Case Review

New Delhi metallo- β -lactamase-1 producing enterotoxigenic *Escherichia coli* in childhood diarrhoea from the Andaman Islands, India

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Introduction: Carbapenems have long been the dependable last line of treatment for multidrug-resistant Gram-negative bacteria.

Case presentation: We report here two cases of enterotoxigenic *Escherichia coli* harbouring the New Delhi metallo- β -lactamase-1 (NDM-1) in childhood diarrhoea. Two paediatric patients aged 1.5 and 5 years were admitted to hospital with acute diarrhoea. The patients both had symptoms of fever, fatigue and anorexia. A molecular assay confirmed the presence of NDM-1 in both the isolates.

Conclusion: To our knowledge, this is the first report of NDM-1-producing enterotoxigenic *E. coli* in paediatric patients suffering from acute diarrhoea in the Andaman Islands and worldwide. Rapid molecular characterization of NDM-1 of Enterobacteriaceae isolates needs to be undertaken in the future, especially when encountered with carbapenem resistance with conventional challenging, to tackle this public health problem.

Keywords: acute diarrhoea; antibiotic; carbapenem.

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Introduction

Multidrug-resistant bacteria pose a major public health threat, due to the limited success in the search for alternative antibiotics. The carbapenem group of antibiotics plays a vital role in the management of Gram-negative bacterial infections, because of their broad spectrum of activity and stability against most of the β -lactamases. Carbapenems have long been the dependable last line of treatment for multidrug-resistant Gram-negative bacteria. However, several new mechanisms of resistance such as a new type of carbapenem resistance conferred by the gene *bla*_{NDM-1} (Yong *et al.*, 2009) have been documented recently. New Delhi metallo- β -lactamase (NDM-1) was first detected in *Klebsiella pneumoniae* isolated in 2008 from a Swedish patient of Indian origin (Muir & Weinbren, 2010). Since then, increasing numbers of infections due to strains possessing the *bla*_{NDM-1} gene have been reported in patients in India, Pakistan, the UK and occasionally from other parts of the world (Kumarasamy *et al.*, 2010).

Subsequently, NDM-1 was detected from many paediatric patients with cystitis, respiratory distress, sepsis, and nosocomial and other infections (Birgy *et al.*, 2011; Mochon *et al.*, 2011; Roy *et al.*, 2011). The presence of NDM-1 has not yet been reported in bacteria causing childhood diarrhoea, although its presence has been detected in bacteria causing several other infections in patients in the paediatric age group. Andaman and Nicobar Islands, an archipelago of more than 500 islands situated in the Bay of Bengal about 1200 km southeast of peninsular India (92–948'E; 6–148'N), is a Union Territory of India. The existence of NDM-1-producing *Proteus mirabilis* has been reported in these Islands from cases with urinary tract infection (Bhattacharya *et al.*, 2013). In this report we describe two cases of childhood diarrhoea caused by NDM-1-producing enterotoxigenic *Escherichia coli* in the Andaman and Nicobar Islands. To our knowledge, this is the first report of NDM-1-producing enterotoxigenic *E. coli* from diarrhoea cases in the paediatric age group.

Abbreviations: ESBL, extended spectrum β -lactamase; NDM-1, New Delhi metallo- β -lactamase-1.

The GenBank/EMBL/DDBJ accession number for the sequence of the NDM-1 gene determined in this study is KF577585.

Case reports

Case one

A 1.5-year-old male child patient was admitted to the Chirayu Child Care Centre, a private paediatric hospital at Port Blair, on 30 August 2013 with history of acute diarrhoea and abdominal cramps. The patient had fever, fatigue, anorexia, tachycardia and polypnea, and more than six episodes of watery diarrhoea with blood. The patient was dehydrated with reduced tears, xerostomia, reduced skin elasticity and sunken eyes. Prior to the onset of the illness, the child had travelled by ship from Kolkata, where he was staying with his relatives for a month for a vacation. The patient was started on intravenous fluids and metronidazole infusion (75 mg), but the response was poor. On examination, the patient was conscious with a pulse rate of 112 min⁻¹, respiratory rate of 20 min⁻¹ and blood pressure of 105/90 mmHg. His haemoglobin concentration was 6.8 g dl⁻¹ and the total leucocyte count was 13 000 mm⁻³ with 67 % polymorphs, 18 % lymphocytes, 2 % eosinophils and 3 %, monocytes. The blood urea was 20 mg dl⁻¹, serum glucose was 90 mg dl⁻¹ serum creatinine was 0.9 mg dl⁻¹ and serum bilirubin was 0.4 mg dl⁻¹. A stool specimen was collected on the day of admission to the hospital.

Case two

A 5 year-old female child patient was admitted to Gobind Ballab Pant Hospital, Port Blair (Andaman and Nicobar state referral hospital), on 3 September 2013 with complaints of watery diarrhoea. The patient had fever, fatigue, anorexia and eight to nine episodes of watery diarrhoea with blood and mucous per day, together with vomiting and abdominal pain. She had dehydration evidenced by oliguria, xerostomia, reduction in tears and sunken eyes, and had dysphagia, lethargy and weight loss. On examination, the patient was conscious, had a pulse rate of 100 min⁻¹, respiratory rate of 25 min⁻¹ and blood pressure of 110/90 mmHg. Her haemoglobin concentration was 6.1 g dl⁻¹ and her total leucocyte count was 13 800 mm⁻³ with 78 % polymorphs, 16 % lymphocytes, 2 % eosinophils and 4 % monocytes. The blood urea was 23 mg dl⁻¹, serum glucose was 100 mg dl⁻¹, serum creatinine was 0.9 mg dl⁻¹ and serum bilirubin was 0.4 mg dl⁻¹. She did not have a history of recent travel and none of her family members had reported diarrhoea episodes in the recent past. She was treated with ceftriaxone (500 mg in 20 ml) and amikacin (75 mg) intravenously. The fever subsided within 2 days, but watery diarrhoea continued with decreased frequency. A stool specimen was collected for microbiological investigation on the day of admission to the hospital.

Laboratory investigations

The stool specimens collected were screened for bacterial aetiology by culture following standard protocols. Isolation, identification and serotyping confirmed the

presence of *E. coli* serotype O114 in the stool specimens of both cases. A PCR test for the *elt* toxic gene (Amisano *et al.*, 2011) showed the presence of the gene in both the isolates, thus confirming that the isolates were enterotoxigenic *E. coli* producing heat-labile toxin. An antibiotic susceptibility test was performed using the Kirby–Bauer disk diffusion method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012). The MICs for fluoroquinolones (ciprofloxacin, ofloxacin, norfloxacin and nalidixic acid) and third-generation cephalosporins (ceftazidime, cefotaxime and ceftriaxone) were $\geq 256 \mu\text{g ml}^{-1}$ for both isolates. The MICs for the carbapenem group of antibiotics (imipenem, meropenem, ertapenem and doripenem) were tested, as these are the last line of treatment for multidrug-resistant gram negative bacteria. The MICs for these antibiotics for the first case isolate were 12, 8, 8 and 4 $\mu\text{g ml}^{-1}$, respectively, and 12, 32, 8 and 4 $\mu\text{g ml}^{-1}$, respectively, for the isolate from the second case.

The isolates were tested for metallo- β -lactamase detection using Etest MBL Strips (AB Biodisk) and both were positive. Both isolates gave positive results in a modified Hodge test using meropenem and ertapenem.

DNA was extracted from the isolates and fragments of the *bla*_{NDM-1}, *bla*_{SHV}, *bla*_{OXA-1} and *bla*_{CTX-M-3} genes were amplified by PCR using published primers and programs (Bhattacharya *et al.*, 2013; Maynard *et al.*, 2003). Both the isolates were positive for *bla*_{NDM-1} and for the extended-spectrum- β -lactamase (ESBL) genes *bla*_{OXA-1} and *bla*_{CTX-M-3}. In addition, the isolate from the first case was positive for the ESBL gene *bla*_{SHV} and the second isolate for *bla*_{TEM}. The amplified fragments were sequenced and the sequence of *bla*_{NDM-1} was compared with published sequences using BLAST. The sequences of *bla*_{NDM-1} of the isolates were identical and showed 100 % nucleotide identity with NDM-1.

The second patient, who did not recover with the antibiotic regimen she was started on, was later treated with cilenem (300 mg in 20 ml normal saline) over 5 h for 2 days. With this medication, the patient's condition improved and she was discharged on day 6 after admission.

Discussion

NDM-1-positive strains can destroy carbapenem antibiotics such as meropenem, imipenem, doripenem and ertapenem by breaking down the carbapenem groups of these antibiotics, which have been serving as the basis for the treatment of antibiotic-resistant bacterial infections. The NDM-1 gene encodes an enzyme that breaks down and neutralizes the activity of carbapenem antibiotics. Therefore, the spread of pathogenic micro-organisms carrying the NDM-1 gene is a potential threat to public health globally. Prior to 2013, none of the isolates obtained as part of our hospital-based diarrhoea surveillance showed resistance to imipenem. The Andaman and Nicobar Islands are visited by tourists all year round from

other parts of the country and world. The emergence of enterotoxigenic *E. coli* harbouring the NDM-1 gene in these remote islands of India is a public health concern. The mainstay of antibiotic therapy against bacterial diarrhoea in the Andaman and Nicobar Islands is fluoroquinolones. Fluoroquinolone resistance has been rapidly emerging in diarrhoea pathogens in recent years and therefore third-generation cephalosporins are being used as the second line of drug treatment. However, ESBL-producing strains of enteric pathogens resistant to newer generations of cephalosporins are rapidly emerging, and in this scenario, imipenem could be the only therapeutic alternative. The presence of NDM-1 enzyme in these organisms has other implications, as there is a possibility of this resistance gene spreading to other bacterial pathogens circulating in the region. This is a serious concern because resistance to other groups of antibiotics is rapidly emerging and imipenem is the only group of effective antibiotics left for treatment of infections with multidrug-resistant bacterial strains.

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