Prevalence of faecal carriage of NDM-1-producing bacteria among patients with diarrhoea in Bangladesh

The increasing incidence of infections caused by NDM-1-producing Enterobacteriaceae is a major concern to the international medical community, as such strains may display resistance to many, if not all, available antibiotics. Although there is much evidence to suggest that NDM-1-producing organisms are widespread in the population of the Indian subcontinent, reports on the emergence of these organisms have come from geographically diverse regions of the globe including Asia, Australia, the Far East, the USA, Canada, the Middle East and many countries in Europe. Whilst the majority of the reports have confirmed isolation of NDM-1-producing organisms from clinical specimens, faecal carriage of these organisms has rarely been reported. Recently, Johnson & Woodford (2013) published a review in this journal. Referring to our work and that of others, the authors mentioned that gut colonization was reported from Bangladesh and Pakistan (Islam et al., 2012; Perry et al., 2011): ‘In the Bangladesh study, screening of consecutive clinical samples over a 1-month period in late 2010 yielded 403 Gram-negative isolates, of which 14 (3.5 %) were positive for NDM-1.’ However, in our study we did not report gut colonization of NDM-1-producing Enterobacteriaceae. The 3.5 % prevalence of NDM-1-producing organisms was found among all Gram-negative organisms isolated from clinical specimens, including endotracheal tube specimens, sputum, throat, tracheal aspirate, catheter tips, urine, pus and wound specimens. We did not include stool samples in our study, and thus gut colonization was not investigated. In a subsequent study, we tested a large number of Escherichia coli and Shigella spp. isolated from stool samples during 2007–2009 to detect the NDM-1 positives, but none of the isolates was positive (Islam et al., 2013). In Pakistan, however, a high prevalence (18.3 %) of faecal carriage of NDM-1-producing organisms has been reported (Perry et al., 2011). A separate study reported that NDM-1-producing Enterobacteriaceae can be found in patients from all major provinces of Pakistan and that chromID CARBA (bioMérieux) is a useful tool for the isolation of carbapenem-resistant organisms (Day et al., 2013). As we were not aware of similar data from Bangladesh, we carried out a study to determine the prevalence of faecal carriage of NDM-1-producing organisms among hospitalized patients with diarrhoea and outpatients attending Dhaka hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) in Bangladesh. Between September and October 2012, we collected 100 consecutive stool samples. Of these samples, 30 were collected from outpatients presenting at the outpatient services of icddr,b and 70 from patients with diarrhoea attending Dhaka hospital (hospital surveillance samples). We inoculated stool samples directly onto chromID CARBA agar plates using a sterile inoculation loop. The inoculated plates were incubated at 37 °C for 18–24 h. We observed the plates for bacterial growth and colony characteristics. In the case of heavy growth including colonies having a typical colony morphology (pink to burgundy/ bluish-green to bluish-grey), we randomly picked five colonies and subcultured these on MacConkey agar supplemented with meropenem (0.5 mg l⁻¹). We extracted DNA from these colonies and performed a PCR specific for the NDM-1 gene (Islam et al., 2012). We identified the NDM-1-positive isolates using the API 20E system (bioMérieux).

A total of 34 samples out of 100 (eight outpatient and 26 hospital surveillance samples) showed growth on chromID CARBA agar plates. PCR screening showed nine samples (9 %) positive for the blaNDM-1 gene, of which six were from hospitalized patients and three were from outpatients. From these nine samples, we isolated 13 NDM-1-producing organisms of which six were identified as Escherichia coli, four as Klebsiella pneumoniae and one each as Pantoea spp., Acinetobacter baumannii and Enterobacter cloacae. All 13 isolates were resistant to multiple antibiotics, although some remained susceptible to minocycline (8/13, 62 %), amikacin (5/13, 38 %), gentamicin (3/13, 23 %), tobramycin (4/13, 31 %), ciprofloxacin (1/13, 8 %) and aztreonam (1/13, 8 %). All isolates were susceptible to tigecyclin and colistin. PCR for extended-spectrum β-lactamase genes showed that all 13 isolates were positive for blaTEM, 12 for blaCTXM-1, 11 for blaOXA-1, 47 and three for blaSHV. Only two isolates were positive for the quinolone resistance gene qnrB, and one isolate each was positive for rmtB, rmtC and armA encoding 16S rRNA methylase. None of the isolates was positive for the AmpC β-lactamase gene blaCMY-2 and class D β-lactamase gene blaOXA-48.

In one patient, we detected three NDM-1-producing organisms, namely Enterobacter cloacae, K. pneumoniae and A. baumannii. In two other patients, we isolated multiple NDM-1-producing Escherichia coli and K. pneumoniae isolates. Isolates within a species were different from each other by antibiotic resistance profiles, PFGE patterns and resistance gene profiles. One of the reasons for obtaining multiple NDM-1-producing isolates from the same patient might be intra-gut spread of the gene in the complex microbial flora. It has been described previously that dissemination of resistance involves not just the spread of the resistant organisms but also the inter-strain, inter-species or even inter-genus spread of the resistance genes (Johnson & Woodford, 2013). For NDM-1-producing organisms, this explains the presence of blaNDM-1 in a wide range of species and genera of Gram-negative bacteria, and in a diverse range of clones and strains within individual
species. Gene spread among bacteria can be mediated by a range of genetic mechanisms including transformation, transduction and conjugative plasmid transfer (Sykes, 2010). Observations to date implicate only plasmid transfer in the spread of blaNDM genes. Plasmid profile analysis of the 13 isolates showed that all the isolates contained plasmids of different sizes ranging from 140 MDa (~220 kb) to 1.4 MDa (~2.2 kb). The majority (n=11) contained more than one plasmid. However, in contrast to previous studies, none of the plasmids in isolates from the present study was found to be self-transmissible via conjugation (Islam et al., 2012, 2013).

In contrast to our previous study, *Escherichia coli* appeared to be the predominant species isolated from stool samples (Islam et al., 2012, 2013). This is not surprising as *Escherichia coli* is the main aerobic organism in the human intestinal tract. PFGE analysis of the six *Escherichia coli* isolates revealed three different patterns (Fig. 1, A, B and C). Three isolates belonged to pattern A. The strains were isolated from three different patients. Similarly, two isolates belonging to pattern B were isolated from two patients. PFGE patterns of three *K. pneumoniae* isolates also differed widely.

In conclusion, we report a prevalence of faecal carriage of NDM-1-producing organisms of 9% in patients from Bangladesh. This observation is rather alarming, as it occurs against a population density of up to 100 000 per square mile in Dhaka slums, a lack of clean drinking water, a lack of basic sanitary infrastructure, and an easy access to and over-the-counter sales and misuse of antimicrobial drugs that are produced locally and are therefore cheap and possibly of inferior quality. This is an ideal setting for the rapid spread of antimicrobial resistance in the population and the environment. Similar events have been described in India (Walsh et al., 2011). A prospective surveillance in the general population and the environment is thus necessary to determine the spread of resistance and risks for public health.

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<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Source</th>
<th>Organism sensitive to:</th>
<th>Resistance gene profile</th>
</tr>
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<tbody>
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<td>HS</td>
<td>CO, TGC, TM, AK</td>
<td>TEM, OXA-1, CTXM15</td>
</tr>
<tr>
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<td>TEM, OXA-1, CTXM15</td>
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Fig. 1. Dendrogram of PFGE fingerprints from NDM-1-producing *Escherichia coli* isolated from stool samples of patients with diarrhoea. The percentage of genetic identity between banding patterns is indicated. The source of the isolates, antibiotic sensitivity pattern and presence of antibiotic resistance genes are indicated on the right. HS, Hospital surveillance; OP, outpatient; CO, colistin; TM, tobramycin; TGC, tigecycline; GM, gentamicin; AK, amikacin; MC, minocycline.

**Table 1.** Strain ID, source, and resistance gene profile for NDM-1-producing *Escherichia coli* isolates from diarrhoea patients in Bangladesh.
