antimicrobial stewardship, prevention and infection control to reduce HAIs, particularly AMR in ICUs in Vietnam and potentially other low-resource settings.

Little is known about colonisation with AROs in Vietnamese ICUs. The reason behind is multifactorial, but partly associated with a lack of active screening which is rarely available or affordable. The limited data available previously suggests that colonisation with AROs in the Vietnamese population is high: 25% of healthy people had nasopharyngeal MRSA colonisation [180], and 46.2% were colonised with ESBL-producing *E. coli* [181]. Moreover, the average daily prevalence rates of MRSA, ESBL-producing *Enterobacteriaceae*, *P. aeruginosa*, gentamicin-resistant *K. pneumoniae*, and amikacin-resistant *Acinetobacter* spp. in tetanus patients admitted to an ICU were 34%, 61.3%, 53.4%, 65.7% and 57.1%, respectively [179]. Thus, my findings are consistent with this previous work. The results detailed in chapter 3 confirmed the high rate of AROs colonisation of 63.1% (529/838) among adult patients on ICU admission. The colonisation frequency with AROs in ICU patients admitted from the community was comparable to those transferred from other hospitals. Moreover, the study showed a dramatic increase in rate of nasal MRSA colonisation in the HTD (2.9% in 2004 - 2006

[179] to 8.6% in 2014 - 2016, my study) and that of 55.2% for ESBL-producing *E. coli* and *K. pneumoniae*, which may suggest that HTD in particular, and Vietnam in general is becoming a new AROs hotspot in the world. Additionally, antimicrobial-resistant infections accounted for 10.5% of ICU patients (8.3% for CAIs and 15.1% for HCAIs) on admission. In daily clinical practice, one quarter of ICU patients with CAIs and HCAIs received inappropriate empirical antimicrobial therapy. Therefore, guidelines for antimicrobial treatment in the HTD should be revised periodically to adapt to a new era of AMR with changing drug susceptibility patterns.

The results described in chapter 4 provided important detailed information about HAIs in an ICU setting. At the time of this study was conducted, there was no national surveillance system for the antimicrobial-resistant infections and HAIs in Vietnam. The little data available on HAIs came from small number of studies performed in some tertiary hospitals, but they reported a wide range of HAIs prevalence from 5.2% to 60.9% between ICUs [175-178]. The proportion of patients with HAIs in my ICU was 23.4% (85/364). My study is particularly valuable as it is the first to include a well-defined ICU population and a prospective longitudinal study design. This ensured that the incidence and prevalence estimates were not made on selected populations, and that the results may be generally applicable to other healthcare setting where similar infection control measures are in place. During ICU stay, I found that 61.3% of ICU patients acquired colonisation with AROs. The rate of AROs acquisition in my study was significantly higher than reported data in other geographic areas [49,212,213,254,265], which again suggest that HTD in particular, and Vietnam in general is more likely to be considered a new endemic area for AROs. Moreover, increased Charlson Comorbidity Index score and receipt of antimicrobial therapy on ICU admission were found to be a significant risk factor for AROs acquisition in ICU. These data emphasize the need for active surveillance of AROs in targeted population in Vietnam immediately.

Additionally, AROs accounted for 41.5% (44/106) of pathogens causing HAIs further underlining the importance of infection control and prevention in my ICU. I also identified vascular catheters (central venous, arterial and hemofiltration catheter) as a risk factor independently associated with ICU-acquired BSI. Moreover, among the patients who developed HAIs due to any of the specified bacteria, 57.1% had prior colonisation with the same organism, suggesting that prior colonisation was an initial stage in the development of HAIs. These findings are consistent with data from other settings where prior colonisation is suggested to be an initial step in infection. However, these have been in settings with generally lower levels of AROs or antimicrobial use, and therefore my findings are important. This improved understanding of pathogenesis of HAIs in a setting of high AMR and broad-spectrum antimicrobial use.

Remarkably, results detailed in chapter 5, proved that use of conventional microbiology falsely overestimated *S. aureus* colonisation and infections. Molecular typing revealed 2 patients were actually colonised and infected with *S. argenteus,* not *S. aureus*. The fact is that *S. argenteus* can not be distinguished from *S. aureus* using conventional method, even MALDI-TOF MS [297,298]. This is also the first time *S. argenteus* being detected in Vietnam and sequenced as ST2250. Therefore, further studies to fully elucidate the clinical and molecular epidemiology of *S. argenteus* are warranted, and sequencing method is an indispensable tool. Furthermore, sequencing method performed better than conventional one for antimicrobial susceptibility testing. The AMR phenotype and gene presence are not exclusively linked in my study. A potential explanation for this inconsistency could be the lack of expression of some resistance genes, which can be influenced by numerous genetic and environmental conditions. Therefore, more detailed studies of sources and movement of AMR genes are crucial to fight *S. aureus* or *K. pneumoniae* infections.

In our ICU, ST188 was the predominant *S. aureus* clone circulating and this is also the first time ST188 has been detected in southern Vietnam. ST188 was initially reported in northern Vietnam by Bich et al. (2016) [311]. I found that ST188 in my study had the most MRSA isolates (38/39), whereas ST188 in northern Vietnam had the most MSSA isolates (about 24/27) [311]. I suggest continued surveillance to monitor for the presence of multidrug-resistant STs, such as ST188, which has not, as of yet, been widely identified in Vietnam. My study is also one of the few to investigate and confirm the role of prior *S. aureus* colonisation as the source of subsequent *S. aureus* infection in Vietnamese ICU patients with the use of whole-genome SNP typing. A clear cluster of colonizing and infecting *S. aureus* ST188 isolates was observed. This finding has further improved human understanding of the pathogenesis from *S. aureus* colonisation to infection, which could enable novel diagnostics and therapeutics to prevent and rapidly treat *S. aureus* infections. So far, *S. aureus* decolonisation of the nares and other body sites has greatly expanded over the past decade. The most favorable results are reported with the use of nasal mupirocin, which is active against staphylococci, including MRSA, streptococci, and some Gram-negative bacteria [112,113]. Therefore, new preventive strategies based on nasal mupirocin adjunct to routine infection control measures in the containment of *S. aureus* infections in ICUs deserve more attention.

Similarly, the WGS data also confirmed a clear phylogenetic cluster between colonizing and infecting *K. pneumoniae* strains. It is of note that patients with BSI had concordant pairs based on genome comparison of blood and rectal swab isolates. ETA and nasal swab isolates from patients with pneumonia also demonstrated perfect concordance. This strong concordance suggests that characterization of colonizing *K. pneumoniae* isolates to inform infection prevention strategies or treatment decisions is feasible. So far, selective digestive decontamination has been studied extensively in ICU patients to prevent or eradicate the oropharyngeal and intestinal abnormal carriage of pathogenic

organisms, such as *E. coli*, *K. pneumoniae*, *P. aeruginosa* and MRSA [114]. This is among the few interventions in ICUs which has shown reductions in infection rates in critically ill patients and improved outcome. Their use is limited to a minority of European ICUs, but it is more likely a promising research direction in LMIC settings, like Vietnam where AMR is on the rise and there are not many effective solutions.

Through this study, I also found that the acquisition of virulence factors (siderophore and *rmpA*) was superior to antimicrobial susceptibility/resistance for the prognostication of *K. pneumoniae* infection in humans. Crucially, siderophores are considered the key function of iron acquisition which enhances the ability of bacteria to survive and replicate within the host, so that *K. pneumoniae* isolates carrying acquired siderophores are highly pathogenic and can cause invasive diseases [153,222-224]. Moreover, the "regulator of mucoid phenotype" *rmpA*, which can up-regulate capsule production, has been identified as a virulence factor in *K. pneumoniae* [225]. In comparion with sequencing method, the gene presence is not exclusively linked with AMR phenotype. This inconsistency could be the lack of expression of some resistance genes, which can be influenced by numerous genetic and environmental conditions. So, clinicians should be aware of the hypervirulence *K. pneumoniae* and efforts should be made to investigate the sources of infection.

Noticeably, WGS helped to disprove patient-to-patient transmission events indicated by MLST analysis, and identified one additional transmission related to *S. aureus* which was missed by using conventional methods in my study. Currently, WGS is applied for bacterial pathogen characterization in the diagnostic microbiology laboratory: identification, typing, resistance detection, and virulence gene detection. More recently, the invention of high-throughput 'next-generation' sequencing technology, with relatively simple bench-top technology and efficient library preparation protocols, has significantly improved the capacity to perform low-cost and efficient WGS. These

properties have given WGS the potential to replace conventional typing methods, and to enhance infection control practice on local, national and international scales. Therefore, I highlight the potential of WGS in the analysis of *S. aureus* or *K. pneumoniae* colonisation, acquisition and infection in ICU settings to direct better targeting of infection control resources.

My study has some limitations. Firstly, nearly one quarter of study patients were treated with antimicrobials within 24 hours before ICU admission which may have had a negative impact on microbiological culture of all samples. Therefore, more sensitive organisms may not have been detected, biasing results in favor of more resistant ones. Secondly, many environmental factors, like workload, hand hygiene compliance, room cleaning protocols, and patient-related factors were not evaluated for the risk of acquired colonisation and infections. Hence, further comprehensive research may be needed to understand their role in transmission of AROs within the ICU. Thirdly, some colonizing and infecting *S. aureus or K. pneumoniae* strains were not retrieved from storage, it is possible that the proportion of matching colonisation/infection pairs underestimates the contribution of colonisation to infection and misses some instances of transmission events. Fourthly, genomic comparison was applied for only *S. aureus* ST188 and *K. pneumoniae* ST17, ST23 and ST86, which can impede the characterization of the burden of *S. aureus or K. pneumoniae* colonisation and infections in ICU. Additionally, my study was performed in a single Vietnamese center, so the findings may not be representative. However, our findings should be generally applicable to ICUs where similar infection control measures are in place. Moreover, my study is considered as a baseline study to be repeated after implementing infection control measures, strengthening laboratory diagnosis and setting regional surveillance networks.

In conclusion, my study provides evidence of the high burden of AMR in Vietnam along with high rate of colonisation and infections with AROs for both Gram-positive and negative bacteria, not only in ICU but also in the community. It is noteworthy that prior *S. aureus* colonisation plays a role as a source of infections in ICU patients with MRSA being an important pathogen of infections in ICU, and perhaps most surprising was the presence of *S. argenteus* strains. Moreover, ICU patients often become infected with their colonizing *K. pneumoniae* isolates, and some hypervirulent *K. pneumoniae* clones associated with life-threatening human infectious diseases are circulating in Vietnam. These findings were only apparent through the use of WGS. Therefore, wide-scale surveillance of *S. aureus* and *K. pneumoniae*, and establishment of a strategy to prevent its further spread are urgently needed in ICU setting.

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Appendix 1. Summary of criteria for diagnosis of some infection types [191]

Criteria	Pneumonia	Urinary tract infection	Bloodstream infection
Signs / Symptoms	At least one of the following: • Fever >38°C • WBC <4,000/mm^3 or ≥12,000/mm^3 • For adults ≥70 years old, altered mental status (with no other cause) AND At least two of the following: • New purulent sputum, or change in character • New onset or worsening cough, or dyspnea, or tachypnea • Rales or bronchial breath sounds • Worsening gas exchange (desaturations, PaO_2/FiO_2 ≤240, increased FiO_2, or ventilator demand) • A deterioration in ventilation following a period of stability: ≥2 days of stable or decreasing daily minimum PEEP followed by a rise in daily minimum PEEP of ≥3cmH_2O, sustained ≥2 calendar days; or ≥2 days of stable or decreasing daily minimum FiO_2 followed by a rise in daily minimum FiO_2 ≥ 0.15 points, sustained ≥2 calendar days	At least one of the following: fever >38°C, urgency, frequency, dysuria, suprapubic tenderness, costo-vertebral angle pain or tenderness (with no other cause)	At least one of the following: fever >38°C, chills, or hypotension

Laboratory	One of these on ≥2 chest X-ray for patients with underlying diseases or ≥1 chest X-ray for patients without: • New or progressive and persistent infiltrate • Consolidation • Cavitation	At least one of the following: • Positive dipstick for nitrite • Urine specimen with ≥10 WBC/mm^3 of unspun urine or >5 WBC/high power field of spun urine OR A positive urine culture of ≥10^5 CFU/ml with no more than 2 species of microorganisms	A recognized pathogen cultured from ≥1 blood culture AND Organism cultured from blood is not related to an infection at another site
Treatment	The clinician starting antibiotics within 2 days of these features developing		

Appendix 1. Summary of criteria for diagnosis of some types of infection (continued) [191]

Criteria	Skin and soft tissue infection	Surgical site infection	Bacterial meningitis
Signs / Symptoms	At least one of the following: • Purulent drainage, pustules, vesicles, or boils • Pain or tenderness, localized swelling, redness, or heat	Infection occurs within 30 or 90 days after the operative procedure AND At least one of the following: • Fever >38°C, pain or tenderness, localized swelling, redness, heat AND incision is deliberately opened by a surgeon, attending physician or other designee • Purulent drainage from the incision • Diagnosis of SSI by the surgeon or attending physician or other designee; or an abscess or other evidence of infection involving the deep incision that is detected on direct examination, or by imaging test	At least one of the following: fever >38°C, headache, stiff neck, meningeal signs, cranial nerve signs, or irritability (with no other cause)
Laboratory	At least one of the following: • Organisms cultured from aspirate or drainage from affected site • Organisms cultured from blood	Organisms isolated from an aseptically-obtained culture of fluid or tissue from the incision	At least one of the following: • Increased white cells, elevated protein, and decreased glucose in cerebral spinal fluid (CSF) • Organisms seen on Gram's stain of CSF • Organisms cultured from blood • Organisms cultured from CSF

Appendix 1. Summary of criteria for diagnosis of some types of infection (continued) [191]

Criteria	Endocarditis	Gastrointestinal tract infection	Cholangitis [193]
Signs / Symptoms	At least two of the following: fever >38°C, new or changing murmur, embolic phenomena, skin manifestations (i.e., petechiae, splinter hemorrhages, painful subcutaneous nodules), congestive heart failure, or cardiac conduction abnormality (with no other cause)	At least two of the following: fever >38°C, nausea, vomiting, abdominal pain or tenderness, or diarrhea (with no other cause)	Clinical manifestations of Charcot's triad: fever and/or chills, abdominal pain (right upper quadrant or epigastric), and jaundice
Laboratory	At least one of the following: • Organisms cultured from ≥2 blood cultures • Evidence of new vegetation seen on echocardiogram	At least one of the following: • Organisms cultured from blood • Evidence of pathologic findings on imaging test	Evidence of inflammation and biliary obstruction on laboratory data and imaging test

Appendix 1. Summary of criteria for diagnosis of some types of infection (continued) [191]

Criteria	Spontaneous bacterial peritonitis [194–196]	Spontaneous bacterial pleuritis [197]
Signs / Symptoms	At least one of the following: • Fever >38°C, hypotension, leukocytosis, acidosis, or hypothermia • Abdominal pain/tenderness, vomiting, diarrhea, or paralytic ileus • Hepatic encephalopathy • Renal failure (new onset) • Worsening of liver function OR An ascitic fluid absolute polymorphonuclear leukocyte count ≥250 cells/mm^3	At least one of the following: • A positive pleural fluid bacterial culture, and a pleural fluid absolute polymorphonuclear leukocyte count ≥250cells/μL • A negative pleural fluid bacterial culture, and a pleural fluid absolute polymorphonuclear leukocyte count >500cells/μL
Laboratory	At least one of the following: • A positive ascitic fluid bacterial culture without an intraabdominal surgically treatable source of infection • A negative ascitic fluid bacterial culture in the absence of pancreatitis or recent receipt of antimicrobial therapy	No radiographic evidence of pneumonia

Appendix 2. Informed consent form

INFORMATION SHEET FOR PATIENTS AND THEIR RELATIVES
INFECTION CONTROL IN ADULT INTENSIVE CARE UNIT

What is this study about?
You are being asked to participate in a study about the bacteriological surveillance in Adult Intensive Care Unit, Hospital for Tropical Diseases, Ho Chi Minh City. If you are considering consent on behalf of your child or relative, please read this form in their consideration.

Antibiotics are used to treat many infections. Unfortunately, many bacteria are becoming resistant to different kinds of antibiotics. This means that some antibiotic drugs can no longer be used to treat infections, and there is a real risk that we will run out of antibiotics to use as more and more bacteria become resistant. We are trying to understand why this problem happens in order to find the best solution.

We all have bacteria on our skin and in our bodies and most of them are helpful to us. However, sometimes these bacteria become more of a problem, especially if they do not respond to normal medicine treatment. We would like to see what is happening to a person's normal bacteria during a hospital stay.

What will happen if you take part in this study?
Today and two times per week you will have a swab taken from your nose, rectum and ventilation tube (if you have one). Today we will also take a small amount of blood (2mls only once) to check diabetes. This does not hurt although may be slightly uncomfortable. The risks of these procedures are very small. Information will be collected from your hospital file. The samples and information will be labelled only with a number and we will not use your name or personal information on any information collected.

If you agree, we would like to store any leftover samples for future medical research.

What are your rights if you take part in this study?
Your participation is voluntary. You may refuse to participate or may stop participating at any time without penalty. Choosing not to take part in or withdraw from the study will not affect the quality of your health care in future. If you decide to stop the study, just tell your doctor or a member of the study team.

What benefits / risks if you take part in this study?
Your participation in this study will help us understand resistance to antibiotics. The results of our research may help your doctor to decide the most appropriate and safe treatment for you. These tests are free so you do not pay anything for them.

Questions or concerns
If you have any questions about the swabs we are taking, what will happen to them or why we are taking them, please ask any of the nurses or doctors on the ward.

If you have any questions about your rights as a subject in this program or about what will happen to the samples taken, you may talk MD. DUONG BICH THUY at 0989937381, or if you want to speak to someone outside of the study you may contact the Ethics Committee at the Ho Chi Minh City Hospital for Tropical Diseases at 0967341010.

Appendix 2. Informed consent form

CONSENT FORM FOR PATIENTS AND THEIR RELATIVES
INFECTION CONTROL IN ADULT INTENSIVE CARE UNIT

Participant ID [__] [__] [__] [__] [__]

• I have read the participant information sheet for this study, and I have been told about the purpose, possible risks and benefits of taking part in this study.
• I have had a chance to discuss with study staff, and have got all answers I consent to study staff collecting and processing my (or my relative's) information.
• I freely agree that I (or my relative) will take part in this study.
• I understand that I (or my relative) may withdraw from this study at any time, and that if I (or my relative) do leave the study, it will not affect my (or my relative's) future care. If I (or my relative) decide to leave the study, I agree that all personal information collected up to the point may continue to be used.

PLEASE CHECK THE APPROPRIATE BOX BELOW:

□ I AGREE **OR** □ I DO NOT AGREE that samples from me (or my relative) may be stored and that further research on these samples may be undertaken in the future, including tests done by researchers outside Viet Nam.

Signature of participant:	Full name:	Date of signature:
x_____	x_____	____/____/_____

If the participant is under 18 or can not decide independently, their representative will sign this form on their behalf:

Signature of person giving consent:	Full name:	Relationship to participant:	Date of signature:
x_____	x_____	x_____	____/____/_____

I, the undersigned, have fully explained the relevant information of this program to the person named above and will provide her/him with a copy of this signed and dated informed consent form.

Signature of investigator:	Full name:	Date of signature:
x_____	x_____	____/____/_____

If the person giving consent cannot read the form themselves, a witness must be present and sign here:
I was present throughout the entire informed consent process with the participant. This form was read accurately to the volunteer, all questions from the volunteer were answered and the volunteer has agreed to take part in the research.

Signature of witness:	Full name:	Date of signature:
x_____	x_____	___/____/_____

Appendix 3. Case report form Study code: [__|__]-[__|__|__|__]

Information	ICU admission	Mon	Thu	Discharge or Ward transfer
Informed consent form	M			
Demographic				
Past medical history	M			
Diagnosis	M			M
Charlson Comorbidity	M			
APACHE II score	M			
Glycemia	M			
HbA1c	M			
Nasal swab	M	M	M	
Rectal swab	M	M	M	
Endotracheal aspirate	M	M	M	
Other clinical samples	O			
Treatment	M			
HAIs	O			
Outcome	M			
Medical cost				M

M (Mandatory)
O (Optional)

Appendix 3. Case report form	Study code: [__|__]-[__|__|__|__]

Inclusion criteria (It must be "YES")		
1. ICF	○ Yes	○ No

General information	
1. Patient's name (Initials)	[__|__|__|__|__|__]
2. Hospital code	[__|__|__|__|__|__]/[__|__|__|__]
3. Gender ○ Male ○ Female	
4. Age [__|__|__]	
5. Job [_____]	
6. Address [_____]	
7. Health insurance ○ Yes ○ No	
8. Weight [__|__|__] Kg	9. Height [__|__|__] cm

Admission to ICU	**DATE** (dd/mm/yy)	**Hours**
1. Admission to HTD	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]
2. Admission to ICU	[__|__]/[__|__]/[__|__]	[__|__]:[__|__]
3. Bed number	[__|__|__]	
4. Presence of invasive medical devices on admission to ICU		
a. Intubation	○ Yes	○ No
b. Tracheostomy	○ Yes	○ No
c. Mechanical ventilation	○ Yes	○ No
d. Arterial catheter	○ Yes	○ No
e. Central venous catheter	○ Yes	○ No
f. Peripheral venous catheter	○ Yes	○ No
e. Urinary catheter	○ Yes	○ No
g. Nasogastric catheter	○ Yes	○ No
h. Other invasive procedures	[_____] [_____]	

Appendix 3. Case report form Study code: [__|__]-[__|__|__|__]

Past medical history			
1. Working with or living lose to livestock	○ Yes [_____]	○ No	○ Unknown
2. Smoking habits			
a. Heavy smoker >20 cigarettes/day	○ Yes	○ No	○ Unknown
b. Light to moderate smoker)	○ Yes	○ No	○ Unknown
c. Non-smoker	○ Yes	○ No	○ Unknown
3. Drinking habits			
a. Heavy drinking	○ Yes	○ No	○ Unknown
b. Light to moderate drinking	○ Yes	○ No	○ Unknown
c. Non- drinking	○ Yes	○ No	○ Unknown
4. IV drug user	○ Yes	○ No	○ Unknown
5. Myocardial infraction	○ Yes (1)	○ No	○ Unknown
6. Congestive heart failure	○ Yes (1)	○ No	○ Unknown
7. Peripheral vascular disease	○ Yes (1)	○ No	○ Unknown
8. Cerebrovascular disease	○ Yes (1)	○ No	○ Unknown
9. Hemiplegia	○ Yes (2)	○ No	○ Unknown
10. Dementia	○ Yes (1)	○ No	○ Unknown
11. Chronic pulmonary disease	○ Yes (1)	○ No	○ Unknown
12. Conjunctive tissue disease	○ Yes (1)	○ No	○ Unknown
13. Gastro-jejunal ulcer	○ Yes (1)	○ No	○ Unknown
14. Mild chronic liver disease	○ Yes (1)	○ No	○ Unknown
15. Severe chronic liver disease	○ Yes (3)	○ No	○ Unknown
16. Chronic kidney disease (>175µmol/L)	○ Yes (2)	○ No	○ Unknown
17. Diabetes mellitus without end organ damage	○ Yes (1)	○ No	○ Unknown
18. Diabetes mellitus with end organ damage	○ Yes (2)	○ No	○ Unknown
19. Non-metastatic solid tumor (within 5 years)	○ Yes (2)	○ No	○ Unknown
20. Leukaemia or lymphoma	○ Yes (2)	○ No	○ Unknown
21. Metastatic solid tumor	○ Yes (6)	○ No	○ Unknown
22. Immunosuppression			
a. Steroids	○ Yes	○ No	○ Unknown
b. Traditional medicines	○ Yes	○ No	○ Unknown
c. Chemotherapy	○ Yes	○ No	○ Unknown
d. Radiotherapy	○ Yes	○ No	○ Unknown
23. AIDS	○ Yes (6)	○ No	○ Unknown
24. Charlson Comorbidity Index	[__|__|__]		

Appendix 3. Case report form	Study code: [__|__]-[__|__|__|__]

Recent admission			
1. Hospitalization history 90 days prior to admission	○ Yes	○ No	○ Unknown
a. General ward	○ Yes	○ No	○ Unknown
b. ICU	○ Yes	○ No	○ Unknown
c. Diagnosis	[_____]		
2. How long does it take from the most recent admission to this hospitalization?	[__|__|__] days		

Current admission			
1. Hospitalization prior to admission	○ Yes	○ No	○ Unknown
a. If "YES": ○ Central hospital	○ City hospital	○ District hospital	
b. General ward	○ Yes	○ No	○ Unknown
c. ICU	○ Yes	○ No	○ Unknown
d. Diagnosis	[_____]		
e. Length of stay in the previous hospital	[__|__|__] days		

History of antibiotics use			
1. Use of antibiotics 90 days prior to admission	○ Yes	○ No	○ Unknown
2. Use of antibiotics for this time	○ Yes	○ No	○ Unknown

Antibiotics	**Start day (dd/mm/yy)**	**Stop day (dd/mm/yy)**
Ceftriaxone	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Ceftazidime	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Amikacin	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Levofloxacine	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Ciprofloxacin	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Ticarcillin – Acid clavulanic	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Piperacillin – Tazobactam	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Ertapenem	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Imipenem	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Meropenem	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Colistin	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Oxacilline	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Vancomycin	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Other [_____]	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Other [_____]	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Other [_____]	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]

Diagnosis - Treatment	
1. Diagnosis in ICU	☐ Tetanus ☐ Dengue infection ☐ Severe sepsis ☐ Septic shock ☐ Pneumonia ☐ Urinary tract infection ☐ Spontaneous bacterial infection ☐ Cellulitis ☐ Fulminant hepatitis due to HBV ☐ Others [_____]
2. Glycemia (mg%)	[_____]
3. HbA1c %	[_____]

4. APACHE II score

	+6	+5	+4	+3	+2	+1	0	+1	+2	+3	+4
Body temperature (^oC)			≥ 41	39-40.9		38.5-38.9	36-38.4	34-35.9	32-33.9	30-31.9	≤ 29.9
Mean arterial pressure			≥ 160	130-159	110-129		70-109		50-69		≤ 49
Heart rate			≥ 180	140-179	110-139		70-109		55-69	40-54	≤ 39
Respiratory rate			≥ 50	35-49		25-34	12-24	10-11	6-9		≤ 5
$AaPO_2$ (If FiO_2 > 50%) or **PaO_2 (If FiO_2 < 50%)**			≥ 500	350-499	200-349		< 200 > 70	61-70		55-60	< 55
Arterial pH or **HCO_3^-**			≥ 7.7 ≥ 52	7.6-7.69 41-51.9		7.5-7.59 32-40.9	7.33-7.49 23-31.9		7.25-7.32 18-21.9	7.15-7.24 15-17.9	< 7.15 < 15
Serum Na^+			≥ 180	160-179	155-159	150-154	130-149		120-129	111-119	≤ 110
Serum K^+			≥ 7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		< 2.5
Serum Creatinine			≥ 3.5	2-3.4	1.5-1.9		0.6-1.4		< 0.6		
Hematocrit			≥ 60		50-59.9	46-49.9	30-45.9		20-29.9		< 20
White Blood Cell count			≥ 40		20-39.9	15-19.9	3-14.9		1-2.9		< 1
Glasgow Coma Score (GCS) **➔ 15 – GCS = [_____]**	**E [_____]** Eye response: spontaneously (+4), to verbal command (+3), to pain (+2), no eye response (+1) **V [_____]** Verbal response: oriented (+5), confused (+4), inappropriate words (+3), incomprehensive sounds (+2), no verbal response (+1) **M [_____]** Motor response: obeys commands (+6), localizes pain (+5), withdrawal from pain (+4), flexion to pain (+3), extension to pain (+2), no motor response (+1)										
Age	≥ 75	65-74		55-64	45-54		≤44				
Chronic health problem		Immune compromised	Chronic dialysis	Severe COPD (hypercapnia, home O_2, pulmonary hypertension)	NYHA Class IV	Biopsy proven cirrhosis					
Operation		Non-surgical or emergent operation			Elective operation						
Total score											

5. Antibiotics	○ Yes ○ No
Ceftriaxone	○ Yes ○ No
Ceftazidime	○ Yes ○ No
Amikacin	○ Yes ○ No
Levofloxacine	○ Yes ○ No
Ciprofloxacin	○ Yes ○ No
Ticarcillin – Acid clavulanic	○ Yes ○ No
Piperacillin – Tazobactam	○ Yes ○ No
Ertapenem	○ Yes ○ No
Imipenem	○ Yes ○ No
Meropenem	○ Yes ○ No
Colistin	○ Yes ○ No
Oxacilline	○ Yes ○ No
Vancomycin	○ Yes ○ No
Other	[_____]
Other	[_____]
Other	[_____]
7. Other treatment	Nasal/mask Oxygen ○ Yes ○ No Intubation ○ Yes ○ No Tracheostomy ○ Yes ○ No Non-invasive Ventilation ○ Yes ○ No Invasive Ventilation ○ Yes ○ No Continuous hemofiltration ○ Yes ○ No Vasopressors ○ Yes ○ No Transfusion ○ Yes ○ No Abdominal paracentesis ○ Yes ○ No Pleural paracentesis ○ Yes ○ No Other [_____] [_____] [_____]

Appendix 3. Case report form Study code: [__|__]-[__|__|__|__]

Date	___/___/___	___/___/___	___/___/___	___/___/___
1. Bed number	[__|__|__]	[__|__|__]	[__|__|__]	[__|__|__]
2. Antibiotics use	O Yes O No	O Yes O No	O Yes O No	O Yes O No
3. Indications				
Severe sepsis	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Septic shock	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Pneumonia	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Urinary tract infection	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Spontaneous bacterial peritonitis	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Hospital-acquired infections	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Ventilator associated pneumonia	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Non-ventilated pneumonia	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Bloodstream infection	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Urinary tract infection	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Other	[_____]	[_____]	[_____]	[_____]
4. Antibiotic change	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Reason: Inappropriate	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Escalation	O Yes O No	O Yes O No	O Yes O No	O Yes O No
De-escalation	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Combination	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Antibiotics				
Ceftriaxone	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Ceftazidime	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Amikacin	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Levofloxacin	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Ciprofloxacin	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Ticarcillin – Acid clavulanic	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Piperacillin – Tazobactam	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Ertapenem	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Imipenem	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Meropenem	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Colistin	O Yes O No	O Yes O No	O Yes O No	O Yes O No
Oxacilline	O Yes O No	O Yes O No	O Yes O No	O Yes O No

Vancomycin	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Other	[_____]	[_____]	[_____]	[_____]
Other	[_____]	[_____]	[_____]	[_____]
5. Invasive medical devices				
Intubation	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Tracheostomy	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Mechanical ventilation	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Arterial catheter	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Central venous catheter	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Peripheral venous catheter	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Urinary catheter	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Nasogastric catheter	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
Others	[_____] [_____]	[_____] [_____]	[_____] [_____]	[_____] [_____]
6. Taking samples				
Nasal swab	___/___/___	___/___/___	___/___/___	___/___/___
Rectal swab	___/___/___	___/___/___	___/___/___	___/___/___
Endotracheal aspirate	___/___/___	___/___/___	___/___/___	___/___/___
Blood culture	___/___/___	___/___/___	___/___/___	___/___/___
Urine culture	___/___/___	___/___/___	___/___/___	___/___/___
Abdominal fluid culture	___/___/___	___/___/___	___/___/___	___/___/___
Arterial line's tip culture	___/___/___	___/___/___	___/___/___	___/___/___
Central line's tip culture	___/___/___	___/___/___	___/___/___	___/___/___
Other samples [_____]	___/___/___	___/___/___	___/___/___	___/___/___

Appendix 3. Case report form **NASAL SWAB**

Study code: [__|__]-[__|__|__|__]

Date of taking samples	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Antibiotics use before taking samples	O Yes O No	O Yes O No
Culture result	O Negative O Positive	O Negative O Positive
Bacterial identification		
Staphylococcus aureus	O Yes [____] O No	O Yes [____] O No
Escherichia coli	O Yes [____] O No	O Yes [____] O No
Klebsiella spp	O Yes [____] O No	O Yes [____] O No
Klebsiella pneumoniae	O Yes [____] O No	O Yes [____] O No
Pseudomonas spp	O Yes [____] O No	O Yes [____] O No
Pseudomonas aeruginosa	O Yes [____] O No	O Yes [____] O No
Acinetobacter spp	O Yes [____] O No	O Yes [____] O No
Acinetobacter baumanii	O Yes [____] O No	O Yes [____] O No
Antibiotic resistant enzymes ESBL Carbapenemase	 O Negative O Positive O Negative O Positive	 O Negative O Positive O Negative O Positive
Antibiogram		
Penicilline	O Sensitive O Resistant	O Sensitive O Resistant
Amox + A.clavulanic	O Sensitive O Resistant	O Sensitive O Resistant
Oxacilline	O Sensitive O Resistant	O Sensitive O Resistant
Ceftazidime	O Sensitive O Resistant	O Sensitive O Resistant
Ceftriaxone	O Sensitive O Resistant	O Sensitive O Resistant
Cefepime	O Sensitive O Resistant	O Sensitive O Resistant
Vancomycin	O Sensitive O Resistant	O Sensitive O Resistant
Erythromycine	O Sensitive O Resistant	O Sensitive O Resistant
Ciprofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Ofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Amikacine	O Sensitive O Resistant	O Sensitive O Resistant
Co-trimoxazol	O Sensitive O Resistant	O Sensitive O Resistant
Imipenem	O Sensitive O Resistant	O Sensitive O Resistant
Rifampicin	O Sensitive O Resistant	O Sensitive O Resistant
Tazocine	O Sensitive O Resistant	O Sensitive O Resistant
Ticarcillin/Clavulanic acid	O Sensitive O Resistant	O Sensitive O Resistant
Ertapenem	O Sensitive O Resistant	O Sensitive O Resistant
Levofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Meropenem	O Sensitive O Resistant	O Sensitive O Resistant
Colistin	O Sensitive O Resistant	O Sensitive O Resistant
Clindamycin	O Sensitive O Resistant	O Sensitive O Resistant
Cefoxitin	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant

Appendix 3. Case report form **NASAL SWAB**

Study code: [__|__]-[__|__|__|__]

Date of taking samples	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Antibiotics use before taking samples	O Yes O No	O Yes O No
Culture result	O Negative O Positive	O Negative O Positive
Bacterial identification		
Staphylococcus aureus	O Yes [____] O No	O Yes [____] O No
Escherichia coli	O Yes [____] O No	O Yes [____] O No
Klebsiella spp	O Yes [____] O No	O Yes [____] O No
Klebsiella pneumoniae	O Yes [____] O No	O Yes [____] O No
Pseudomonas spp	O Yes [____] O No	O Yes [____] O No
Pseudomonas aeruginosa	O Yes [____] O No	O Yes [____] O No
Acinetobacter spp	O Yes [____] O No	O Yes [____] O No
Acinetobacter baumanii	O Yes [____] O No	O Yes [____] O No
Antibiotic resistant enzymes		
ESBL	O Negative O Positive	O Negative O Positive
Carbapenemase	O Negative O Positive	O Negative O Positive
Antibiogram		
Penicilline	O Sensitive O Resistant	O Sensitive O Resistant
Amox + A.clavulanic	O Sensitive O Resistant	O Sensitive O Resistant
Oxacilline	O Sensitive O Resistant	O Sensitive O Resistant
Ceftazidime	O Sensitive O Resistant	O Sensitive O Resistant
Ceftriaxone	O Sensitive O Resistant	O Sensitive O Resistant
Cefepime	O Sensitive O Resistant	O Sensitive O Resistant
Vancomycin	O Sensitive O Resistant	O Sensitive O Resistant
Erythromycine	O Sensitive O Resistant	O Sensitive O Resistant
Ciprofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Ofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Amikacine	O Sensitive O Resistant	O Sensitive O Resistant
Co-trimoxazol	O Sensitive O Resistant	O Sensitive O Resistant
Imipenem	O Sensitive O Resistant	O Sensitive O Resistant
Rifampicin	O Sensitive O Resistant	O Sensitive O Resistant
Tazocine	O Sensitive O Resistant	O Sensitive O Resistant
Ticarcillin/Clavulanic acid	O Sensitive O Resistant	O Sensitive O Resistant
Ertapenem	O Sensitive O Resistant	O Sensitive O Resistant
Levofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Meropenem	O Sensitive O Resistant	O Sensitive O Resistant
Colistin	O Sensitive O Resistant	O Sensitive O Resistant
Clindamycin	O Sensitive O Resistant	O Sensitive O Resistant
Cefoxitin	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant

Appendix 3. Case report form **RECTAL SWAB**

Study code: [__|__]-[__|__|__|__]

Date of taking samples	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Antibiotics use before taking samples	O Yes O No	O Yes O No
Culture result	O Negative O Positive	O Negative O Positive
Bacterial identification		
Staphylococcus aureus	O Yes [____] O No	O Yes [____] O No
Escherichia coli	O Yes [____] O No	O Yes [____] O No
Klebsiella spp	O Yes [____] O No	O Yes [____] O No
Klebsiella pneumoniae	O Yes [____] O No	O Yes [____] O No
Pseudomonas spp	O Yes [____] O No	O Yes [____] O No
Pseudomonas aeruginosa	O Yes [____] O No	O Yes [____] O No
Acinetobacter spp	O Yes [____] O No	O Yes [____] O No
Acinetobacter baumanii	O Yes [____] O No	O Yes [____] O No
Antibiotic resistant enzymes ESBL Carbapenemase	 O Negative O Positive O Negative O Positive	 O Negative O Positive O Negative O Positive
Antibiogram		
Penicilline	O Sensitive O Resistant	O Sensitive O Resistant
Amox + A.clavulanic	O Sensitive O Resistant	O Sensitive O Resistant
Oxacilline	O Sensitive O Resistant	O Sensitive O Resistant
Ceftazidime	O Sensitive O Resistant	O Sensitive O Resistant
Ceftriaxone	O Sensitive O Resistant	O Sensitive O Resistant
Cefepime	O Sensitive O Resistant	O Sensitive O Resistant
Vancomycin	O Sensitive O Resistant	O Sensitive O Resistant
Erythromycine	O Sensitive O Resistant	O Sensitive O Resistant
Ciprofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Ofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Amikacine	O Sensitive O Resistant	O Sensitive O Resistant
Co-trimoxazol	O Sensitive O Resistant	O Sensitive O Resistant
Imipenem	O Sensitive O Resistant	O Sensitive O Resistant
Rifampicin	O Sensitive O Resistant	O Sensitive O Resistant
Tazocine	O Sensitive O Resistant	O Sensitive O Resistant
Ticarcillin/Clavulanic acid	O Sensitive O Resistant	O Sensitive O Resistant
Ertapenem	O Sensitive O Resistant	O Sensitive O Resistant
Levofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Meropenem	O Sensitive O Resistant	O Sensitive O Resistant
Colistin	O Sensitive O Resistant	O Sensitive O Resistant
Clindamycin	O Sensitive O Resistant	O Sensitive O Resistant
Cefoxitin	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant

Appendix 3. Case report form **RECTAL SWAB**

Study code: [__|__]-[__|__|__|__]

Date of taking samples	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Antibiotics use before taking samples	O Yes O No	O Yes O No
Culture result	O Negative O Positive	O Negative O Positive
Bacterial identification		
Staphylococcus aureus	O Yes [____] O No	O Yes [____] O No
Escherichia coli	O Yes [____] O No	O Yes [____] O No
Klebsiella spp	O Yes [____] O No	O Yes [____] O No
Klebsiella pneumoniae	O Yes [____] O No	O Yes [____] O No
Pseudomonas spp	O Yes [____] O No	O Yes [____] O No
Pseudomonas aeruginosa	O Yes [____] O No	O Yes [____] O No
Acinetobacter spp	O Yes [____] O No	O Yes [____] O No
Acinetobacter baumanii	O Yes [____] O No	O Yes [____] O No
Antibiotic resistant enzymes ESBL Carbapenemase	 O Negative O Positive O Negative O Positive	 O Negative O Positive O Negative O Positive
Antibiogram		
Penicilline	O Sensitive O Resistant	O Sensitive O Resistant
Amox + A.clavulanic	O Sensitive O Resistant	O Sensitive O Resistant
Oxacilline	O Sensitive O Resistant	O Sensitive O Resistant
Ceftazidime	O Sensitive O Resistant	O Sensitive O Resistant
Ceftriaxone	O Sensitive O Resistant	O Sensitive O Resistant
Cefepime	O Sensitive O Resistant	O Sensitive O Resistant
Vancomycin	O Sensitive O Resistant	O Sensitive O Resistant
Erythromycine	O Sensitive O Resistant	O Sensitive O Resistant
Ciprofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Ofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Amikacine	O Sensitive O Resistant	O Sensitive O Resistant
Co-trimoxazol	O Sensitive O Resistant	O Sensitive O Resistant
Imipenem	O Sensitive O Resistant	O Sensitive O Resistant
Rifampicin	O Sensitive O Resistant	O Sensitive O Resistant
Tazocine	O Sensitive O Resistant	O Sensitive O Resistant
Ticarcillin/Clavulanic acid	O Sensitive O Resistant	O Sensitive O Resistant
Ertapenem	O Sensitive O Resistant	O Sensitive O Resistant
Levofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Meropenem	O Sensitive O Resistant	O Sensitive O Resistant
Colistin	O Sensitive O Resistant	O Sensitive O Resistant
Clindamycin	O Sensitive O Resistant	O Sensitive O Resistant
Cefoxitin	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant

Appendix 3. Case report form **ENDOTRACHEAL ASPIRATE**

Study code: [__|__]-[__|__|__|__]

Date of taking samples	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Antibiotics use before taking samples	O Yes O No	O Yes O No
Culture result	O Negative O Positive	O Negative O Positive
Bacterial identification		
Staphylococcus aureus	O Yes [____] O No	O Yes [____] O No
Escherichia coli	O Yes [____] O No	O Yes [____] O No
Klebsiella spp	O Yes [____] O No	O Yes [____] O No
Klebsiella pneumoniae	O Yes [____] O No	O Yes [____] O No
Pseudomonas spp	O Yes [____] O No	O Yes [____] O No
Pseudomonas aeruginosa	O Yes [____] O No	O Yes [____] O No
Acinetobacter spp	O Yes [____] O No	O Yes [____] O No
Acinetobacter baumanii	O Yes [____] O No	O Yes [____] O No
Antibiotic resistant enzymes ESBL Carbapenemase	 O Negative O Positive O Negative O Positive	 O Negative O Positive O Negative O Positive
Antibiogram		
Penicilline	O Sensitive O Resistant	O Sensitive O Resistant
Amox + A.clavulanic	O Sensitive O Resistant	O Sensitive O Resistant
Oxacilline	O Sensitive O Resistant	O Sensitive O Resistant
Ceftazidime	O Sensitive O Resistant	O Sensitive O Resistant
Ceftriaxone	O Sensitive O Resistant	O Sensitive O Resistant
Cefepime	O Sensitive O Resistant	O Sensitive O Resistant
Vancomycin	O Sensitive O Resistant	O Sensitive O Resistant
Erythromycine	O Sensitive O Resistant	O Sensitive O Resistant
Ciprofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Ofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Amikacine	O Sensitive O Resistant	O Sensitive O Resistant
Co-trimoxazol	O Sensitive O Resistant	O Sensitive O Resistant
Imipenem	O Sensitive O Resistant	O Sensitive O Resistant
Rifampicin	O Sensitive O Resistant	O Sensitive O Resistant
Tazocine	O Sensitive O Resistant	O Sensitive O Resistant
Ticarcillin/Clavulanic acid	O Sensitive O Resistant	O Sensitive O Resistant
Ertapenem	O Sensitive O Resistant	O Sensitive O Resistant
Levofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Meropenem	O Sensitive O Resistant	O Sensitive O Resistant
Colistin	O Sensitive O Resistant	O Sensitive O Resistant
Clindamycin	O Sensitive O Resistant	O Sensitive O Resistant
Cefoxitin	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant

Appendix 3. Case report form **ENDOTRACHEAL ASPIRATE**

Study code: [__|__]-[__|__|__|__]

Date of taking samples	[__|__]/[__|__]/[__|__]	[__|__]/[__|__]/[__|__]
Antibiotics use before taking samples	O Yes O No	O Yes O No
Culture result	O Negative O Positive	O Negative O Positive
Bacterial identification		
Staphylococcus aureus	O Yes [____] O No	O Yes [____] O No
Escherichia coli	O Yes [____] O No	O Yes [____] O No
Klebsiella spp	O Yes [____] O No	O Yes [____] O No
Klebsiella pneumoniae	O Yes [____] O No	O Yes [____] O No
Pseudomonas spp	O Yes [____] O No	O Yes [____] O No
Pseudomonas aeruginosa	O Yes [____] O No	O Yes [____] O No
Acinetobacter spp	O Yes [____] O No	O Yes [____] O No
Acinetobacter baumanii	O Yes [____] O No	O Yes [____] O No
Antibiotic resistant enzymes ESBL Carbapenemase	 O Negative O Positive O Negative O Positive	 O Negative O Positive O Negative O Positive
Antibiogram		
Penicilline	O Sensitive O Resistant	O Sensitive O Resistant
Amox + A.clavulanic	O Sensitive O Resistant	O Sensitive O Resistant
Oxacilline	O Sensitive O Resistant	O Sensitive O Resistant
Ceftazidime	O Sensitive O Resistant	O Sensitive O Resistant
Ceftriaxone	O Sensitive O Resistant	O Sensitive O Resistant
Cefepime	O Sensitive O Resistant	O Sensitive O Resistant
Vancomycin	O Sensitive O Resistant	O Sensitive O Resistant
Erythromycine	O Sensitive O Resistant	O Sensitive O Resistant
Ciprofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Ofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Amikacine	O Sensitive O Resistant	O Sensitive O Resistant
Co-trimoxazol	O Sensitive O Resistant	O Sensitive O Resistant
Imipenem	O Sensitive O Resistant	O Sensitive O Resistant
Rifampicin	O Sensitive O Resistant	O Sensitive O Resistant
Tazocine	O Sensitive O Resistant	O Sensitive O Resistant
Ticarcillin/Clavulanic acid	O Sensitive O Resistant	O Sensitive O Resistant
Ertapenem	O Sensitive O Resistant	O Sensitive O Resistant
Levofloxacin	O Sensitive O Resistant	O Sensitive O Resistant
Meropenem	O Sensitive O Resistant	O Sensitive O Resistant
Colistin	O Sensitive O Resistant	O Sensitive O Resistant
Clindamycin	O Sensitive O Resistant	O Sensitive O Resistant
Cefoxitin	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant
Other [_____]	O Sensitive O Resistant	O Sensitive O Resistant

Appendix 3. Case report form Study code: [__|__]-[__|__|__|__]

1. Date of filling the form: [__|__]/[__|__]/[__|__] *(dd/mm/yy)*
2. Time: [__|__]:[__|__]
3. Study investigator (Initials): [_____]

STUDY COMPLETION		
4. **Hospital-acquired infections**	○ Yes ○ No	Number of HAIs [__|__]
	Ventilator-associated pneumonia	○ Yes ○ No
	Non-ventilated pneumonia	○ Yes ○ No
	Bloodstream infection	○ Yes ○ No
	Urinary tract infection	○ Yes ○ No

DISCHARGE / WARD TRANSFER	
5. Day of ward transfer	[__|__]/[__|__]/[__|__]
6. Day of death	[__|__]/[__|__]/[__|__]
7. Day of discharge	[__|__]/[__|__]/[__|__]
8. Length of ICU stay	[__|__|__] days
9. Length of hospital stay	[__|__|__] days

Outcome			
10. Discharged alive	○ Yes	11. Transferred to other hospital	○ Yes
12. Died at home	○ Yes	13. Died in hospital, due to respiratory distress	○ Yes
14. Died in hospital, not due to respiratory distress or unknown reason			○ Yes

ICU cost
15. Total medical cost [_____]
16. Payment by health insurance [_____]

Final diagnosis
☐ Tetanus
☐ Dengue infection
☐ Severe sepsis
☐ Septic shock
☐ Pneumonia
☐ Urinary tract infection
☐ Spontaneous bacterial peritonitis
☐ Cellulitis
☐ Fulminant hepatitis due to HBV
☐ Others [_____]

Antibiotics use			
Antibiotics	Indications	Days of treatment	Being used
Ceftriaxone			☐ Yes
Ceftazidime			☐ Yes
Amikacin			☐ Yes
Levofloxacine			☐ Yes
Ciprofloxacin			☐ Yes
Ticarcillin – Acid clavulanic			☐ Yes
Piperacillin – Tazobactam			☐ Yes
Ertapenem			☐ Yes
Imipenem			☐ Yes
Meropenem			☐ Yes
Colistin			☐ Yes
Oxacillin			☐ Yes
Vancomycin			☐ Yes
Others [_____]			

Other medications	
17. Sedation	○ Yes ○ No
18. Muscle relaxants	○ Yes ○ No
19. Antacid or proton-pump inhibitor	○ Yes ○ No
20. Insulin	○ Yes ○ No
21. Steroids	○ Yes ○ No
22. Vasopressors	○ Yes ○ No
Medical procedures	
23. Nasal/mask Oxygen	○ Yes ○ No
24. Intubation	○ Yes ○ No
25. Tracheostomy	○ Yes ○ No
26. Non-invasive ventilation	○ Yes ○ No
27. Invasive ventilation	○ Yes ○ No
28. Duration of invasive ventilation	[_____] days
29. Continuous heamofiltration	○ Yes ○ No
30. Vasopressors	○ Yes ○ No
31. Transfusion	○ Yes ○ No
32. Abdominal paracentesis	○ Yes ○ No
33. Pleural paracentesis	○ Yes ○ No
34. Others [_____]	