

non-bacteremic IED were characterized by similar clinical features and MDR phenotypes.

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# A NOVEL METHOD FOR IN - VITRO BETA-LACTAMASE QUANTIFICATION: PROOF-OF-CONCEPT

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**Intro:** Extended – spectrum beta-lactamase (ESBL) producing Enterobacteriaceae pose a therapeutic challenge across all ages and demographics. ESBL infections are associated with increased antimicrobial resistance and greater morbidity and mortality worldwide. There are limited methods to quantify the production of ESBL enzymes as part of phenotypic antimicrobial susceptibility testing (AST). With widespread empiric broad- spectrum  $\beta$ -lactam use creating selective pressure, quantification of ESBL produced by organisms could improve the precision with which we select and prescribe antimicrobial therapy. We aim to develop a method to quantify ESBL production in-vitro and correlate this with minimum inhibitory concentrations and clinical outcomes.

**Methods:** Gold electrodes were coated with iridium oxide to create a pH sensitive sensor. A range of beta lactamase concentrations (0–10mg/mL) were prepared using ESBL powder (Sekisui Diagnostics) diluted in Mueller Hinton broth (ThermoFisher). Sensors were placed in each enzyme concentration and allowed to stabilise before adding 5ml Penicillin G (512mg/L). After 5 minutes, open circuit potentials were recorded. Data was analysed, plotting enzyme concentration against potential. Triplet repeats of experiments were performed and sensors were calibrated in PBS between each run to check durability and stability over time.

**Findings:** Sensors remained stable throughout the experiments with calibrations measured in PBS solutions of different pH between 4.5 and 8. The slope of the line was Nernstian. Across 3 repeats the sensors measured a positive correlation between enzyme concentration and potential. The sensors were able to measure mean absolute changes of 0.01323V.

**Conclusion:** The data suggests that an iridium oxide-based pH sensor can detect a change in ESBL concentration in standard in-vitro experimental conditions. This could provide a novel method of ESBL quantification as part of in-vitro AST. Future work will apply this methodology to in-vitro assessment of Enterobacteriaceae phenotype.

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# KNOWLEDGE, ATTITUDES AND PRACTICES ON ANTIBIOTIC USE AND ANTIMICROBIAL RESISTANCE IN PATIENTS AND PHYSICIANS OF PRIMARY HEALTH CARE CENTERS IN NORTHERN LIMA, 2014-2015

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**Intro:** Incorrect use of antibiotics is a public health problem that leads to antimicrobial resistance. Objective: To describe the

frequency of antibiotic use and to obtain information about antibiotic prescription, in two health centers in Northern Lima, Peru.

**Methods:** Cross-sectional study using a self-administered questionnaire for patients and physicians.

**Findings:** 1961 surveys were considered, mainly of women (n=1565, 79.21%) and high school degree (n=1144, 58.34%). Two thirds (67.2%, n=1319) received antibiotics in the last year, to obtain these drugs, 76.08% (n=1492) were prescribed by a physician, 17.9% (n=350) by the drug store dealer, 2.6% (n=52) were self-prescribed and 1% (n=20) were recommended by other people. Many physicians agreed in the importance of adequate use of antibiotics and 85.1% (n=23) prescribed antibiotics more than once every day. Most of them (85.1%, n=23) indicated the internet is a useful resource of information and many physicians suggested the development of educative programs about use of antibiotics.

**Conclusion:** There is insufficient knowledge about adequate use of antibiotics in the community, more than a half of participants received an antibiotic prescription by the physician and three quarters would go to the health care center if they presented upper respiratory tract symptoms. Almost all physicians prescribed antibiotics more than once every day, they agreed that antimicrobial resistance is a public health problem, and it is necessary to reinforce training in antibiotic prescription.

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# HAND, FOOT AND MOUTH DISEASE IN SOUTHERN VIETNAM DURING 2015 – 2021

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**Intro:** Hand, foot and mouth disease (HFMD) continues to challenge Asia with pandemic potential. In Vietnam, there have been two major outbreaks occurring during 2011–2012 (>200,000 hospitalizations and >200 deaths) and more recently in 2018 (>130,000 hospitalizations and 17 deaths). Given the high burden and the complex epidemic dynamics of HFMD, synthesizing its clinical and epidemiological data remains essential to develop appropriate interventions and apply public health measures.

**Methods:** From 2015–2021, clinical samples were collected from patients enrolled in a HFMD study conducted at three referral hospitals in Ho Chi Minh City, Vietnam. Enterovirus diagnosis and serotypes determination was carried out using a combination of PCR and sequencing approaches. All EV-A71 positive cases were then subtyped by Sanger sequencing and/or whole-genome sequenced using next-generation based approach

**Findings:** A total of 19 enterovirus serotypes were detected in 1660 HFMD patients enrolled in the study during 2015–2021. EV-A71 (26.2%, n=435) remains the leading cause of HFMD in Vietnam, followed by coxsackievirus A6 (CV-A6, 17.8%, n=296), CV-A16 (11%, n=184) and CV-A10 (7.1%, n=118). There are two main EV-A71 subgenogroups, C4 and B5, and their prevalence interchanges

over the years. EV-A71 C4 displayed low activity during 2015 – early 2018 and then emerged in late 2018, early 2019 and late 2020. Compared with B5, C4 was more likely to be associated with severe HFMD. During the study period, the proportion of CV-A6 and CV-A16 increased in 2017 followed by a drop in 2018, and then went up again between 2019 and 2021

**Conclusion:** Our data have provided significant insights into important aspects of HFMD over seven years (2015–2021) in Vietnam, and emphasize active surveillance for pathogen circulation remains essential to inform the local public health authorities in the development of appropriate intervention strategies to reduce the burden of this disease. Multivalent vaccines are urgently needed to control HFMD

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### LONG TERM CIRCULATION OF SARS-COV-2 RELATED LINEAGES IN BATS IN CAMBODIA

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**Intro:** Recent evidence shows the Greater Mekong Subregion to be a hotspot for Sarbecoviruses in bats, especially insectivorous Horseshoe bats (genus *Rhinolophus*). However, prevalence, maintenance, and evolution of these viruses in *Rhinolophids* is still poorly understood. Sampling efforts are still limited and generally only cover cross-sectional surveillance at single points in time. Following the detection of Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2)-related viruses in *Rhinolophus shameli* from 2010 in Steung Treng, Cambodia, further active longitudinal surveillance in the same area between 2020–2021 continued the detection of these viruses.

**Methods:** Live bat capture and sampling has been implemented in several sites located in Stung Treng province. All rectal swabs of bats were tested for the detection of SARS-CoV-2 or Sarbecoviruses by real time RT-PCR. RNA samples from positive RT-PCR bats were then sequenced using a highly multiplexed PCR amplicon approach using new designed primers set guided by the ARTIC Network multiplex PCR primers set (<https://artic.network/ncov-2019>), on Oxford Nanopore technology.

**Findings:** The sarbecoviruses were detected in four *Rhinolophus shameli* bats, a percentage of similarity ranging at the nucleotide level between 98.8% – 99.1% when compared to two other Cambodian bat sarbecoviruses from 2010 and between 92.4% – 94.5% when compared to human SARS-CoV-2 across the whole genome.

**Discussion:** The bat SARS-CoV-2 related virus recently detected in four positive bats in 2020–2021 are genetically homologous with the virus detected in 2010, indicating a geographically/host limited population that is stable over time in the past ten years.

**Conclusion:** Overall, our findings indicate further complexity in the diversity and evolution of sarbecoviruses and add intricacy to the search for the origins of the Coronavirus Disease 2019 (COVID-19) pandemic.

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### SIDEROPHORE RECEPTOR PROTEIN FROM KLEBSIELLA PNEUMONIAE AS A PROMISING IMMUNOGEN FOR SEROTYPE-INDEPENDENT THERAPEUTIC LEAD DEVELOPMENT

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**Intro:** *Klebsiella pneumoniae* causes wide range of infections including urinary tract infections, sepsis, bacteremia, pneumonia and liver abscesses. The emergence of multi drug resistance in this bacterium led to a major setback for clinical management. WHO also endorsed need for finding alternative therapy to antibiotics for the treatment of these infections. Development of vaccines and passive antibody therapy has been proven as a potent alternative to antibiotics in case of MDR, XDR and PDR *Klebsiella* infections.

**Methods:** Antigen isolation, characterisation and identification through ultracentrifugation, SDS-PAGE and Triple-TOF Mass spectrometry. Cloning and expression of Fep A gene was done. Mice immunisation and challenge study with *Klebsiella pneumoniae* bacteria accomplished.

**Findings:** Clinical strains of *Klebsiella pneumoniae* were grown in iron deficient conditions and the iron regulated outer membrane proteins were extracted and characterized through mass spectrometry for specific identification. The gene for identified protein was cloned in pET- 28a vector and expressed in *E. coli*. The native protein and the recombinant protein were isolated and purified and used as antigens for generation of immune response in BALB/c mice. The native protein of *Klebsiella pneumoniae* grown in iron deficient condition was identified as FepA (Ferrienterobactin receptor) and other siderophore receptors. This 80 kDa protein generated a significant immune response in BALB/c mice. The antiserum from mice after subsequent booster doses was collected and showed binding with FepA protein in western blot and phagocytic uptake assay of *K. pneumoniae*. From animal studies after bacterial challenge post immunisation in mice, significant bacterial clearance was observed. The antiserum from mice showed binding and clearance of the *Klebsiella pneumoniae* bacteria in vitro and in vivo.

**Conclusion:** The antiserum from immunised mice with FepA showed binding and clearance of the *Klebsiella pneumoniae* bacteria in vitro and in vivo. This study demonstrates the potential significance of FepA as an immunogen or a therapeutic agent.

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