Transmission of imipenem resistance determinants during the course of an outbreak of NDM-1 *Escherichia coli* in a sick newborn care unit

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Received 29 April 2011; returned 8 June 2011; revised 18 August 2011; accepted 19 August 2011

**Objectives:** This study reports a cluster of septicaemic newborns with imipenem-resistant *Escherichia coli* in the blood and delineates the possible mechanisms of transmission of imipenem resistance with respect to the New Delhi metallo-β-lactamase (NDM-1) gene.

**Methods:** During a point prevalence survey, attempts were made to isolate Gram-negative bacilli (GNB) from the environment of a sick newborn care unit (SNCU) and body sites of neonates. Subsequently, four fresh newborns admitted to the SNCU developed sepsis with *E. coli*. *E. coli* isolates from body sites and blood of the newborns were analysed in terms of clonality, carbapenemases, integrons, virulence factors, porins and transmissibility.

**Results:** During the survey, both imipenem-resistant and imipenem-susceptible *E. coli* were isolated from the body sites of neonates, but none from the environment. None of these neonates developed sepsis. The freshly admitted septicaemic neonates had imipenem-resistant *E. coli* in their blood, which were similar to the imipenem-susceptible *E. coli* obtained from the body sites (during the survey) in terms of clonality, phylogroup, virulence and other resistance genes, except possession of *bla*NDM-1. Imipenem-resistant *E. coli* from blood and body sites were not clonal, though both had *bla*NDM-1. Besides *E. coli*, other GNB isolated from the environment and body sites also harboured *bla*NDM-1. Imipenem-resistant and imipenem-susceptible *E. coli* from the blood and body sites respectively, possessed a novel AmpC β-lactamase, *bla*CMY-59. The plasmid carrying *bla*NDM-1 was transferable.

**Conclusions:** The time frame of isolation and clonal identity indicated a possible transfer of *bla*NDM-1 from imipenem-resistant GNB to the imipenem-susceptible *E. coli*, which subsequently caused septicaemia. This establishes the promiscuous nature of *bla*NDM-1 and emphasizes the need for the early recognition of similar isolates.

**Keywords:** neonatal sepsis, Gram-negative bacilli, carbapenem resistance, CMY-59, India

**Introduction**

An overwhelming proportion of neonatal deaths occur in developing countries. Resource-poor settings, high population density and hygiene conditions pose a great challenge for the neonatal healthcare system. Though the development of sick newborn care units (SNCUs) at rural hospitals in India has made a difference in infant survival rates, infection control still remains a concern. The options for the treatment of nosocomial infections are being severely compromised due to the increasing prevalence of carbapenem resistance in Gram-negative bacilli (GNB), limiting the choice of antibiotics to a few highly selected and costly ones.

Carbapenem resistance can occur due to either carbapenemase production or due to other mechanisms of resistance, such as alteration of outer membrane permeability along with extended-spectrum β-lactamase (ESBL) production or overexpression of AmpC type β-lactamases. Resistance due to carbapenemases can be because of either metallo-β-lactamases (MBLs) or non-MBLs. The production of carbapenemases is generally a more potent mechanism of carbapenem resistance compared with the other mechanisms. Recently, the emergence of a new MBL, designated New Delhi MBL 1 (NDM-1), has intensified the problem of drug resistance. Earlier studies have extensively assessed the prevalence of this gene in India. Most studies have reported sporadic cases of infection caused by NDM-1-producing isolates in adult patients. Moreover, NDM-1-producing isolates have been recently reported by the current authors in two neonates from a tertiary care hospital in India.
In November 2009, carbapenem-resistant GNB were isolated during a point prevalence survey carried out on a single day in the SNCU of a rural hospital in West Bengal, India. Shortly after the survey, a cluster of bloodstream infections with carbapenem-resistant *Escherichia coli* were detected in the same unit. The present study attempts to describe the transmission of the carbapenem resistance gene in GNB isolated from the environment of the SNCU and the body sites of the neonates during the survey. Further, bacteria isolated during the survey and shortly after the survey from the blood of the septicemic neonates were evaluated with respect to clonality, virulence factors, the blaNDM-1 gene, porins, integrons and transmissibility.

**Materials and methods**

**Setting and description of the point prevalence survey**

The point prevalence survey was carried out on a single day in a 20 bed level II SNCU in a rural hospital, West Bengal, India. The Department of Neonatology, IPGMER and SSKM Hospital, West Bengal, acts as a nodal centre for the rural SNCUs. In 2005, the nodal centre took the initiative to develop level II SNCUs in various districts. In March 2009, arrangements were made for blood cultures from the Birbhum SNCU to be performed at the nodal centre. In the event of a high prevalence of multidrug resistance, it was decided to conduct a point prevalence survey of the unit, its environment and the healthcare providers in November 2009. Shortly after this survey, four fresh neonates admitted to the SNCU developed septicemia.

**Collection of specimens**

During the survey, four body sites of the neonates were sampled: umbilicus; groin; mouth; and rectum (henceforth referred to as body site). In addition, hand-wash specimens from doctors and nursing staff in the unit, and environmental specimens (wash basin, washing area, water dripping from air conditioner, radiant warmer, medicine tray, stethoscope and suction apparatus) were collected. Disinfectants, intravenous (iv) cannulae, syringes, unused iv fluids (10% dextrose and normal saline) and coconut oil (a source of medium-chain triglyceride for pre-term neonates) were identified using ID 32 E or ID 32 GN kits (bioMérieux, Marcy l’Étoile, France).

**Laboratory procedures**

All GNB isolated from the environment of the SNCU, body sites and bloods of the neonates were identified, and antibiotic susceptibility profiles were evaluated. Detailed molecular characterization was carried out for the isolates possessing blaNDM-1.

**Identification of the strains**

Survey specimens and blood cultures were plated onto MacConkey agar or 5% sheep blood agar (BD Diagnostics, Franklin Lakes, USA). Isolates were identified using ID 32 E or ID 32 GN kits (bioMérieux, Marcy l’Étoile, France).

**Antimicrobial susceptibility testing and MIC**

Antibiotic susceptibility testing was performed as per CLSI criteria and the MIC value (mg/L) was determined using Etest (AB Biodisk, Solna, Sweden). MICs were also determined with 40 mg/L phenylalanine arginine β-naphthylamide (PABN) (Sigma–Aldrich, St Louis, MO, USA) to investigate the possible role of efflux pumps in imipenem resistance.

**PFGE, phylogenetic analysis and virulence genotyping**

The clonality of all *E. coli* isolates was determined by PFGE following PulseNet standardized procedures (http://www.cdc.gov/pulsenet/protocols.htm) and interpreted according to the criteria of Tenover et al. Further analysis of the genetic makeup of the *E. coli* isolates was done by evaluation of the phylogroup (A, B1, B2 and D) and virulence factors (hly, papC, sfa, iroN, cnf1, iucC and ibeA).

**Detection of β-lactamases phenotypes**

The production of ESBLs, carbapenemases and MBLs was evaluated using a cephalosporin/clavulanic acid combination disc test, modified Hodge test and imipenem–EDTA double-disc combination test, respectively. AmpC β-lactamase was also investigated according to the previous study.

**SDS–PAGE and immunodetection of OmpA and porins**

Whole-cell extracts of *E. coli* isolates were separated on 11% SDS–polyacrylamide gels and transferred to Immobilon-P filters (Millipore) following standard procedures. Porins and outer membrane proteins were detected using polyclonal anti-OmpC/E and polyclonal anti-OmpA antibody, separately.

**Molecular characterization of β-lactamases**

PCR was carried out for β-lactamase genes (blaPER, blaSHV-TEM,OXA-1,CTX-M, blaOXA-6, blaOXA-23,blaOXA-48,blaOXA-51,blaOXA-58, blaVIM, IMP, SPM-1,GIM-1, SIM-1,7, blaKPC,4, blaOXA-23, OXA-24, OXA-48, OXA-51, OXA-58 and ampicillin) and also for 16S rRNA methylase-encoding genes in all *E. coli* isolates. NDM-1 was detected using primers reported previously. Amplified products were further sequenced. The other carbapenem-resistant GNB isolated were screened for blaNDM-1 by PCR, and those found positive were then screened for other β-lactamases and 16S rRNA methylase-encoding genes.

**Detection of integrons, transfer of blaNDM-1 and plasmid analysis**

Plasmid analysis and detection of integrons were carried out for representative isolates possessing blaNDM-1 (Table 2). Conjugal transfer of blaNDM-1 to the sodium azide-resistant *E. coli* J53 recipient was attempted by a broth mating assay. Transconjugants were selected with 4 mg/L ceftoxime and 100 mg/L sodium azide. Plasmids of the transconjugants were analysed. The presence of blaNDM-1, blaOXYG alleles and integrons in the transconjugants was confirmed by PCR.

**Ethical clearance**

The study was approved by the Institutional Ethics Committee (IPGMER and SSKM Hospital); Ethical Clearance Memo No. 317 (dated 31 March 2008).
Results
Description of the isolates
The GNB isolated from the environmental specimens were Pseudomonas aeruginosa (two isolates), Klebsiella pneumoniae (three) and Acinetobacter junii (four). Cultures from the body sites of the neonates contained E. coli (16 isolates), Acinetobacter baumannii (7), Stenotrophomonas maltophilia (3), P. aeruginosa (2), Enterobacter cloacae (2) and K. pneumoniae (1). E. coli (n=16, EC 1–16) was the most common aerobic GNB found during the survey and was isolated from the body sites of 10 neonates (Table 1). No E. coli were isolated from environmental specimens. None of the neonates whose body sites yielded E. coli during the point prevalence survey later developed sepsis. Shortly after the survey, four fresh neonates developed septicaemia with E. coli (EC 17–20). These four neonates were admitted to the SNCU after the point prevalence survey was conducted and, hence, no specimens had been collected from their body sites.

Clinical presentation of the neonates
All the four neonates with septicaemia due to imipenem-resistant E. coli weighed >2000 g, all were delivered at term and the majority were extramural births (3/4). All four infected neonates had been hospitalized for ≥2 days before they developed signs of sepsis. None of these neonates was ventilated or had a central vascular catheter in place. Of the four neonates, three had perinatal risk factors (delayed cry, or foul-smelling or meconium-stained liquor) for sepsis.

Among the 10 neonates whose body sites yielded E. coli during the survey, 6 weighed <1500 g, 2 weighed 1500–2000 g and 2 weighed >2000 g. The majority of the neonates were pre-term and intramural births (Table 1).

Laboratory findings
Antimicrobial susceptibility testing, MIC determination and phenotypic detection of β-lactamases
All 20 E. coli isolates (16 body site and 4 blood) were found to be multidrug resistant (Table 2). Eighteen (14 body site and 4 blood) of the 20 isolates were imipenem resistant; only 2 body site isolates of E. coli were imipenem susceptible (Table 2). The MIC value of imipenem for imipenem-resistant isolates was ≥32 mg/L and PAβN had no effect on imipenem MICs.

The production of ESBL and AmpC was confirmed in all 20 E. coli isolates. Imipenem-resistant E. coli isolates (18) were found to produce MBLs.

Phylogenetic groups and virulence genes of the E. coli isolates
Assessment of the phylogroups and virulence patterns of the E. coli isolates (from body sites and blood) revealed that phylogroup A was the most frequent, accounting for 14 (imipenem-resistant body site isolates) of the 20 E. coli isolates, followed by group D with 6 isolates (4 imipenem-resistant blood isolates and 2 imipenem-susceptible body site isolates) (Table 2). Septicaemia was caused by E. coli of phylogroup D. Most virulence factors were not detected in the isolates, with the notable exception of papC, which was only detected in 5/20 isolates (Table 2). papC is a P fimbriae gene conferring mannose-resistant (MR) adherence to intestinal epithelial cells. MR adhesins are well-known virulence factors in urinary tract infection, septicaemia and meningitis.

Genetic diversity
PFGE analysis of the E. coli isolates revealed that the isolates belonged to two different clones (X, with subtypes X1 and X2, and Y1) (Figure 1 and Table 2). The patterns obtained with four blood isolates (EC 17–20) and one of the two imipenem-susceptible isolates (EC 3) were indistinguishable (PFGE pattern X1); these five isolates possessed the same virulence factor (papC). The other imipenem-susceptible isolate, EC 2, obtained from the same patient as EC 3, showed a closely related pattern to X1, designated as X2. The 14 imipenem-resistant body site isolates were different from the bloodstream isolates and their PFGE patterns were designated as Y1. The PFGE results correlated with the PCR phylogrouping (Table 2). All the E. coli body site isolates, except two (EC 2 and EC 3), were indistinguishable (pattern Y1), imipenem resistant and belonged to phylogroup A. However, all isolates that caused septicaemia had a pattern (X1) indistinguishable from that of EC 3 (X1) and closely related to EC 2 (X2), and belonged to phylogroup D.

Genetic context and DNA sequencing
Exploration for carbapenem resistance genes showed the presence of blaNDM-1 in the 18 imipenem-resistant E. coli isolates, irrespective of their clonality. The other β-lactamase genes identified were blaCTX-M group 1, blaOXA-1 and/or blaTEM along with blacIT genes. Sequence analysis of the isolates identified blCTX-M-15, blaTEM-1, blaCMY-59 and blaCMY-42 (Table 2). CMY-59 was identified in imipenem-resistant and imipenem-susceptible E. coli from blood and body sites, respectively. CMY-42 was identified in imipenem-resistant E. coli from body sites. The DNA sequence and deduced amino acid sequence of NDM-1 and CMY-59 from E. coli have been deposited in DDBJ/EMBL/GenBank under accession nos. AB571289 and AB587082, respectively. All NDM-1–harbouring E. coli isolates showed the presence of the rmtB gene. It was noted that the imipenem-susceptible E. coli from body sites and the imipenem-resistant E. coli from blood had identical resistance genes (Table 2), except for rmtB and blaNDM-1.

Western blot analysis of the outer membrane proteins
Porin (OmpC or OmpF) loss was observed in the imipenem-resistant E. coli isolates (EC 1, EC 4–16) from body sites. We believe the lost porin to be OmpF, which is generally lost or has reduced expression in most ESBL-producing E. coli. OmpF also runs above OmpC in the gel system used. Isolates retained normal levels of OmpA, a structural protein. All porins were detected in the imipenem-susceptible E. coli body site isolates (EC 2 and EC 3) and the imipenem-resistant E. coli blood isolates (EC 17–20) (Figure S1 (available as Supplementary data at JAC Online) and Table 2). Hence, imipenem resistance in the blood isolates was only due to the presence of blaNDM-1 and not porin loss.
Table 1. Demographic data and clinical features of the neonates (n=14) from whom *E. coli* were isolated either from body sites or blood

<table>
<thead>
<tr>
<th>Patient</th>
<th>Isolate no./site of isolation</th>
<th>Date of birth</th>
<th>Date of admission to SNCU</th>
<th>Date of sampling</th>
<th>Stay in SNCU (days) before sampling</th>
<th>Sex</th>
<th>Birth weight (g)</th>
<th>Gestational age (weeks)</th>
<th>Intramural/ extramural birth</th>
<th>Mode of delivery</th>
<th>Primary cause of admission to SNCU</th>
<th>Total duration (days) of hospital stay</th>
<th>Outcome</th>
<th>Date of outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>EC 1/rectum</td>
<td>25.09.09</td>
<td>26.09.09</td>
<td>04.11.09</td>
<td>38</td>
<td>M</td>
<td>1176</td>
<td>32</td>
<td>extramural birth</td>
<td>SVD</td>
<td>pre-term, vlbw</td>
<td>44</td>
<td>discharge</td>
<td>09.11.09</td>
</tr>
<tr>
<td>P2</td>
<td>EC 2/mouth, EC 3/rectum</td>
<td>25.09.09</td>
<td>26.09.09</td>
<td>04.11.09</td>
<td>38</td>
<td>M</td>
<td>1136</td>
<td>32</td>
<td>extramural birth</td>
<td>LUCS</td>
<td>pre-term, vlbw</td>
<td>44</td>
<td>discharge</td>
<td>09.11.09</td>
</tr>
<tr>
<td>P3</td>
<td>EC 4/rectum</td>
<td>25.09.09</td>
<td>26.09.09</td>
<td>04.11.09</td>
<td>38</td>
<td>M</td>
<td>1276</td>
<td>34</td>
<td>intramural birth</td>
<td>SVD</td>
<td>pre-term, vlbw</td>
<td>44</td>
<td>discharge</td>
<td>09.11.09</td>
</tr>
<tr>
<td>P4</td>
<td>EC 5/rectum</td>
<td>13.10.09</td>
<td>13.10.09</td>
<td>04.11.09</td>
<td>30</td>
<td>M</td>
<td>1250</td>
<td>28</td>
<td>intramural birth</td>
<td>SVD</td>
<td>pre-term, vlbw</td>
<td>38</td>
<td>discharge</td>
<td>20.11.09</td>
</tr>
<tr>
<td>P5</td>
<td>EC 6/groin, EC 7/rectum</td>
<td>13.10.09</td>
<td>01.11.09</td>
<td>04.11.09</td>
<td>3</td>
<td>M</td>
<td>2986</td>
<td>34</td>
<td>extramural birth</td>
<td>SVD</td>
<td>birth asphyxia</td>
<td>6</td>
<td>discharge</td>
<td>07.11.09</td>
</tr>
<tr>
<td>P6</td>
<td>EC 8/groin, EC 9/rectum, EC 10/mouth</td>
<td>21.10.09</td>
<td>28.10.09</td>
<td>04.11.09</td>
<td>7</td>
<td>M</td>
<td>1649</td>
<td>36</td>
<td>intramural birth</td>
<td>SVD</td>
<td>birth asphyxia</td>
<td>14</td>
<td>discharge</td>
<td>11.11.09</td>
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<tr>
<td>P7</td>
<td>EC 11/rectum</td>
<td>23.10.09</td>
<td>23.10.09</td>
<td>04.11.09</td>
<td>12</td>
<td>M</td>
<td>1250</td>
<td>30</td>
<td>intramural birth</td>
<td>SVD</td>
<td>pre-term, vlbw</td>
<td>36</td>
<td>discharge</td>
<td>28.11.09</td>
</tr>
<tr>
<td>P8</td>
<td>EC 12/groin, EC 13/rectum</td>
<td>25.10.09</td>
<td>25.10.09</td>
<td>04.11.09</td>
<td>10</td>
<td>M</td>
<td>3250</td>
<td>37</td>
<td>intramural birth</td>
<td>SVD</td>
<td>jaundice</td>
<td>13</td>
<td>discharge</td>
<td>07.11.09</td>
</tr>
<tr>
<td>P9</td>
<td>EC 14/groin, EC 15/mouth, EC 16/rectum</td>
<td>27.10.09</td>
<td>28.10.09</td>
<td>04.11.09</td>
<td>6</td>
<td>M</td>
<td>1946</td>
<td>38</td>
<td>intramural birth</td>
<td>SVD</td>
<td>jaundice</td>
<td>11</td>
<td>discharge</td>
<td>08.11.09</td>
</tr>
<tr>
<td>P10</td>
<td>EC 17/blood</td>
<td>29.10.09</td>
<td>01.11.09</td>
<td>04.11.09</td>
<td>3</td>
<td>M</td>
<td>1248</td>
<td>32</td>
<td>intramural birth</td>
<td>SVD</td>
<td>pre-term, vlbw</td>
<td>23</td>
<td>discharge</td>
<td>24.11.09</td>
</tr>
<tr>
<td>P11</td>
<td>EC 18/blood</td>
<td>14.11.09</td>
<td>14.11.09</td>
<td>16.11.09</td>
<td>2</td>
<td>M</td>
<td>2576</td>
<td>36</td>
<td>extramural birth</td>
<td>SVD</td>
<td>RDS</td>
<td>4</td>
<td>death</td>
<td>18.11.09</td>
</tr>
<tr>
<td>P12</td>
<td>EC 19/blood</td>
<td>15.11.09</td>
<td>15.11.09</td>
<td>19.11.09</td>
<td>4</td>
<td>M</td>
<td>2759</td>
<td>37</td>
<td>intramural birth</td>
<td>SVD</td>
<td>birth asphyxia</td>
<td>5</td>
<td>death</td>
<td>20.11.09</td>
</tr>
<tr>
<td>P13</td>
<td>EC 20/blood</td>
<td>16.11.09</td>
<td>17.11.09</td>
<td>19.11.09</td>
<td>2</td>
<td>M</td>
<td>2440</td>
<td>37</td>
<td>extramural birth</td>
<td>SVD</td>
<td>jaundice</td>
<td>4</td>
<td>death</td>
<td>21.11.09</td>
</tr>
<tr>
<td>P14</td>
<td>EC 21/blood</td>
<td>16.11.09</td>
<td>17.11.09</td>
<td>19.11.09</td>
<td>2</td>
<td>M</td>
<td>2104</td>
<td>37</td>
<td>extramural birth</td>
<td>SVD</td>
<td>birth asphyxia</td>
<td>4</td>
<td>death</td>
<td>21.11.09</td>
</tr>
</tbody>
</table>

M, male; SVD, spontaneous vaginal delivery; LUCS, low uterine Caesarean delivery; vlbw, very low birth weight (<1500 g); RDS, respiratory distress syndrome.

*Pre-term, gestational age <37 weeks.
### Table 2. Phylogenetic groups, virulence factors, PFGE patterns, antibiotic susceptibility, molecular characterization of β-lactamases and plasmid profiles of all E. coli and other imipenem-resistant isolates harbouring bla<sub>NDM-1</sub>

<table>
<thead>
<tr>
<th>Isolate no., site of isolation</th>
<th>Phylogroup</th>
<th>Presence of virulence genes</th>
<th>PFGE patterns</th>
<th>Antibiotic susceptibility</th>
<th>Presence of β-lactamase and 16S rRNA methylase genes</th>
<th>Calculated size of different megaplasmids (kb) and presence of integron</th>
<th>Calculated size of megaplasmid containing bla&lt;sub&gt;NDM-1&lt;/sub&gt; gene in transconjugant</th>
<th>Presence of porins (OmpC and OmpF) and outer membrane protein OmpA</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC 1 (E. coli), rectum</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 2 (E. coli), mouth</td>
<td>D</td>
<td>X2</td>
<td>type IV</td>
<td>bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-59&lt;/sub&gt;</td>
<td>ND</td>
<td>ND</td>
<td>OmpF, OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 3 (E. coli), rectum</td>
<td>D</td>
<td>papC</td>
<td>X1</td>
<td>type IV</td>
<td>bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-59&lt;/sub&gt;</td>
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<td>ND</td>
<td>OmpF, OmpC, OmpA</td>
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<tr>
<td>EC 4 (E. coli), rectum</td>
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<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 5 (E. coli), rectum</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 6 (E. coli), groin</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 7 (E. coli), rectum</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 8 (E. coli), groin</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 9 (E. coli), rectum</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 10 (E. coli), mouth</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
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<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
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<tr>
<td>EC 11 (E. coli), rectum</td>
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<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
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<tr>
<td>EC 12 (E. coli), groin</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
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<td>OmpC, OmpA</td>
<td></td>
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<td>EC 13 (E. coli), rectum</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 14 (E. coli), groin</td>
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<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
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<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 15 (E. coli), mouth</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 16 (E. coli), rectum</td>
<td>A</td>
<td>Y1</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
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<td>ND</td>
<td>OmpC, OmpA</td>
<td></td>
</tr>
<tr>
<td>EC 17 (E. coli), blood</td>
<td>D</td>
<td>papC</td>
<td>X1</td>
<td>type I</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-59&lt;/sub&gt;, rmtB</td>
<td>ND</td>
<td>ND</td>
<td>OmpF, OmpC, OmpA</td>
</tr>
<tr>
<td>EC 18 (E. coli), blood</td>
<td>D</td>
<td>papC</td>
<td>X1</td>
<td>type I</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-59&lt;/sub&gt;, rmtB</td>
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<td>83</td>
<td>OmpF, OmpC, OmpA</td>
</tr>
<tr>
<td>EC 19 (E. coli), blood</td>
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<td>papC</td>
<td>X1</td>
<td>type I</td>
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<td>83, 49, integron 1</td>
<td>83</td>
<td>OmpF, OmpC, OmpA</td>
</tr>
<tr>
<td>EC 20 (E. coli), blood</td>
<td>D</td>
<td>papC</td>
<td>X1</td>
<td>type I</td>
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<td>ND</td>
<td>ND</td>
<td>OmpF, OmpC, OmpA</td>
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<tr>
<td>AB 1 (A. baumannii), mouth</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>type III</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-23&lt;/sub&gt;, bla&lt;sub&gt;OXA-51&lt;/sub&gt;, armA</td>
<td>470, integron 1</td>
<td>not successful</td>
<td>ND</td>
</tr>
<tr>
<td>SM 1 (S. maltophilia), umbilicus</td>
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<td>NA</td>
<td>NA</td>
<td>type II</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-42&lt;/sub&gt;, rmtB</td>
<td>600, 83, integron 1</td>
<td>83</td>
<td>ND</td>
</tr>
<tr>
<td>KP 1 (K. pneumoniae), wash basin</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>type III</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, rmtC</td>
<td>385, 98, 54, 36, integron 1</td>
<td>not successful</td>
<td>ND</td>
</tr>
<tr>
<td>KP 2 (K. pneumoniae), wash basin</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>type III</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, bla&lt;sub&gt;TEM-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-1&lt;/sub&gt;, bla&lt;sub&gt;OXA-59&lt;/sub&gt;, rmtB</td>
<td>385, 98, 54, 36, integron 1</td>
<td>not successful</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined; NA, not applicable.

Antibiotics used were ampicillin, amikacin, gentamicin, ciprofloxacin, gatifloxacin, cefpodoxime, ceftriaxone, cefotaxime, ceftazidime, cefepime, imipenem, piperacillin, aztreonam, trimethoprim/sulphamethoxazole, piperacillin/tazobactam, netilmicin, ampicillin/subbactam, minocycline, doxycycline, tigecycline, tetracycline and colistin. Type I, isolates were resistant to all antibiotics, but susceptible to minocycline, colistin, tigecycline and doxycycline; type II, isolates were resistant to all antibiotics, but susceptible to minocycline, colistin, tigecycline, doxycycline and tetracycline; type III, isolates were resistant to all antibiotics, but susceptible to minocycline, colistin, tigecycline, doxycycline, and triparental; type IV, isolates were resistant to all antibiotics, but susceptible to minocycline, colistin, tigecycline, doxycycline, and triparental. 'a' indicates that none of the virulence genes was present.

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Detection of blaNDM-1 in imipenem-resistant GNB apart from E. coli

Imipenem-resistant GNB from the environment and body sites were screened for blaNDM-1 by PCR. Four isolates (one A. baumanii and one S. maltophilia from body sites, and two K. pneumoniae from two different wash basins) showed the presence of the blaNDM-1 gene. The DNA sequence and deduced amino acid sequence of NDM-1 from S. maltophilia have been deposited in DDBJ/EMBL/GenBank under accession no. AB614355. Other β-lactamase genes and 16S rRNA methylase genes were also present in these isolates (Table 2).

Detection of integrons, analysis and transfer of plasmid DNA

Integron 1 was detected in all isolates that possessed blaNDM-1. Megaplasmids of different sizes were also isolated from the GNB harbouring the blaNDM-1 gene (Table 2). Imipenem resistance was found to be transferable from E. coli (from body sites and blood) and S. maltophilia, but not from K. pneumoniae and A. baumanii. The blaNDM-1 and blaCMY genes, and integron 1 were detected in the transconjugants [Figure S2 (available as Supplementary data at JAC Online) and Table 2].

Discussion

This study describes the sequence of events that happened in the clinical setting of an SNCU, and makes an attempt to understand the dynamics between the neonates, the environment and the clinical setting of an SNCU, and makes an attempt to understand the interplay between the isolates. Imipenem-resistant isolates that caused septicaemia were clonally and phylogenetically similar to the imipenem-susceptible E. coli isolates (from body sites), and possessed the same virulence gene papC (average stay of 18 days), waiting to attain the weight criteria for discharge. There was no sharing of beds or any article between the sick neonates. Shortly after the survey, imipenem-resistant E. coli were isolated from the blood of four neonates who were admitted to the SNCU after the survey. Though these four neonates were admitted to the SNCU after the survey, it is evident from the dates of admission and discharge that there was an overlap in hospital stay between the neonates whose body sites yielded E. coli and those who developed septicaemia. These neonates were treated with cefotaxime and amikacin as the pre-emptive antimicrobial therapy for clinically suspected cases of septicaemia. However, this therapy could not prevent fatality in the neonates, as the E. coli isolates were later found to be imipenem resistant. After the cases of septicaemia with imipenem-resistant E. coli were detected, standard precautions were reinforced. Strict attention was paid to hand-washing with liquid soap and water. In addition, hand hygiene with chlorhexidine/alcohol was introduced after this outbreak.

Detailed analysis of the E. coli isolates (from body sites and blood) with respect to their genotypes, phylogeny, resistance to carbapenem and virulence factors gave us an insight into the interplay between the isolates. Imipenem-resistant isolates that caused septicaemia were clonally and phylogenetically similar to the imipenem-susceptible E. coli isolates (from body sites), and possessed the same virulence gene papC and an identical set of resistance genes, except for blaNDM-1. This indicated that the imipenem resistance must have been acquired by these imipenem-susceptible isolates at some point in time, probably during the period between the survey and when the neonates became infected with E. coli.

The factors responsible for carbapenem resistance were evaluated. Analysis of porins showed that the imipenem-resistant blood isolates had intact porin profiles, though β-lactamases were present. This indicated that carbapenem resistance was probably acquired. The imipenem-resistant E. coli from the body sites could be a probable source, but other imipenem-resistant GNB could not be disregarded. The carbapenem resistance gene found in all the imipenem-resistant E. coli was blaNDM-1. Since E. coli were not isolated from any environmental specimens, screening for the blaNDM-1 gene from the other imipenem-resistant GNB from the environment and body sites...
Transmission of \( \text{bla}_{\text{NDM-1}} \) in a sick newborn care unit

was carried out. This study yielded two isolates of \( K. \text{pneumoniae} \) from two different wash basins, and \( A. \text{baumannii} \) and \( S. \text{maltophilia} \) from the body sites as harbouring \( \text{bla}_{\text{NDM-1}} \). NDM-1-mediated resistance to carbapenems has been recently found in \( E. \text{coli} \), \( K. \text{pneumoniae} \), \( A. \text{baumannii} \) and \( S. \text{maltophilia} \) in many parts of India.\(^6\) In accordance with previous studies, the NDM-1 producers of this study also co-produced a 165 rRNA methylase (ArmA, RmtB or RmtC).\(^8\)\(^,\)\(^12\)

NDM-1-positive isolates in this study carried large plasmids (~80 to >500 kb), corresponding to the size range reported for \( \text{bla}_{\text{NDM-1}} \) plasmids by other authors.\(^6\) The possible transfer of \( \text{bla}_{\text{NDM-1}} \) was substantiated when integrons and the transfer of plasmids were analysed. Integron 1 was probably associated with the \( \text{bla}_{\text{NDM-1}} \) gene, as all imipenem-resistant GNB and their transconjugants harbouring \( \text{bla}_{\text{NDM-1}} \) possessed integron 1. In concordance with earlier studies, the transmissibility of a megaplasmid was confirmed in \( E. \text{coli} \) isolates and in \( S. \text{maltophilia} \).\(^13\) These results lead us to conclude that the promiscuous \( \text{bla}_{\text{NDM-1}} \) gene had disseminated in the SNCU via different GNB (\( E. \text{coli} \), \( A. \text{baumannii} \), \( S. \text{maltophilia} \) and/or \( K. \text{pneumoniae} \)) harbouring \( \text{bla}_{\text{NDM-1}} \). Subsequently, the gene was transferred to the imipenem-susceptible \( E. \text{coli} \) body site isolates, which later caused septicaemia (Figure S3, available as Supplementary data at JAC Online). Hence, the imipenem-resistant \( E. \text{coli} \) that caused septicaemia were found to be clonally identical to the imipenem-susceptible \( E. \text{coli} \) from body sites.

Earlier studies showed the presence of AmpC \( \beta \)-lactamases along with \( \text{bla}_{\text{NDM-1}} \).\(^6\)\(^–\)\(^9\) The present study reports for the first time the presence of \( \text{bla}_{\text{CMY-59}} \), which is a novel AmpC \( \beta \)-lactamase, along with \( \text{bla}_{\text{NDM-1}} \) in imipenem-resistant blood isolates. The deduced amino acid sequence of CMY-59 differed time the presence of \( \text{bla}_{\text{CMY-59}} \).

This study demonstrates the transmission of \( \text{bla}_{\text{NDM-1}} \) in a real-life situation. It is shown that colonization does not always lead to infection, but that the transfer of resistance to certain bacterial species can cause fatal infection. \( E. \text{coli} \) are a leading cause of septicaemia in neonates, both in developing and developed countries.\(^13\) The emergence of newer mechanisms of resistance in resource-poor facilities is worrisome. Early, cost-effective and adequate detection of such new mechanisms of resistance is crucial for infection control and prevention. The experience also highlights that even in resource-poor settings, point prevalence surveys can help to understand the emergence of newer antibiotic resistance mechanisms.

Acknowledgements
We extend our thanks to Dr Thammarai Schneiders for critically reviewing the manuscript prior to submission, Anne Marie Queenan and Yunsoo Chong for providing the control strains, George A. Jacoby for \( E. \text{coli} \) JS3 and Heinz Schwarz for the antibodies.

References


Supplementary data

Figures S1, S2 and S3 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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We extend our thanks to Dr Thammarai Schneiders for critically reviewing the manuscript prior to submission, Anne Marie Queenan and Yunsoo Chong for providing the control strains, George A. Jacoby for \( E. \text{coli} \) JS3 and Heinz Schwarz for the antibodies.

Funding

The study was partially supported by the Department of Science & Technology, Government of India. The Collaborative Research Centre of Okayama University for Infectious Diseases in India gave a fellowship to S. R., and R. V. was a recipient of the Women Scientist Scholarship Scheme for Societal Programmes (WOS-B), Department of Science & Technology, Government of India.

Transparency declarations

None to declare.


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