We report two cases of infantile diarrhea due to multidrug-resistant, NDM-1 metallo-β-lactamase-producing *Salmonella enterica* serovar Agona from Pakistan. This study alerts toward possible risk of NDM-1 transmission to enteric fever pathogens and encourages microbiologists to consider active screening of carbapenem resistance in nontyphoidal *Salmonella* isolates.

**CASE REPORT**

Case 1 is a 9-month-old child from Nosheroferoz, a small town in Sindh, Pakistan, who presented to Aga Khan Hospital, Karachi, with high-grade fever, vomiting, diarrhea, and abdominal pain. Stool microscopy revealed 10 leukocytes (WBC)/low-power field with mucous threads. The patient was admitted and started on 10 mg intravenous ciprofloxacin/kg of body weight twice a day. The patient became afebrile after 2 days of treatment and was discharged from the hospital on 10 mg oral ciprofloxacin/kg twice a day for 5 days. Stool culture yielded carbapenem-resistant *Salmonella* sp. (isolate 1a) that was susceptible only to azithromycin, fosfomycin, and colistin. On the follow-up visit, the child was still passing loose stools 5 or 6 times/day. A repeat stool culture yielded a *Salmonella* sp. isolate (isolate 1b) with the same susceptibility profile as the first. Treatment was changed to 250 mg oral fosfomycin three times daily, and the patient finally improved.

Case 2 is a 1-year-old child from Karachi who was admitted to the Aga Khan Hospital, Karachi, with history of diarrhea and vomiting for 2 days. Stool microscopy revealed >20 WBC/low-power field with mucous threads. The patient was admitted and started on 65 mg intravenous ceftriaxone/kg once a day. Diarrhea subsided within 3 days of admission, and the patient was discharged on 10 mg oral ciprofloxacin/kg twice a day for 5 days. Stool culture grew a carbapenem-resistant *Salmonella* sp. (isolate 2) susceptible only to azithromycin, fosfomycin, and colistin. This patient had no further follow-up at our hospital.

Gastroenteritis caused by nontyphoidal *Salmonella* species (NTS) is a major public health problem worldwide. Children less than 2 years old are the main sufferers of enteritis caused by NTS (1). The majority of these infections are self-limiting; therefore, antimicrobial therapy is reserved for treatment of serious infections. Some patients may develop prolonged enteritis, and septicaemia and extraintestinal complications are more commonly seen in immunocompromised and malnourished populations, with an associated case fatality of 20 to 25% (2).

Diarrheal diseases are very common in Pakistan. Laboratory-based surveillance data from our laboratory showed a 13% isolation rate of enteric bacterial pathogens, including *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., and *Vibrio cholerae*, from clinically suspected cases of diarrhea (3). The frequency of isolation of *Salmonella* spp. among stool pathogens is 18.4%, and it is the third most commonly isolated enteric pathogen in our setting (4). Reported rates of antimicrobial resistance in NTS vary geographically. Resistance also varies between different serotypes and different antibiotics. Generally, low rates of resistance to ampicillin, chloramphenicol, and ciprofloxacin have been reported in *Salmonella enterica* serovar Enteritidis. In contrast, a higher rate of resistance has been reported in *S. enterica* serovar Typhimurium (5).

With the emergence globally of ceftriaxone and quinolone resistance in NTS, empirical therapy for severe gastroenteritis and invasive infections has become challenging. Moreover, since the emergence of the NDM metallo-β-lactamase enzyme in enteric organisms, there has been a continuous threat of its spread into NTS. With the emergence of the NDM-1-producing *Salmonella* sp. isolate (isolate 1b) from Pakistan, there is an increasing concern for the spread of NDM-1 metallo-β-lactamase producing *Salmonella* isolates to other regions, especially in Europe and India (6).

In 2011 and 2012, two studies from the United States and Reunion Island reported colonization with NDM-1-positive *S. enterica* serovars Senftenberg and Westhampton, respectively; however, the clinical significance of the reported isolates was questionable (9). Only one clinically significant infection has been reported, an NDM-1-producing *S. enterica* serovar Stanley from China (10). Our report is the first report of clinically significant NDM-1-producing *S. enterica* from the Indian subcontinent and the first report of this enzyme in *S. enterica* serovar Agona.

Bacterial culture, identification, susceptibility testing, and preliminary serotyping of all three isolates were performed at the clinical laboratory of the Aga Khan University Hospital, while

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TABLE 1 MIC of Salmonella enterica serovar Agona isolates 1a and 1b (case 1) and isolate 2 (case 2), against various antibiotics determined by the Vitek 2 Compact system.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml)</th>
<th>Isolate 1a</th>
<th>Isolate 1b</th>
<th>Isolate 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>320</td>
<td>320</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

All these isolates were tested for carbapenemase production by using a modified Hodge test (14) and were also positive for blaNDM PCR, using primers 5’-GGG CAG TCG TCT CCA ACG GT-3’ and 5’-GTA GTG CTC AGT GTC GGC AT-3’ (15). Further confirmation for the presence of an NDM carbapenemase gene and screening for extended-spectrum-beta-lactamase (ESBL) genes were performed using the commercial Check-MDR CT102 ESBL-carbapenemase microarray (Check-Points Health BV, Wageningen, The Netherlands), which detected NDM carbapenemase, a group 1 CTX-M ESBL, and a non-ESBL TEM gene. Gene sequencing identified the NDM variant as NDM-1 in all three isolates. So far, NDM-1 has also been reported in other diarrheal agents, like Shigella spp. and Vibrio cholerae, from the Indian subcontinent, from the environmental water samples with potential contamination from the sewage system (16). However, no NDM-1-positive Shigella spp. and Vibrio cholerae have been reported from Pakistani. Similarly, this gene has not yet been identified in typhoidal salmonellae. As the NDM-1 gene is already spreading in community Enterobacteriaceae isolates, like Escherichia coli and Klebsiella pneumoniae (4), there is real potential for this gene to spread into enteric fever isolates.

When analyzed by pulsed-field gel electrophoresis, the three isolates shared identical profiles, as shown in Fig. 1. Moreover, S. enterica serovar Agona isolates of these two cases had similar antibiograms, suggesting they originated from a common source. However, because of the lack of laboratory-based data and active surveillance of diarrheal diseases in the community, whether these two isolates were part of a larger outbreak cannot be established.

This study alerts toward possible risk of NDM-1 transmission to enteric fever pathogens and emphasizes microbiologists to consider active screening of NTS isolates. Any carbapenem-resistant isolates should be further investigated for the presence of NDM enzymes and other carbapenemase genes. In addition, clinicians are alerted to the emergence of these isolates, especially when treating relapsing infections with Salmonella spp.

In conclusion, there is need for active surveillance and screen-
ing of resistant Salmonella spp. in the region to avoid emergence of NDM-1-positive typhoidal salmonella strains.

REFERENCES


