abstract
Microbiologists agree that now-a-days multidrug-resistant Gram negative aetiology is predominant, with Enterobacteriaceae, being the most common. The occurrence of carbapenem-resistant bacteria in the health care settings significantly limits treatment options for some of the most critically ill patients, generating a whole new class of gram-negative “superbugs”. The situation becomes more worrisome in case of neonates as they have only limited treatment options. Treatment of neonatal sepsis has become a challenge with the emergence of carbapenemase-producing bacteria. Therefore, a necessary first step in devising treatment strategies is to understand how the genotypes of these complex antibiotic resistance phenotypes emerge and spread in the clinical settings. This study is the first to review the carbapenem resistance patterns in neonatal sepsis over an extended period of time. In this study we investigated (i) carbapenem resistance pattern in septicaemic neonatal isolates (ii) presence of transmissible carbapenemase genes and its transmission (iii) other resistant determinants associated with carbapenem resistance (iv) occurrence of carbapenem resistance through non-transmissible mechanisms.

We studied the trend of carbapenem susceptibility in Enterobacteriaceae that caused septicaemia in neonates over a five year period (2007–2011) and the molecular characterisation of Enterobacteriaceae resistant to carbapenems and cephalosporins. Hundred and five Enterobacteriaceae including *Escherichia coli* (n = 27), *Klebsiella pneumoniae* (n = 68) and *Enterobacter* spp. (n = 10) were isolated from blood of septicaemic neonates followed by antibiotic susceptibility tests, determination of MIC values, phenotypic and genotypic detection of β-lactamases. Carbapenem was the most active antimicrobial tested after tigecycline. Study of the extended spectrum β-lactamases (ESBLs) showed the predominance of CTX-Ms (82%) over this period. Other β–lactamases were also identified which included TEM, OXA and SHV types (TEM-1, OXA-1, SHV-1, SHV-28, SHV-11, SHV-37, SHV-61, SHV-167). AmpCs (CMY-4, CMY-6, CMY-42, ACT-7 and ACT-16)
were not frequent and were mostly associated with New Delhi Metallo-β-lactamase-1 (NDM-1)-carrying isolates. Two novel β-lactamases were identified in this study, a new SHV-type, SHV-167 (GenBank accession no. AB733453) and an AmpC gene, ACT-16 (GenBank accession no. AB737978).

NDM-1, which is a recent addition to the carbapenemase list, was the only carbapenemase identified in our setting. Fourteen percent of the isolates possessed blaNDM-1. Carbapenem non-susceptibility was first observed in 2007 and it was due to loss of Omp F/Ompk35 in combination with the presence of ESBLs and/or AmpCs. NDM-1 first emerged in *E. coli* during 2008; later in 2010, the resistance was detected in *K. pneumoniae* and *E. cloacae* isolates.

NDM-1-producing isolates were resistant to other broad-spectrum antibiotics and possessed ESBLs, AmpCs, 16S-rRNA methylases, AAC(6’)-Ib-cr and class 1 integron. These β-lactamases (ESBLs and AmpCs) were masked in phenotypic tests, as NDM-1 interferes with the interpretation of ESBL/AmpC detection, facilitating inter- and intraspecies dissemination of these enzymes silently. In this study a combination disk assay was established using cefotaxime, cefotaxime/clavulanic acid, cefotaxime/clavulanic acid/cloxacillin, cefoxitin and cefoxitin/phenylboronic acid/cloxacillin on Mueller Hinton agar supplemented with dipicolinic acid for determination of β-lactamases in the presence of NDM-1.

The study also showed that there was a significantly higher incidence of sepsis caused by NDM-1-harbouring isolates in the male sex, in neonates with low birth weight and neonates born at an extramural centre. However, sepsis with NDM-1-harbouring isolates did not result in a higher mortality rate.

Pulsed field gel electrophoresis of the NDM-1-producing isolates indicated that the isolates were clonally diverse. This indirectly established the horizontal transmission of carbapenem
resistance among these isolates and not cross-transmission among the neonates. The efficient transmission of \( \text{bla}_{\text{NDM-1}} \) can be attributed to the mobile genetic elements. Therefore, we carried out an investigation of the mobilizable elements associated with \( \text{bla}_{\text{NDM-1}} \) in Enterobacteriaceae. As this study involved isolates from one unit which were collected sequentially, an attempt was also made to understand whether there was a pattern in the temporal acquisition of \( \text{bla}_{\text{NDM-1}} \) within the unit.

Carbapenem resistant isolates successfully transferred \( \text{bla}_{\text{NDM-1}} \) to \( E. \text{coli J53}^{\text{AzR}} \). \( \text{bla}_{\text{NDM-1}} \) was associated with different plasmid scaffolds (IncFII, IncL/M, IncN, IncR, IncHIB-M/FIB-M), IncF being the prevalent one. The most frequently represented addiction systems were \( \text{pndAC, ccdAB, vagCD and hok/sok} \) but association of addiction system with NDM-1-harbouring plasmid was not frequently observed; \( \text{ccdAB} \) and \( \text{hok/sok} \) were associated with transferable plasmids.

Genetic structures surrounding \( \text{bla}_{\text{NDM-1}} \) showed association with at least a remnant of \( \text{IS}_{\text{Aba125}} \) at its 5’-end and \( \text{ble}_{\text{MBL}} \) at its 3’-end, though downstream sequence of \( \text{bla}_{\text{NDM-1}} \) in the study isolates, varied immensely. The entire \( \text{IS}_{\text{Aba125}} \) element was identified upstream of the \( \text{bla}_{\text{NDM-1}} \) gene in most isolates. However, \( \text{IS}_{\text{S}5} \) was present in three cases. The spread of NDM-1 was not related to class 1 integron which possessed resistance determinants against trimethoprim (\( \text{dfrA12, dfrA1, dfrA5} \)), streptomycin (\( \text{aadA2, aacA4} \)) and rifampicin (\( \text{arr-3} \)).

RFLP pattern showed that three isolates possessed the same FII/FIIs plasmid; two (\( E. \text{cloacae} \) and \( E. \text{coli} \)) of these three isolates were isolated from a neonate within an interval of one week. The temporal isolation of two pathogens from the neonate, similar genetic determinants, similar replicon type and RFLP pattern of the plasmid indicate the transmission of \( \text{bla}_{\text{NDM-1}} \) via the plasmid from \( E. \text{cloacae} \) to \( E. \text{coli} \) within the same patient. To the best of
our knowledge, this report is the first to inform the in vivo interspecies plasmid transfer event of $bla_{NDM-1}$ in a neonate.

The predominance of F plasmids and ISAb125 along with $bla_{NDM-1}$ in the NICU indicates that the F-type plasmids and ISAb125 may have played the role of acquisition and spread of NDM-1 in our unit, but no specific pattern in the temporal acquisition of mobile genetic elements could be identified.

This study goes beyond the genotypes or pulsotypes of strains and evaluates the mobile genetic elements in a hospital setting to understand the emergence of resistance. In trying to understand whether interspecies transmission of mobile genetic elements happened in a real life situation, this study could unravel the diversity of the mobile genetic elements in the carbapenem-resistant strains. However, it should be noted that, though there is diversity in the mobilizable elements associated with NDM-1, the predominance of FII plasmids and ISAb125 cannot be ignored. IncFII is a narrow host range plasmid and it is quite widespread in Enterobacteriaceae. The class 1 integrons are not associated with NDM-1 but its frequent presence in the strains and its diversity is a reason for further concern. The emergence of a new resistance mechanism such as $bla_{NDM-1}$ is evidenced in the hospital environment only after there is a sudden change in the resistance profile of the infecting organisms. It is difficult to exactly pinpoint how such genes gain access to the hospital environment but it can be certainly said that mobilizable elements like plasmids, transposons and integrons play a leading role in the drama of emerging resistance. Many questions remain to be answered about these elements, particularly, why certain genes such as CTX-M and NDM can spread extensively compared to some other similar genes and efforts in this direction has potential for better health outcomes.