Prevalence of Multidrug Resistant Salmonellae with Increasing Frequency of *Salmonella enterica* Serovars Kentucky and Virchow among Hospitalized Diarrheal Cases in and around Delhi, India

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SUMMARY: Non-typhoidal salmonellae (NTS) are a major cause of acute diarrhea with characteristic multidrug resistance (MDR). In a hospital-based study, 81 NTS were isolated and tested for serotypes and antimicrobial resistance (AMR). *Salmonella enterica* isolates were classified into 7 different typable serovars, however, 19 (23%) isolates remained untypable. The most common serovars were *S.* Kentucky (48%), and *S.* Virchow (22%). Most of the NTS isolates displayed resistance to nalidixic acid (NA) (73%), ciprofloxacin (CIP) (48%), ampicillin (AM) and norfloxacin (NOR) (36% each), and gentamicin (CN) (31%). The AMR profiles for CN and NA; and AM, CIP, NA and NOR, were found to be high in *S.* Virchow (83%) and *S.* Kentucky (43%), respectively. Analysis of the pulsed-field gel electrophoresis patterns of *S.* Kentucky revealed 3 clusters. *S.* Kentucky has clones closely related to become prominent in recent years in Delhi. The AMR appears to be consistent with the change in MDR patterns during 2014-2017. The observed prevalence of *S.* Kentucky and *S.* Virchow in large numbers of diarrheal cases is novel. The NTS are mostly resistant to fluoroquinolones, which is the current drug of choice for treating diarrheal cases. MDR is very common among clonally related *S.* Kentucky.

INTRODUCTION

Diarrheal disease is still a major problem in low-income countries, with high morbidity and mortality rates. In 2015 alone, the estimated diarrheal death among children under 5-years-of-age was 499,000. More than 90,000 deaths were due to *Salmonella enterica* infection, which was the third leading cause of infection-related death after rotavirus (199,000) and shigellae (164,000) (1). *Salmonella is* one of the most common causes of bacterial gastroenteritis and is a major public health problem.

Over 2,700 *Salmonella* serovars have been identified. All can cause disease in humans (2). Serovars causing human infections other than Typhi, Paratyphi A, and Paratyphi B are collectively known as non-typhoidal salmonellae (NTS). The average global yearly incidence of NTS is approximately 153 million cases of gastroenteritis, with 57,000 deaths (3). This large prevalence of NTS is due to the poor water quality and sanitation in developing countries, and also the involvement of foodborne infections, as observed in industrialized countries. *S.* Enteritidis and *S.* Typhimurium are the most frequently isolated human pathogenic *Salmonella* serovars. However, the emergence or replacement of several serovars has been frequently reported, as their endemicity is dynamic (4).

Although the gastro-enteric salmonellae commonly cause self-limiting diarrhea, they might cause systemic infections requiring antimicrobial treatment. Since invasive NTS are intracellular pathogens, fluoroquinolones are recommended for their treatment due to their ability to penetrate epithelial cells and inhibit bacterial DNA synthesis (5). Most salmonellae are susceptible to many antimicrobials. However, the emergence of multidrug resistance (MDR) has been widely reported (6,7). Understanding the antimicrobial susceptibility pattern of salmonellae is important in determining an effective drug for the management of diarrhea.

This study investigated the prevalence, serotype distribution, and antimicrobial susceptibility of *Salmonella* spp. among diarrheal patients admitted to Maharishi Valmiki Infectious Diseases Hospital (MVIDH), Delhi, India, from 2011 to 2017.

MATERIALS AND METHODS

Collection of stool specimens: Fecal specimens were collected in McCartney bottles or rectal swabs in Cary Blair medium (Difco, Sparks, MD, USA) before antibiotic treatment. Diarrhea was defined as more than 3 episodes of loose or liquid stools within 24 hours, accompanied by other associated clinical symptoms,
isolates were evaluated using cluster analysis with the Martens-Latem, Belgium). The similarities between

Numerics software version 4.0 (Applied Maths, Sint-

strain H9812 in each gel, and were analyzed using Bio-

chloramphenicol (C; 30 μg), ceftriaxone (CRO; 30 μg), imipenem (IMP; 10 μg), gentamicin (CN; 10 μg), norfloxacin (NOR; 10 μg), ciprofloxacin (CIP; 5 μg), nalidixic acid (NA; 30 μg), and trimethoprim/sulfamethoxazole (SXT; 1.25 μg/23.75 μg). Escherichia coli ATCC 25922 was used as the control.

Pulsed-field gel electrophoresis (PFGE): PFGE analysis of Xbal-digested genomic DNA of S. Kentucky as the representative was performed using a CHEF-Mapper (Bio-Rad Laboratories, Hercules, CA, USA) following the PulseNet standardized protocol for subtyping (10). PFGE images captured using the Gel Doc XR system (Bio-Rad) were normalized by aligning the peaks of the Xbal size standard of S. Braenderup strain H9812 in each gel, and were analyzed using Bio-

unweighted-pair group method with arithmetic mean (UPGMA) method and the Dice correlation coefficient with a position tolerance of 1.5%.

Ethical considerations: The MVIDH Ethics Committee approved this study (No. 1120/MVIDH). Each participant, or parent in the case of children, gave their written informed consent.

RESULTS

From 2011 to 2017, 8,757 patients were admitted to MVIDH for treatment of diarrhea. Stool specimens collected from these patients were screened for the presence of major bacterial pathogens in this region, i.e., V. cholerae, Salmonella spp., and Shigella spp. by conventional culture methods. During this study period, Salmonella spp. was identified in 81 (1.0%) stool specimens (Table 1). In 2014, 30 (2.7%) cases were positive for Salmonella spp. These were sporadic diarrheal cases that were not associated with any cluster or local outbreak. Patients ≥5-years-of-age were markedly more numerous compared to patients ≤5-years-of-age (66% vs 34%, Table 2). Most of the patients infected with Salmonella spp. were ≥5-years-of-age compared to those ≤5-years-of-age (67% vs 33%, Table 3).

S. enterica isolates were serologically classified into 7 different typable serovars and 19 (23%) isolates remained untypable (Table 1). Of the total of 81 isolates, the most common serovars were Kentucky (48%) followed by Virchow (22%) (Table 1). S. Virchow was
MDR Salmonella with Emerging Serovars

Table 3. Age group distribution of S. Kentucky, S. Virchow and other serovars

<table>
<thead>
<tr>
<th>Age</th>
<th>Salmonella serovars (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. Kentucky</td>
<td>S. Virchow</td>
</tr>
<tr>
<td>≤ 5 years</td>
<td>14 (36)</td>
<td>6 (33)</td>
</tr>
<tr>
<td>≥ 5 years</td>
<td>25 (64)</td>
<td>12 (67)</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>18</td>
</tr>
</tbody>
</table>

more prevalent among diarrheal cases during 2014, whereas S. Kentucky was isolated throughout the study period with maximal isolation during 2012 and 2013 (Table 1). Both serovars were predominantly isolated from patients ≥ 5-years-old than from patients ≤ 5-years-old (> 60% vs > 30%, Table 3). Co-infection of NTS and V. cholerae O1 was evident in 16 patients; the majority of these (~60%) patients were ≥ 5-years-old (data not shown).

With a few exceptions, most of the Salmonella spp. displayed resistance to NA (73%), CIP (48%), AM and NOR (36% each), and CN (31%). S. Kentucky was mostly resistant to CIP (95%), NA (90%), AM (72%), and NOR (72%), whereas, S. Virchow displayed resistant to NA (100%), CN (83%), and CIP (61%). Overall, 25 antibiotic resistance profiles were recorded (Table 4), indicating a large variation in the resistant patterns. The CN-NA, AM-CIP, and NA-NOR AMR

Table 4. Antimicrobial resistance (AMR) profiles of Salmonella spp.

<table>
<thead>
<tr>
<th>AMR profile</th>
<th>Salmonella enterica serovar (nos)</th>
<th>Salmonella enterica untypable serogroup (nos)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kentucky (39)</td>
<td>Virchow (18)</td>
</tr>
<tr>
<td>Sensitive</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AM C NA NOR</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CTX CN NA</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>CN NA</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>CIP</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CIP CTX CN NA</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>CIP CTX CRO NA</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: AM, ampicillin; C, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; CN, gentamicin; CTX, cefotaxime; NA, nalidixic acid; NOR, norfloxacin.

Underlined drugs exhibited reduced susceptibility (intermediate).
profiles were very frequent in S. Virchow (83%) and S. Kentucky (43%) (Table 4).

Clonal relatedness of 24 randomly selected S. Kentucky isolates, covering all the years of isolation, was investigated using PFGE. Analysis of the PFGE patterns of the isolates revealed three clusters (A to C) with > 80% similarity coefficient (Fig. 1). Cluster A contained 3 AM-susceptible S. Kentucky isolates that had 90-96% similarity with the identical AMR profile (CIP-NA-NOR). Thirteen isolates in Cluster B had a similarity coefficient of 94%. In this cluster, 7 out of 13 isolates were resistant or displayed reduced susceptibility to CTX. One isolate (33183) was susceptible to all the tested antibiotics. Cluster C was represented by 8 isolates with an overall similarity coefficient of 93%. Six of these S. Kentucky isolates were resistant to gentamicin.

**DISCUSSION**

The prevalence of salmonellae among diarrheal cases was low (~1%) in this study. Although most of the NTS in this study were isolated from patients ≥ 5-years-of-age, a considerable number of patients were ≤ 5-years-of-age. A similar trend has been reported in other Asian countries (11-14).

In other parts of India, S. Worthington, S. Typhimurium, and S. Senftenberg are the major NTS serovars associated with human infections (15-17). S. Weltevreden reportedly caused several foodborne outbreaks in several states of India (18-22). The present frequent occurrence of S. Virchow and S. Kentucky serovars from diarrheal cases in India is a novel finding. The first reported isolation of S. Kentucky in India was in 1969 (23). The increasing prevalence of the S. Kentucky and S. Virchow serovars in Ethiopia was reported from 2012 to 2014 (24). Diarrheal outbreaks associated with S. Virchow rank among the top five serovars in several countries (25-31). The World Health Organization’s Global Foodborne Infections Network has also identified S. Virchow among the top 20 serovars in South-East Asia, Europe, and Oceania (32). In the United States, a multistate outbreak was linked to S. Virchow infections (33). This serovar has also been reported from clinical and food sources in India (34-36).

We found that majority of the NTS isolates were MDR; this trend has been recently evident in Delhi and neighboring regions. NTS isolated in India and other countries are generally susceptible to many antimicrobials (12,13,37). Except for S. Kentucky, the other NTS isolates from diarrheal patients from 2011 to 2014 in Manipal, south of India, were susceptible to most of the tested antimicrobials (38). S. Kentucky encountered in this study was highly resistant to many of antimicrobials and the resistance profiles increased in four years from 2014 to 2017. Globally, the emergence of fluoroquinolones resistance in S. Kentucky has been reported since 2002, and the resistance exceeded 80% by 2011 (39).

Most of the MDR S. Virchow isolates showed high rates of resistance to NA acid and reduced susceptibility to CTX and fluoroquinolone. Similar observations were reported in Europe and Asia (28,40,41). Globally, CIP resistance in S. Virchow is often increased with treatment failures (42). Moreover, increase in CTX-resistant S. Virchow was reported in South Korea in 2011 (43). Several studies have described the overall increase in CN resistance among NTS (44-46). Generally, resistance to CN is high in salmonellae isolates from food animal sources (47).

PFGE revealed an overall genetic similarity of S. Kentucky isolates that exceeded 80%. A previous study that analyzed a global collection of S. Kentucky isolates also revealed a close clonal resemblance (48). The genetic relatedness of the isolates could be correlated with the AMR profiles with the appearance of CTX and CN resistance during 2012 and 2014, respectively.

In conclusion, the present data clarify serovar prevalence and antibiotic resistance among NTS from humans with salmonellosis in and around Delhi, India. The identification of the S. Kentucky serovar is novel in India. A noteworthy observation is the clonal lineages in S. Kentucky that infected all patients; these lineages have an increased ability to acquire new antibiotic resistance.

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**Conflict of interest** None to declare.

**REFERENCES**


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