


Prevalence of MDR organism (MDRO) carriage in children and their household members in Siem Reap Province, Cambodia

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Background: The rising incidence of infections caused by MDR organisms (MDROs) poses a significant public health threat. However, little has been reported regarding community MDRO carriage in low- and middle-income countries.

Methods: We conducted a cross-sectional study in Siem Reap, Cambodia comparing hospital-associated households, in which an index child (age: 2–14 years) had been hospitalized for at least 48 h in the preceding 2–4 weeks, with matched community households on the same street, in which no other child had a recent history of hospitalization. Participants were interviewed using a survey questionnaire and tested for carriage of MRSA, ESBL-producing Enterobacteriales (ESBL-E) and carbapenemase-producing Enterobacteriales (CPE) by culture followed by antibiotic susceptibility testing. We used logistic regression analysis to analyse associations between collected variables and MDRO carriage.

Results: Forty-two pairs of households including 376 participants with 376 nasal swabs and 290 stool specimens were included in final analysis. MRSA was isolated from 26 specimens (6.9%). ESBL-producing *Escherichia coli* was detected in 269 specimens (92.8%) whereas ESBL-producing *Klebsiella pneumoniae* was isolated from 128 specimens (44.1%), of which 123 (42.4%) were co-colonized with ESBL-producing *E. coli*. Six (2.1%) specimens tested positive for CPE (4 *E. coli* and 2 *K. pneumoniae*). The prevalence ratios for MRSA, ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* carriage did not differ significantly in hospital-associated households and hospitalized children compared with their counterparts.

Conclusions: The high prevalence of ESBL-E across both household types suggests that MDRO reservoirs are common in the community. Ongoing genomic analyses will help to understand the epidemiology and course of MDRO spread.

Introduction

The rising prevalence in carriage of MDR organisms (MDROs) in the community and increasing incidence of community-associated drug-resistant infections pose a significant threat to public health.^{1,2} This threat is particularly high in Southeast Asia, which is considered a global hotspot for emergence and spread of antimicrobial resistance (AMR).³ However, weak surveillance systems and lack of community data make it difficult to estimate the extent of drug resistance in the region.⁴ MRSA has been ranked high and ESBL-producing Enterobacteriales

(ESBL-E) and carbapenemase-producing Enterobacteriales (CPE) have been ranked critical in the WHO priority pathogen list to guide antibiotic development.⁵ MRSA and ESBL-E can cause a range of infections that result in extended hospital stays and higher socioeconomic costs.⁶

Typically, MDROs are linked to hospitals where they emerge due to selective pressure from increased use of broad-spectrum antibiotics.^{7,8} MRSA remains a common cause of nosocomial infections, while also increasing in prevalence as a cause of infections in healthy individuals in the community.^{9,10} Evolution of MRSA during

carriage,^{11,12} and horizontal gene transfer of key virulence determinants, may drive MRSA epidemic waves.¹³

Over the last two decades, the rates of ESBL-E carriage and infection have been rising in individuals in the community.^{1,14} Prior studies have strongly linked hospitalization history with ESBL and CPE gastrointestinal carriage.^{15,16} Based on genomic analysis, a Spanish study reported that ESBL producing organisms were found in 16.7% of household contacts of discharged inpatients.¹⁷ In another study from Switzerland, ESBL-producing *Escherichia coli* carriage was found plausible in 22.7% household contacts of index patients in a study, especially in mother-to-child and child-to-child transmission, suggesting that children play an important role in ESBL epidemiology.¹⁸ Human-to-human transmission in the community has been assessed to be a high risk factor for spread of AMR in Southeast Asia.⁴ Hence, it is necessary to investigate MDRO transmission in household settings especially in settings where there is limited access to water and soap and poor knowledge of hygiene measures. In addition, the rise in community-associated ESBL-E infections is associated with a considerable rise in empirical carbapenem use in hospitals. Over the past decade, there have also been reports of increasing CPE infection and colonization in hospital settings.¹⁹

In Cambodia, MDRO surveillance has focused on hospital-based clinical microbiology specimens.²⁰ Past studies have reported that 13%²¹ and 21.7%²² of bloodstream infections caused by *Staphylococcus aureus* in paediatric and adult inpatients, respectively, were methicillin resistant. A study conducted in an outpatient department in a paediatric hospital reported an overall 3.5% MRSA carriage with particularly high prevalence in children with recent hospitalization (8.5%) compared with children with no recent hospitalization (2.6%).²³ With regards to ESBL-E, 82.1% of all Enterobacteriales-associated bloodstream infections in children were ESBL positive.²¹ In a separate study by the Institut Pasteur du Cambodge, the proportion of *E. coli* from clinical specimens that were ESBL producers increased from 28.8% in 2012 to 48.2% in 2015.²⁴

MDRO carriage data in Cambodian community settings are limited. A study on helminth prevalence in children from outpatient departments reported ESBL-E carriage in 55% of children and adolescents and also found an association with inpatient hospitalization and ESBL-E carriage.²⁵ This study therefore aims to compare MDRO carriage prevalence between households with recently hospitalized children and children who had not been hospitalized in the past 12 months. We hypothesized that (i) carriage of MDRO would be high in recently hospitalized children and that (ii) carriage of MDRO would be higher in households with recently hospitalized children compared with households in which children had not had any hospitalization in the past 1 year, reflecting intra-household transmission of MDRO from the index child.

Methods

Design and setting

We conducted a cross-sectional study from August to November 2019 in Siem Reap district, Siem Reap Province, Cambodia. The study included a hospital-associated arm and a community-associated arm. For the hospital-associated arm, children aged 2–14 years who had been hospitalized for >48 h at Angkor Hospital for Children (AHC)—a non-governmental paediatric referral hospital in Siem Reap town—were identified. Prior to

hospital discharge, informed consent for study participation was obtained from the accompanying relative.

The median duration of carriage of MRSA and ESBL-E has been estimated to be approximately 0.4 and 1.4 months respectively.²⁶ Hence, the hospital-associated households were visited within 14–28 days post-discharge. Consenting residents who had stayed in that household for a minimum of 1 month prior to the recruitment day were interviewed by the data collectors, using a tablet-based questionnaire on the Qualtrics platform. Parental consent was sought for all participants below 18 years of age, and a separate assent was obtained for each child participant aged 7 years and above. Repeated visits were made to each household to recruit remaining household members where necessary. There were no exclusion criteria for other residents of the households. Since there were no baseline data on MDRO carriage from the general community, we aimed to recruit 50 households in each arm.

Households on the same street/village with an age-matched child (± 2 years) to the index child from hospital-associated households were then approached on the same day. Those households in which the age-matched child as well as any other child had no history of hospitalization in the past 1 year were eligible for inclusion as community-associated households. The first eligible community household that consented to participate was recruited in the study.

Questionnaire

The interviewer-administered questionnaire contained seven sections and was prepared using a literature review to collect information regarding common risk factors relevant to MRSA^{27–30} and ESBL-E carriage.^{31–35} Separate questionnaires were prepared for individuals in the working age group (age >14 years) and children (age ≤ 14 years) according to age-relevant risk factors (Supplementary questionnaire, available as [Supplementary data](#) at JAC-AMR Online). Information on household living conditions was obtained from one adult per household. The working age group questionnaire included questions on antibiotic knowledge, attitude, practice³³ and medical history and travel history,³⁶ among others. The child questionnaire included questions on medical history, attendance of school/childcare facilities,³⁷ contact with animals³⁴ and vaccination history.³⁸ Caregivers were interviewed on behalf of young children. Each interview took an average of 15 min and was administered in Khmer. Survey questionnaires were translated to Khmer and back-translated in English to ensure accuracy. No confidential information was collected, and all participant information was saved with a pseudonym.

Specimen collection

Anterior nares sampling was performed by trained study team members on each study participant using a nasal swab (Transwab with Amies charcoal transport medium, Medical Wire and Equipment, Corsham, UK). Each participant was also asked to provide a stool specimen in a sterile universal container (Sterilin, Newport, UK), and to store this in a cool and dry place before returning to the data collection team. Specimens were transported in cool boxes to the AHC microbiology laboratory at the end of each day and frozen prior to culture.

Nasal swab and stool specimens were stored at -80°C in 1 mL skim milk/tryptone soya broth/glucose/glycerol (STGG; nasal swabs) or tryptone soya broth with 10% glycerol (stool) medium immediately on receipt at the laboratory. These specimens were subsequently thawed for interval processing in batches.

Nasal swabs and stool were cultured to detect MRSA as well as ESBL-E and CPE (specifically *E. coli* and *Klebsiella pneumoniae*), respectively, using chromogenic media (MRSA, ESBL and KPC agar; CHROMagar, Paris, France; prepared in house). The identities of suspect colonies were confirmed with MALDI-TOF (VITEK MS, database V3.2; bioMérieux, Marcy l'Étoile, France). Antimicrobial susceptibilities were assessed by disc diffusion and Etest MIC

testing. Zone diameters and MICs were interpreted using the CLSI guidelines, 2019 version.³⁹ The double-disc diffusion test was used to confirm ESBL production. Carbapenemase production was confirmed by the modified carbapenem inactivation method (mCIM).⁴⁰ The AHC microbiology laboratory participates in national (bacterial identification and antimicrobial susceptibility testing; coordinated by the Pacific Pathology Training Centre, New Zealand) and international (WHO Invasive Bacterial—Vaccine Preventable Diseases; coordinated by UK NEQAS) external quality assurance schemes.

Analysis

The bacterial phenotype and antimicrobial susceptibility data were analysed to estimate the prevalence ratio (PR) of MDRO carriage across the hospital and community arms. We investigated the association between each bacterial carriage and survey variables using conditional logistic regression where we matched hospital and community-associated households from the same neighbourhood to control for unmeasured confounders such as environmental conditions. Analysis was carried out at three levels: household, adult individual (aged >14 years) and child individual factors. The alpha level was set to 0.05. The Pearson's χ^2 test was used to test differences in distribution of categorical variables across groups. Statistical analyses were carried out in R version 3.6.3.⁴¹

Ethics

The project was approved by the National Ethics Committee for Health Research-Cambodia (148 NECHR), Oxford Tropical Research Ethics Committee (OxTREC; 551-18), and National University of Singapore Institutional Review Board (NUS-IRB; H-18-069).

Results

A total of 44 households were recruited in each arm, of which two pairs of households were excluded because the community households did not meet the inclusion criteria. The final analysis included 42 households in each arm comprising a total of 376 participants (Figure 1). Of all the hospital-associated households that had showed interest in participation at AHC, 69.8% of households consented to participate when we approached their households

post hospitalization. All the members of each household agreed to participate in the study. The median number of individuals that participated per household was 4 (IQR: 4–5). All recruited participants provided nasal swabs at the time of survey; stool specimens were collected from 290 participants (77.1%). Participants who did not provide stool specimens were similar to participants who did in terms of age and education level, however participants from the community arm and male participants provided significantly fewer specimens compared with participants from the hospital arm and female participants, respectively (Table S6).

Sociodemographic characteristics of each arm were comparable (Table 1). Child participants formed 51.5% of total participants in the hospital-associated arm and 59.6% in the community-associated arm.

The majority (64/84) of households were built with modern housing materials except a few households (11 in the hospital arm and 9 in the community arm) that were built with natural materials such as earth/mud flooring or rudimentary raw materials such as bamboo or unfinished wood planks. Overcrowding was defined as an area of 4.65 m² (50 square feet) or less per resident.⁴² We found overcrowding in 5 hospital arm households and 11 community arm households.

Antibiotic knowledge, attitude and practice

Adult participants (age >14 years) had a mean score of 8 (IQR: 7–9) out of 11 antibiotic knowledge questions (Table S5). A total of 23 households, 11 (26%) in the hospital and 12 (28.6%) in the community arm, had stocked antibiotics at home that were either leftovers from prescribed medicine or were bought without prescriptions. Amoxicillin and co-amoxiclav were the most commonly stocked antibiotics in these households (12/23, 52.2%), followed by ampicillin, cefalexin, ciprofloxacin and lincomycin. Many participants (38.3%) agreed when asked if they would share the prescribed leftover antibiotics with other individuals experiencing similar symptoms. Several participants (11.1%) agreed to sharing the antibiotics used for humans with their animals too. Summary

Table 1. Demographics for hospital and community arms

Characteristics	Community arm	Hospital arm
Age, median/median age range (IQR)		
children (≤ 14 years)	7 (5–11)	8 (5–11)
adults (> 14 years)	31–40 (15–50)	31–40 (15–50)
Sex, n (%)		
male	79 (45.93)	89 (43.63)
female	93 (54.07)	115 (56.37)
Ethnicity, number of households		
Khmer	40	41
Cham	1	1
Vietnamese	1	0
Number of occupants per household, n (%)		
<3 persons	14 (33.33)	6 (14.29)
4–5 persons	24 (57.14)	26 (61.90)
>5 persons	4 (9.52)	10 (23.81)

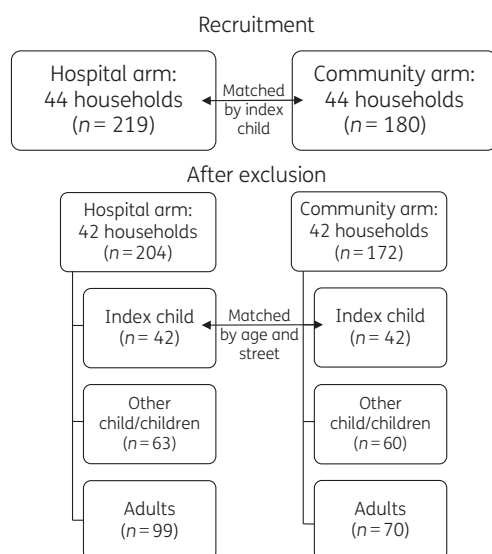


Figure 1. Participant recruitment in the hospital and community arms.

Table 2. Overall prevalence of MDRO across hospital and community arms

Characteristics	Community arm, <i>n</i> (%)	Hospital arm, <i>n</i> (%)	PR ^a (95% CI)
MRSA (376 specimens)			
negative	161 (93.60)	189 (92.65)	
positive	11 (6.40)	15 (7.35)	1.15 (0.02–2.09)
ESBL <i>E. coli</i> (290 specimens)			
negative	6 (4.96)	15 (8.82)	
positive	115 (95.04)	154 (91.12)	0.96 (0.91–1.02)
ESBL <i>K. pneumoniae</i> (290 specimens)			
negative	63 (52.07)	99 (58.58)	
positive	58 (47.93)	70 (41.42)	0.86 (0.67–1.08)
CPE (290 specimens)			
negative	121 (100)	165 (97.63)	
positive	0	4 (2.37)	—

^aPrevalence ratio of MDRO carriage in participants from the hospital arm compared with the community arm.

Table 3. Prevalence of MDRO in index children across hospital and community arms

Characteristics	Community arm, <i>n</i> (%)	Hospital arm, <i>n</i> (%)	PR ^a (95% CI)
MRSA (84 specimens)			
negative	36 (85.71)	37 (88.1)	
positive	6 (14.29)	5 (11.9)	0.87 (0.12–1.48)
ESBL <i>E. coli</i> (64 specimens)			
negative	2 (7.14)	3 (8.33)	
positive	26 (92.86)	33 (91.67)	0.98 (0.87–1.12)
ESBL <i>K. pneumoniae</i> (64 specimens)			
negative	16 (57.14)	23 (63.89)	
positive	12 (42.86)	13 (36.11)	0.84 (0.22–1.35)
CPE (64 specimens)			
negative	28 (100)	35 (97.22)	
positive	0	1 (2.78)	—

^aPrevalence ratio of MDRO carriage in index children from the hospital arm compared with the community arm.

data on antibiotic knowledge, attitude and practice are presented in Table S5.

MRSA carriage

MRSA was isolated from 26 of 376 (6.9%, 95% CI: 4.6–9.9) nasal swabs. The PR of MRSA carriage in all participants from the hospital arm was not significantly higher than the community arm [1.15 (95% CI: 0.02–2.09)] (Table 2), and neither was the PR in index children from the hospital arm when compared with matched children in the community arm [0.87 (95% CI: 0.12–1.48)] (Table 3).

Of the 26 MRSA-positive individuals, 18 were children and 8 were adults. Children had an OR of 3.08 (95% CI : 1.18–8.04, $P = 0.02$) for MRSA carriage compared with adults. Also, individuals who had an animal contact had an OR of 6.32 (95% CI : 1.56–25.59, $P = 0.01$) for MRSA carriage compared with individuals with no animal contact. All the individuals who tested positive for MRSA carriage resided in overcrowded households and signified a very strong association (Table S2), however this association could not

be measured by conditional logistic regression. Other household and individual characteristics were not significantly correlated with MRSA carriage. Following reports of increased *S. aureus* carriage following pneumococcal conjugate vaccine (PCV) introduction,⁴³ we investigated the effects of the 13-valent PCV (PCV13), which was introduced in Cambodia in January 2015, on MRSA carriage in children aged below 5 years.⁴⁴ PCV13 uptake was 67.7% (42/62) and there was no statistically significant difference in MRSA carriage in children across arms with or without pneumococcal vaccination (Table S4).

ESBL-producing *E. coli* carriage

ESBL-producing *E. coli* carriage was isolated from 269 of 290 stool specimens (92.8%, 95% CI: 89.1%–95.5%) (Table S1). Nine additional isolates were third-generation cephalosporin resistant (3GC-R) on screening (i.e. pink colonies on the ESBL CHROMagar plate) but later confirmed as ESBL negative. The PR of ESBL-producing *E. coli* carriage in all participants [0.96 (95% CI: 0.91–1.02)] and index children

[0.98 (95% CI: 0.87–1.12)] were not significantly different between the hospital and community arms (Tables 2 and 3). On analysis of survey variables, none of the household, adult or child characteristics showed any significant association with ESBL-E carriage (Tables S2, S3 and S4).

ESBL-producing *K. pneumoniae* carriage

ESBL-producing *K. pneumoniae* was isolated from 128 of 290 stool specimens (44.1%, 95% CI: 38.3%–50.1%) (Table S1). In 123 specimens, ESBL-producing *E. coli* (42.4%, 95% CI: 36.7%–48.3%) was co-isolated. The PR of ESBL-producing *K. pneumoniae* carriage in the hospital arm compared with the community arm was 0.86 (95% CI: 0.67–1.08). On comparing the index children from both arms, the PR in the hospital arm was 0.84 (95% CI: 0.22–1.35). No factors were significantly correlated with ESBL-producing *K. pneumoniae* carriage (Tables S2, S3 and S4).

CPE carriage

Four carbapenemase-producing *E. coli* and two *K. pneumoniae* [2.07% (95% CI: 0.76–4.45)] were isolated from stool specimens. All CPE were found in the hospital arm, although all belonged to different households and villages. Among the four CPE-positive participants, two were children, of which one was a 6-year-old index child with a recent history of hospitalization and other was an infant. Except for the 6-year-old child, none of the CPE-positive participants had a hospitalization history in the past 12 months. Given the small number of positive isolates, it was not possible to further analyse characteristics associated with CPE carriage.

Discussion

We found that individuals in Siem Reap had a high prevalence of MDRO carriage, regardless of whether they lived in households with a recently hospitalized child or not. The MRSA prevalence of 6.9% was also higher than the previously reported prevalence of 3.5% in outpatient children in Cambodia.²³ The prevalence of MRSA carriage reported in this study is higher than other regional countries of Thailand^{45,46} and China^{47,48} though the upper bound of 95% CI from our study is similar to Vietnam, which reported 7.9% and 8.6% MRSA carriage in two studies.^{49,50} Given that the common mode of MRSA transmission is by person and fomite contact, it is unsurprising to see that all participants with MRSA carriage stayed in overcrowded households, which has also been reported as a risk factor in previous studies.^{27,51,52} Other factors strongly associated with MRSA carriage were age category and contact with animals. Differences in MRSA carriage across age categories are inconsistent in the literature,⁵³ however, MRSA carriage has been consistently associated with intensity of contact with farm and pet animals.^{54,55}

ESBL-producing *E. coli* carriage was pervasive among healthy community participants and, at 92.8%, was much higher than the previously reported carriage rate of ~50% or lower in other Cambodian community studies.^{24,56,57} However, hospital studies on newborns and infants at AHC have reported high prevalence of 3GC-R *E. coli* carriage (63.3%).⁵⁸ The ESBL-E carriage rate in our study was also higher than community prevalence reported in studies from Laos, Vietnam, Thailand, China and

Singapore.^{15,32,33,59} Given the extremely high carriage prevalence, we were unable to identify specific factors associated with ESBL-producing *E. coli* carriage.

The ESBL-producing *K. pneumoniae* carriage of 44.1% in our specimen was also higher compared with other regional studies.^{33,60} However, previous studies in hospital settings at AHC, Cambodia had reported a high prevalence of 3GC-R *K. pneumoniae* carriage (76%) from infants.⁵⁸ High prevalence of co-colonization with ESBL *E. coli* and *K. pneumoniae* suggest that there may be horizontal transfer of ESBL-bearing plasmid and genes across these bacteria, as has been reported in previous studies.⁶¹ The problem of emerging high resistance in *K. pneumoniae* is particularly alarming because of its high genetic diversity and the existence of hypervirulent strains.⁶² A recent study reported emerging *K. pneumoniae* strains that harboured both virulence- and resistance-causing genes, also termed as genotypic MDR-virulence convergence, in bloodstream infections from the Southeast Asian region.⁶³ Though it is unclear whether convergent MDR-virulent *K. pneumoniae* strains will disseminate widely in the community the combination of limited treatment options and highly virulent strains warrants concern and investment in more comprehensive AMR surveillance in Southeast Asia.

The CPE prevalence rate of 2.1% was similar to that found in another Cambodian study (<1%)⁵⁷ but was lower than the prevalence of 4% found recently in Vietnam.⁶⁴ Owing to their high cost, carbapenems are reserved as last-line antibiotics⁶⁵ and not yet used frequently in Cambodia, which may explain the low prevalence of CPE carriage. However, the rising rate of ESBL-E infections and resistance to first-line antibiotics may drive increase in carbapenem consumption and eventual emergence of CPE. Thus, strict antibiotic stewardship programmes and alternative treatment strategies are needed to limit carbapenem use in Cambodia.⁶⁶

There are many potential drivers of AMR in Cambodia. As with many other lower-middle-income countries, strict antibiotic prescription regulations are not enforced, with antibiotics freely available at pharmacy and other stores^{67,68} and patients often practicing self-medication.⁶⁹ There have been reports of inappropriate antibiotic prescribing of broad-spectrum orally administered antibiotics, which may have driven high rates of 3GC-R Enterobacteriaceae.⁷⁰ Much of the food supply comes from integrated agriculture-aquaculture system where humans, vegetable/grain farms, livestock and aquaculture ponds are in close proximity, which eases horizontal transfer of AMR genes/plasmids.³ MDR gene transfer and subsequent spread is also exacerbated by poor sanitation and inadequate sewerage that result in contamination of water sources.⁷¹ Previous studies have described the presence of ESBL-producing Gram-negative bacteria in food⁷² and drinking water,^{73,74} which might explain high carriage rates in the community. Other studies have highlighted that contact with animal manure and slaughtering in one's own house was associated with MDR *E. coli* and *K. pneumoniae* carriage.^{57,75,76} Though it is difficult to quantify the contribution of each driver in the emergence and spread of MDROs, lack of systematic community surveillance makes comparison more difficult, which is important for informing and prioritizing MDRO prevention efforts in the region.⁷⁷ In a review study on factors contributing to global spread of MDROs, it was reported that spread of resistant strains/genes, and not antibiotic consumption, is the dominant contributing factor to the global drug resistance problem.⁷⁸ However, further molecular studies will

be needed to determine the common community reservoirs of MDR strains and/or plasmids by establishing the genetic link.

This study offers a preliminary insight into prevalence of MDRO carriage in Siem Reap Province and will be a useful guide for future studies in the region. A mix of nuclear and extended families were recruited with a good response rate for nasal swabs and stool samples and this is likely to be a good representation of typical households in Cambodia in both urban and rural settings.⁷⁹ One of the major limitations of this study is the small sample size, especially for investigating risk factors associated with high MDRO carriage. Also, we did not sample any other environment source to investigate the community sources of MDRO. Another limitation is that collection and storage of stool specimens in the community may have been suboptimal due to potential for contamination and lack of temperature control before collection by the study team, and this may have affected the stool specimen integrity. However, the study team tried to minimize contamination by repeated instructions and follow-up calls to guide the participants to collect and store stool specimens in good condition.

MDRO carriage appears to be higher in Siem Reap communities compared with neighbouring countries and higher than previous studies in Cambodia, signifying an increase in carriage rates. ESBL-producing *K. pneumoniae* carriage prevalence was especially striking in this community specimen, which is a concern given the regional increase in invasive infections caused by this pathogen. The high prevalence of ESBL-producing *E. coli* and *K. pneumoniae* with uniform carriage across the arms in our study suggests that MDRO reservoirs are not exclusive to hospitals and that these organisms are endemic in the community. The high MDRO carriage rate in asymptomatic individuals may act as reservoirs for further spread in the community. Other concomitant environmental factors such as food, drinking water and exposure to animals also need to be explored to determine community reservoirs of MDR bacteria in Cambodia. Further sequencing-based analyses will be used to identify the diversity of genes and genetic relatedness between the MDROs collected during this study.

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Transparency declarations

None to declare.

Supplementary data

The questionnaire and Tables S1 to S6 are available as [Supplementary data](#) at JAC-AMR Online.

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