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Letter to the Editor

Resistance profiles and resistome mapping of multidrug resistant carbapenem-hydrolyzing *Klebsiella pneumoniae* strains isolated from the nares of preterm neonates

Sir,

Prematurity at birth is a leading cause of death among the under-five-years population across the world. Premature births with different levels of lung development often result in respiratory distress and repeated hospitalizations where aggressive use of antibiotics, exposure to nosocomial pathogens and lymphoid organ prematurity result in high fatality rates with infectious etiology. Here we report the nasal carriage rates, susceptibility profiles and resistome maps of strains of the ESKAPE pathogen, *Klebsiella pneumoniae* isolated from neonate nares admitted with respiratory distress.

Nasal swab specimens were collected between 2017 and 2018 from 38 preterm neonates admitted after birth to the sick neonatal care unit (SNCU), College of Medicine & JNM Hospital, Kalyani, India with respiratory distress (Supplementary Table S1). Specimen enrichment was carried out in Muller Hinton broth (Himedia Labs, Mumbai, India) and singles colonies were grown aerobically on MacConkey agar (Himedia Labs, Mumbai, India) at 37°C. Sixteen (42.11%) had growth and three to four colonies per specimen were sub-cultured. Genomic DNA was purified using the DNAeasy blood and tissue DNA isolation kit (Qiagen, Hilden, Germany). Initial screening for *K. pneumoniae* was carried out by *16S* rDNA amplification and Sanger sequencing using S-D-Bact-0008-c-S-20/S-D-Bact-1391-a-A-17 primer pairs (Eurofins Scientific, Bengaluru, India). Eight specimens (21.05%) were found to be positive (Supplementary Table S1).

To understand the susceptibility profiles, Kirby–Bauer disc diffusion assays were carried out following The Clinical & Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested were cefotaxime (30 μg), piperacillin-tazobactam (100/10 μg), meropenem (10 μg), amikacin (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg) and chloramphenicol (30 μg). All the isolates were found to be resistant to cefotaxime, piperacillin-tazobactam and all but one to meropenem (Table 1).

Presence of *β-lactamase* genes, $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM} and bla_{NDM} were determined by polymerase chain reaction (PCR) and subtyped by Sanger sequencing (Table 1, Supplementary Table S3). The only meropenem-susceptible strain was JNM19C1 which harboured none of the genes. A 186-base-pair (bp) region of the *DNA polymerase I* gene was amplified as an internal control. All the carbapenem-hydrolyzing strains with the exception of JNM25C3 harboured the bla_{NDM} gene (Table 1, Supplementary Fig. S1). Presence of metallo-β-lactamase and chloramphenicol resistance was further confirmed by Etests (Himedia Labs, India) (Supplementary Table S2).

Two of the carbapenem-hydrolyzing strains JNM8C2 and JNM10C3 were subjected to paired-end whole-genome sequencing (2×100 bp) on an Illumina HiSeq2500 platform (Illumina, San Diego, USA). Both *de novo* and reference (NC_009648) guided assembly were carried out using Velvet and Bowtie2, respectively [1,2]. Multilocus sequence and *wzi* typing (capsular polysaccharide type) were achieved by running the sequences against the Bacterial Isolate Genome Sequence Database (BIGSdb). Plasmid replicons were identified using PlasmidFinder [3]. For JNM8C2, after removal of low-quality reads, a total of 16,462,916 (88.12%) reads remained and the alignment was achieved with 13,269,930 (80.61%) reads with chromosome coverage of 93.84%. JNM8C2 chromosome length was determined to be 5,315,966 bp, a multilocus ST-2816, possessing *wzi* allele 22 (CP030857) and carried two plasmids (pKJNM8C2.1 (CP030858) and pKJNM8C2.2 (CP030859)). From JNM10C3 strain sequencing, a total of 7,168,939 (99.52%) reads remained after filtering and alignment was achieved with a total of 5,802,851 (80.94%) reads. The chromosome length was 5,138,694 bp with a coverage of 99.54%. JNM10C3 was an ST-711, with *wzi*_303 allele (CP030877) and carried two plasmids (pKJNM10C3.1 (CP030876) and pKJNM10C3.2 (CP030878)).

Protein coding regions were annotated using RAST [4], NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and the Antimicrobial Resistance Database (ARDB). With the exception of the presence of a *catB3* gene in the chloramphenicol-susceptible strain, JNM8C2, there was a general concordance between antimicrobial resistance and presence of resistance genes. Both JNM8C2 and JNM10C3 were found to harbour equal numbers of chromosomal resistance genes against toxic metals such as cadmium, cobalt, chromium and zinc ($n = 11$), resistance conferring gene against colicin E2 ($n = 1$), adaptation to d-cysteine ($n = 1$), resistance to fosfomycin (n=1; Table 2) and the *β-lactamase* gene, bla_{SHV} (Table 2). The JNM10C3 plasmids were found to carry a greater number of antimicrobial genes against different classes of antibiotics compared with JNM8C2 (Table 2, Supplementary Fig. S1). There were gene copy number variations as well, and the pKJNM8C2.1 plasmid harboured three copies of $bla_{\text{NDM-1}}$ gene. By contrast, both the JNM10C3 plasmids (pKJNM10C3.1, pKJNM10C3.2) harboured a single copy of the $bla_{\text{NDM-7}}$ gene.

The presence of high percentage of multidrug-resistant carbapenem-hydrolyzing *K. pneumoniae* strains in the faeces of neonates admitted to neonatal care units has been reported [5] However, this is the first report on multidrug-resistant carbapenem-hydrolyzing *K. pneumoniae* carriage in the nares of neonates. Further, the resistome profiles of the selected strains also highlight the role that resistome mapping can play in appropriate antibiotic usage in this vulnerable population.

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Table 1
Antimicrobial susceptibility and *β-lactamase* gene profiles of the eight *K. pneumoniae* strains.

Sample ID	List of antibiotics							*β-lactamase* gene variants			
	CTX	P/T	MRP	AK	GEN	CIP	C	bla_{NDM}	bla_{CTX}	bla_{SHV}	bla_{TEM}
JNM8.C2								bla_{NDM-1}	$bla_{CTX-M-15}$	bla_{SHV-11}	-
JNM10.C3								bla_{NDM-7}	$bla_{CTX-M-15}$	$bla_{SHV-187}$	$bla_{TEM-169}$
JNM11.C4								bla_{NDM-7}	$bla_{CTX-M-15}$	bla_{SHV-1}	bla_{TEM-1}
JNM13.CaC1								bla_{NDM-1}	$bla_{CTX-M-15}$	$bla_{SHV-220}$	bla_{TEM-1}
JNM19.C1								-	-	-	-
JNM22.C1								bla_{NDM-5}	$bla_{CTX-M-15}$	bla_{SHV-1}	bla_{TEM-1}
JNM25.C3								-	$bla_{CTX-M-15}$	bla_{SHV-85}	bla_{TEM-1}
JNM28.C1								bla_{NDM-1}	$bla_{CTX-M-15}$	bla_{SHV-85}	bla_{TEM-1}

AK, amikacin; C, chloramphenicol. CIP, ciprofloxacin; CTX, cefotaxime; GEN, gentamicin; MRP, meropenem; P/T, piperacillin-tazobactam. The color red denotes resistance, orange represents intermediate phenotype, and green is sensitivity to an antibiotic.

Table 2
Antimicrobial resistance genes identified in JNM8C2 and JNM10C3 genomes.

Antimicrobial category	JNM8C2	JNM10C3
Aminoglycosides	*aac(3)-III,aac(3)-Ib, aac(6')lb-cr#, aadA2*	*aac(3)-IIc, aadA2, aph(3')-VI, aph(3")-Ib, aph(6)-Id, armA, aph(3')-VI*
Carbapenem, Penicillins+ β-lactamase inhibitors	bla_{NDM-1}	bla_{NDM-7}
Extended-spectrum third generation cephalosporins	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{SHV-11}*	$bla_{CTX-M-15}$, bla_{OXA-1}, $bla_{TEM-169}$, $bla_{SHV-187}$*
Fluoroquinolones	*aac(6')lb-cr#, emrA*, ermB**	*qnrB1*
Folate pathway inhibitors	*dfrA12*	*dfrA12*
Phenicols	*catB3*	*catB3*
Glycopeptides	ble_{MBL}	ble_{MBL}
Macrolides	*mphD, msr(E)*	*msr(E), macA, macB*
Nucleosides		*tmrB*
Phosphonic acids (Fosfomycin)	*fosA6**	*fosA6**
Sulphonamide	*sul1*	*sul1, sul2*
Tetracyclines		*tet(A)*
Multidrug efflux pumps & activator proteins	*acrB*, oqxA*, oqxB*, marA*, marB*, marR mutant*, nodT**	*acrA*, emrE, ompK37*, oqxA**

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Competing interests

None declared.

Ethical approval

The hospital ethical committee provided approval (reference no. F-24/Pr/COMJNMH/IEC/16/536).

Authors' contributions

B.B. designed all the experiments, carried out all the next-generation sequencing analyses, collated all the data and wrote the full paper. T.B. and M.C. ran all the laboratory experiments. M.B. was the clinician who was involved in specimen collection.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2018.12.002.

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