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CASE REPORT

Clinical Isolates of *Salmonella enterica* Serovar Agona Producing NDM-1 Metallo-β-Lactamase: First Report from Pakistan

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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 and 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 only. Some patients may develop prolonged enteritis, and septicemia 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 *S. enterica* (6). Two cases of diarrhea caused by NDM-positive *S. enterica* serovar Agona are presented in this report. A literature search revealed few reports of carbapenem-resistant NTS; however, the genes identified were either bla_{KPC}, bla_{IMP}, or bla_{VIM} (7, 8). 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

Antibiotic	MIC (μg/ml)		
	Isolate 1a	Isolate 1b	Isolate 2
Ampicillin	≥32	≥32	≥32
Amoxicillin-clavulanic acid	≥32	≥32	≥32
Piperacillin-tazobactam	≥128	≥128	≥128
Ceftriaxone	≥64	≥64	≥64
Trimethoprim-sulfamethoxazole	≥320	≥320	≥320
Gentamicin	≥16	≥16	≥16
Ciprofloxacin	2	2	2
Imipenem	≥8	≥8	≥16
Meropenem	≥16	≥16	≥16
Colistin	≤0.5	≤0.5	≤0.5
Azithromycin	4	8	8

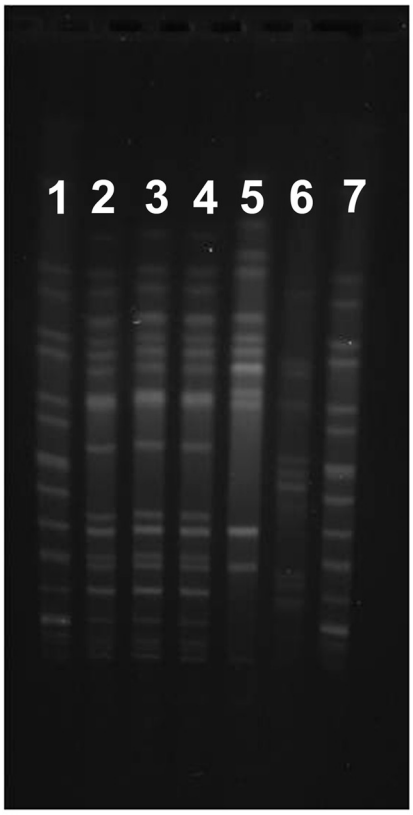
advance serotyping, PCR, sequencing, and pulsed-field gel electrophoresis (PFGE) were performed at Public Health England's *Salmonella* Reference Section, United Kingdom. Samples were processed by direct plating on MacConkey's and xylose-lysine-deoxycholate agar and inoculating selenite F broth as an enrichment medium, which was subcultured the next day on *Salmonella* and *Shigella* agar. Suspected colonies were identified by conventional biochemical methods as *Salmonella* spp. and confirmed by API 20E (bioMérieux, France). Initial serotyping of the isolates revealed that all three isolates belonged to *Salmonella* group B. Further serological identification was performed at Public Health England's *Salmonella* Reference Section, United Kingdom, and isolates were identified as *S. enterica* serovar Agona (4,12:f,g,s:−) by using the Kauffmann-White scheme (11). Antimicrobial susceptibilities were performed by the disk diffusion method and Vitek 2 Compact system (bioMérieux, France) and interpreted according to Clinical and Laboratory Standards Institute guidelines (12), shown in Table 1. For azithromycin susceptibility, British Society of Antimicrobial and Chemotherapy breakpoints (13) for *S. enterica* serovar Typhi were used. The multidrug resistance pattern of these isolates is alarming, as the treatment options are extremely limited. Both isolates were tested for other treatment options, like azithromycin, fosfomycin, and colistin. Both children received treatment for gastroenteritis; however, as the majority of diarrheal infections are self-limiting, treatment success could not be evaluated.

All these isolates were tested for carbapenemase production by using a modified Hodge test (14) and were also positive for bla_{NDM} PCR, using primers 5′-GGG CAG TCG CTT 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 Pakistan. 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-



Lanes 1 and 7 are the control *Salmonella enterica* serovar Braenderup
Lane 2. *Salmonella enterica* serovar Agona (isolate1a)
Lane 3. *Salmonella enterica* serovar Agona (isolate1b)
Lane 4. *Salmonella enterica* serovar Agona (isolate 2)
Lane 5. *Salmonella enterica* serovar Agona type strain
Lane 6. NDM+ve *Salmonella enterica* serovar Senftenberg

FIG 1 PFGE gel showing similarity of all three *Salmonella enterica* serovar Agona isolates. To digest the DNA, restriction enzyme XbaI (New England BioLabs) was used, and the following PFGE conditions were applied: 2 to 64 s at 200 V for 22 h (17).

ing of resistant *Salmonella* spp. in the region to avoid emergence of NDM-1-positive typhoidal salmonella strains.

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