Emergence of NDM-1-producing Enterobacteriaceae in China

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Sir,

The worldwide dissemination of bacteria producing New Delhi metallo-β-lactamase (NDM)- is a major public health issue.[1] Since NDM producers are primarily found among individuals with history of hospitalization or travel to the India subcontinent, many hospitals have implemented microbiological screening of patients with such an epidemiological history.[1,2]

In mid-2011, the stool sample of a one year-old infant was found to have CRE upon admission screening. The infant was admitted because of cough and intermittent fever in the preceding two weeks. The family had travelled to and stayed in Hunan province, China in the preceding month. Following onset of the symptoms, the infant had been admitted to a hospital in Hunan for 3 days. Patient was given a diagnosis of broncholitis and had been treated with a course of intravenous cefoperazone. At presentation to our hospital, patient had fever of 38 ^{o}C. Chest examination was unremarkable. Patient was treated conservatively and the fever resolved without further antibiotic treatment. Patient was discharged 2 days later. In accordance with the screening policy, stool samples were obtained for surveillance culture.

In brief, a red-bean size faecal pellet was suspended in saline. A 10 μL aliquot of the suspension was then removed and plated directly onto one ChromID ESBL plate. In addition, a broth enrichment step was performed by inoculating another 10 μL aliquot of the faecal suspension into nutrient broth with 1 mg/L meropenem, incubated at 37 ^{o}C overnight, and then subcultured onto MacConkey plate with 1 mg/L meropenem. All plates were incubated at 37 ^{o}C in air for 20 h. All distinct colony types recovered from either the chromogenic or the MacConkey media were investigated for evidence of carbapenemase activity using the combined disc method and boronic acid or EDTA as inhibitor.[3] All isolates were identified using VITEK 2 and antimicrobial susceptibility was determined by the CLSI disc diffusion method.[4]

After the patient's stool samples were found to carry CRE. Stool samples from the infant's parents and other family members were also cultured using the same methodology. A total of four CRE isolates were recovered from the faecal samples of the child and her mother (Table 1). Cultures of the faecal samples from the other household members (father, grandfather, grandmother and aunt) were negative. All four isolates (two *Escherichia coli*, one *Klebsiella pneumoniae* and one *Enterobacter aerogenes*) exhibited synergy with EDTA in the combined disc testing. No synergy with boronic acid was observed. PCR and sequencing, using previously described methods confirmed presence of NDM-1 in the four CRE isolates.[2] Additional β-lactamases including other metallo-β-lactamases (IMP, VIM, GIM, SPM, SIM), CTX-M and OXA-48-like genes were also sought by PCR and sequencing.[5] This allowed identification of an additional extended-spectrum β-lactamase (ESBL) gene in the *K. pneumoniae* (CTX-M-65) and the two *E. coli* (CTX-M-57) isolates. Next, we studied the relationship between the two *E. coli* isolates by PFGE after digestion of their genomic DNAs with *XbaI*. The two isolates shared the same PFGE banding pattern, indicating that the two isolates were clonally related. Multilocus sequence typing showed that the two *E. coli* strains and the *K. pneumoniae* strain belonged to ST744 and ST483, respectively. S1-PFGE demonstrated that the strains had one to three plasmids with sizes of 50-90 kb. Hybridization demonstrated that $bla_{\text{NDM-1}}$ gene was harboured on the 50 kb plasmid in all the strains. In conjugation experiments,[2] the 50 kb $bla_{\text{NDM-1}}$ harbouring plasmid could be transferred to J53 *E. coli* recipient at high frequencies (up to 10^{-1} per donor cell). In the transconjugants, there was no co-transfer of resistance to the non-β-lactam antibiotics (amikacin, ciprofloxacin, chloramphenicol, fosfomycin, nalidixic acid, sulphonamides, tetracycline and trimethoprim). Transconjugants with the $bla_{\text{NDM-1}}$ harbouring plasmid as the only plasmid was investigated by PCR-based replicon typing and $bla_{\text{CTX-M}}$ PCR. The finding

showed that the 50 kb plasmid belonged to an untypeable replicon type and was *bla*$_{\text{CTX-M}}$ negative.

Members of this family had no history of travel to the Indian subcontinent. The index patient is the only one with a history of hospitalization. Therefore, the infant has mostly likely acquired the *bla*$_{\text{NDM-1}}$ gene during the hospitalization in Hunan province. This indicates that some hospitals in mainland China could be reservoirs of the *bla*$_{\text{NDM-1}}$ gene,[6] which may or may not initially have reached there from the Indian subcontinent. While both the infant and her mother shared the same *E. coli* strain, it is impossible to tell if there was intra-familial transmission as opposed to the mother and the infant both becoming colonized while in hospital. In China, the burden of CTX-M-producing Enterobacteriaceae is tremendous. Therefore, accumulation of NDM-1 in multiple CTX-M-producing Enterobacteriaceae species is worrying.[1] In conclusion, this report shows the spread of NDM-1 among persons with no established links to the Indian subcontinent and demonstrates the usefulness of admission screening for early identification of NDM-1 for patients who have been treated aboard.

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Transparency declaration

None to declare.

92 Table 1. Characteristics of four carbapenem-resistant Enterobacteriaceae in this study

	Strain (bacterial species)			
	CRE379 (EA)	CRE380 (KP)	CRE396 (EC)	CRE397 (EC)
Specimen source	index	index	index	index's mother
β-lactamase gene content[a]	NDM-1	NDM-1, CTX-M-65	NDM-1, CTX-M-57	NDM-1, CTX-M-57
Plasmid content (size in kb)[b]	50	50	50, 90	50, 80, 90
Inhibition zone diameter (mm, with/without EDTA)				
Ertapenem	24/9	20/6	16/13	24/6
Imipenem	23/12	25/15	25/15	27/11
Meropenem	24/11	24/10	25/13	27/8
Co-resistance pattern				
Amikacin	S	S	S	S
Chloramphenicol	S	R	R	R
Ciprofloxacin	S	R	R	R
Cotrimoxazole	S	R	R	R
Fosfomycin	M	S	S	S
Gentamicin	S	R	S	S
Nitrofurantoin	R	R	S	S
Tigecycline[c]	M	R	S	S

93 EA, *Enterobacter aerogenes*; EC, *Escherichia coli*; KP, *Klebsiella pneumoniae*

94 [a] β-lactamase group-specific PCR was used to assay for presence of NDM, IMP, VIM, KPC,

95 GES and OXA-48-like genes.

96 [b]The plasmid showed to harbour the $bla_{\text{NDM-1}}$ gene by probe hybridization was underlined.

97 [c]The FDA disc breakpoints was used to interpret tigecycline susceptibility results: sensitive

98 ≥19 mm, intermediate 15-18 mm and resistant ≤14 mm.

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