Bauer disc diffusion method. 20 MDR *Shigella spp* were selected in random for further analysis. Quinolone resistance genes (*qnr*A, *qnr*B and *qnr*S) were identified by colony PCR for all isolates. Plasmids were isolated from 20 isolates for confirmation of the presence of *qnr* genes. MDR *Shigella* isolates (n = 2), harbouring plasmid with *qnr*S gene was conjugated with completely susceptible *Salmonella spp.* to observe horizontal gene transfer. Selection of conjugates were done using antibiotic medium. Next generation sequencing (NGS) was performed to confirm the identity of the conjugated plasmid.

Results: 6 out of 20 MDR *Shigella* isolates had *qnr*S genes in addition to one *qnr*B gene in one isolate. The *qnr*S containing plasmid was observed to successfully transfer from MDR *Shigella* isolates (n = 2) to susceptible *Salmonella spp.* through conjugation. PCR of the transferred plasmid confirmed the presence of *qnr*S gene. NGS confirmed that the same *qnr*S harbouring plasmid was transferred from *Shigella spp*. to *Salmonella spp*.

Conclusion: This study clearly demonstrates that the plasmid donated by *Shigella spp.* can be naturally acquired by *Salmonella spp*., which poses a greater threat for rapid spread of fluoroquinolone resistance among enteric pathogens. Continuous surveillance of plasmids containing antimicrobial resistance genes is crucial for control of further spread of fluoroquinolone resistance.

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Molecular binding analysis of aminoglycoside N-acetyltransferase aac(6′)-Ib and its bi-functional fluoroquinolone active variant aac(6′)-Ib-cr active-site with ciprofloxacin and kanamycin: An in-silico approach

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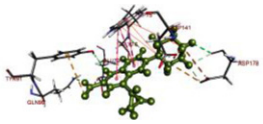
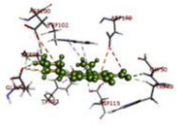
Background: Fluoroquinolone resistance is a serious public health problem. Enzymatic modification of fluoroquinolones is a new phenomenon discovered recently and is mediated by bi-functional fluoroquinolone active cr variant of *aac(6′)-Ib* encoded fluoroquinolone modifying enzyme. The kinetic characterization of *aac(6′)-Ib-cr* and its wild-type parent enzyme has been reported previously however its mode of action is still unclear. Hence, this study was carried out to understand fluoroquinolone acetylation mechanism using the predicted structure of *aac(6′)-Ib-cr* via relevant *in-silico* methods.

Methods & Materials: The structure of *aac(6')-Ib* enzyme, retrieved from PDB database and predicted structure of *aac(6')-Ib-cr* (Yugendran T, Unpublished data) were docked against kanamycin and ciprofloxacin using Schrödinger. The complexes were analysed for protein – ligand interactions using Discovery Studio v3.5.



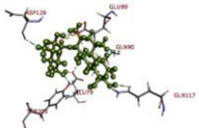
Ciprofloxacin interacting residues of the cr variant

Results: The predicted structure of the mutant enzyme was docked with ciprofloxacin and its interactions were compared with that of kanamycin bound WT enzyme [PDB ID: 1VOC] to understand the mechanism underneath the development of resistance to ciprofloxacin. They were further cross referenced with interactions observed in ciprofloxacin docked WT enzyme. Results based on GLIDE score, complex energy and non-bonded interactions show that ciprofloxacin binds effectively with mutant enzyme and it is positioned close to acetyl-CoA so as to enable acetylation of fluoroquinolone, thereby rendering resistance to the drug. The key residues involved in interaction with ciprofloxacin were W75, G76, Y91, R128 and D141. Comparison with kanamycin bound WT shows that ciprofloxacin engages in more number of strong non-covalent bonds that could be the structural reason for enhancing strong affinity for ciprofloxacin towards mutant enzyme.



Ciprofloxacin interacting residues of the wildtype enzyme

Conclusion: The role of T128R mutation in promoting the interaction with fluoroquinolone molecule has been revealed. The amino-acid residues that bring about the acetylation of fluoroquinolone by interacting with them have also been identified. The mechanism elucidated here ascertains the adaptive potential of aminoglycoside N-acetyltransferase that in this case is presumably driven by the selective pressure of fluoroquinolone use.



Kanamycin interacting residues of the cr variant.

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Combination of NS1 antigen ans anti-NS1 IgA assays in the diagnosis of dengue infection in Asia

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Background: Diagnosis of Dengue infection is essential for clinical care of patients with acute febrile illness. It has been demon-

strated that combining NS1 Ag to an early serologic test improved Dengue diagnosis. If IgM has been extensively evaluated, there are few data about IgA detection in association to NS1.

The purpose of these clinical studies was to evaluate the efficiency of combining dengue NS1 Ag and anti-NS1 IgA antibodies for the detection of Dengue infection during the acute phase in Asian populations.

Methods & Materials: Evaluated kits were Platelia™ Dengue IgA Capture associated to Platelia™ Dengue NS1 Ag for ELISA part and RDT Dengue IgA/IgG associated to Dengue NS1 STRIP for rapid testing. Sensitivity was performed on 216 samples from patients with clinically confirmed Dengue of 3 Asian countries: Cambodia (n=135), India (n= 31) and Singapore (n=50). Specificity was evaluated on 101 sera from 50 healthy Indian donors and 51 febrile patients from Cambodia for which dengue infection has been excluded.

Results: Depending on the population, the sensitivity ranged from 48.5% to 93.3% for Platelia™ Dengue IgA Capture assay versus 48.9% to 90.3% for IgM ELISA and 50% to 100% for the IgA rapid test. When NS1 and IgA tests were combined, the sensitivity reached 93.5% to 98.0% for the ELISAs and 94.1% to 100% for the rapid tests, compared to 94.9% for an "IgM+NS1" combination. The overall specificity of the NS1 and IgA combination in ELISA and rapid test assays were 95,9% and 96,0% respectively. The analysis of NS1 and IgA ELISAs sensitivity related to the sampling time after fever onset in Cambodia population showed that NS1 assay sensitivity was lower and decreased earlier (day 5) in secondary infection than in primary one (day 11).

Conclusion: Detection of anti-NS1 IgA efficiently completes NS1 antigen detection in the diagnosis of acute dengue infection in Asian populations. The combination performs as well on ELISA as on rapid test format and demonstrates similar performance to "IgM+NS1". Moreover, IgA appears to be especially useful in secondary infections and has also been described to be indicative of more severe outcome in primary infections.

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Atypical presentation and nosocomial spread - intensifying the MERS mystery and misery

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Background: Infection control measures to prevent nosocomial transmission of novel pathogens like the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) require strict adherence to guidelines. However, atypical presentations may mislead unwary Emergency Department (ED) physicians, thus posing challenges. We present the investigation of a MERS case with atypical presentation at the King Fahad Medical City (KFMC) in Riyadh in the summer of 2015.

Methods & Materials: The patient's charts and electronic health records covering her two ED visits and subsequent intensive care unit (ICU) admission were reviewed. Adhering to MOH protocols, health care workers (HCWs) exposed to the patient were monitored for possible nosocomial MERS CoV transmission.

Results: The patient was a 77-year-old female with Diabetes Mellitus, Hypertension, chronic kidney disease and chronic myelocytic leukemia who presented twice at the ED, within 4 days. On her first visit, she was febrile (37.9^0C), had abdominal pain and distension (ascites), nausea and vomiting. Four days earlier, she had visited her primary hospital, known to be experiencing a MERS outbreak at that time, for chemotherapy. Biochemical and microbiological testing of drained ascitic fluid were unremarkable. She was discharged the same day after spending 10 hours in the ED. Three days later, she returned to the ED with progressive abdominal distension, worsening fever (38.8^0C) and deteriorating hepatic and renal function. She developed pulseless electrical activity (PEA) and asystole that required resuscitation for 19 minutes. She survived the arrest but clinically worsened and died 4 days in the ICU. Despite 6 intra-hospital transfers (5 prior to MERS CoV confirmation) during her second visit, none of the exposed HCWs (n = 60) developed MERS; included are those who performed high risk procedures (intubation and CPR) on her. However, epidemiological investigation suggests she infected a post-mastectomy patient that shared the waiting room with her while awaiting triage on her first ED visit. Both patients died.

Conclusion: This case of an atypical MERS case with multiple exposures to several HCWs having varying levels of protection on multiple occasions led to only one nosocomial case thus further intensifying the mystery surrounding MERS CoV transmission.

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The impact of increased use of ASHAs on rural immunization coverage in India

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Background: Accredited Social Health Activists (ASHAs) are located in rural Indian villages to promote positive health behaviors and facilitate better health care utilization, including vaccination. We calculated the average coverage of the Bacillus Calmette-Guérin vaccine (BCG); Diphtheria, Pertussis, and Tetanus vaccine (DPT); polio vaccine; measles vaccine, and full vaccine coverage (BCG, 3 doses of DPT, 3 doses of polio, and measles vaccine) across districts in India, and evaluated the impact of expanded ASHA presence on changes in district-level vaccine coverage.

Methods & Materials: We used District Level Household and Facility Survey data, collected in 2007-2008 (DLHS-3) and 2012-2013 (DLHS-4); districts are the unit of analysis. The changes in use of ASHAs and in vaccine coverage over time were calculated as the difference between district-level values in DLHS-3 and DLHS-