The detection and antimicrobial susceptibility profile of Shigella isolates in and around Hyderabad, Telangana

Sanem Soma Sekhar Goud, SV Raghavendra, M Shylaja and A Jagadeesh Babu

Abstract
Shigellosis is an important food borne pathogen of worldwide economic significance. Water is the most common vehicle for this infection in humans. It has been isolated from wide variety of foods such as beef, chicken, egg, milk, fish, vegetables and fruits. The object of this study was to determine Shigella antibiotic resistance pattern against the antibiotics such as ampicillin, erythromycin, ofloxacin, ciprofloxacin, azithromycin, chloramphenicol, tetracycline, co-trimaxazole, ceftriaxone and gentamicin. In this cross-sectional study, a total of 17 Shigella strains were collected from different specimens (farm water, milk, chicken and fish). The isolated strains showed resistance to ampicillin (82.3%), tetracycline (70.6%), erythromycin (47.1%), ofloxacin (47.1%), ciprofloxacin (41.2%), azithromycin (41.2%), chloramphenicol (35.3%), co-trimaxazole (17.7%), ceftriaxone (11.8%) and gentamicin (11.8%).

Keywords: Antimicrobial susceptibility, Shigella, worldwide economic significance

Introduction
Enteric bacteria like Escherichia coli, Shigella spp, Salmonella spp, etc. [42] infect GIT of humans and animals. Shigella belongs to the family Enterobacteriaceae. It is a small, unencapsulated, non-motile, Gram negative, non spore forming, facultative anaerobic bacilli [19]. There are four species of Shigella, classified on the basis of biochemical and serological differences: S.dysenteriae (Group A), S. flexineri (Group B), S.boydii (Group C) and S. sonnei (Group D) [50]. Transmission of Shigella is by the faeco-oral route, and may be via food or water and/ through person to person contact [51]. In some regions, disease rates increase somewhat during the monsoon season, perhaps due to increased faecal contamination of drinking water [24].

Shigella is one of the most important food borne pathogen causing diarrheal disease in both developing and developed countries [51]. Epidemiology repors show that about 140 million people suffer from shigellosis with estimated 600,000 deaths per year worldwide [17]. In United States, an estimated 4,50,000 people are infected each year, majority in children of 1-5 years age group [47]. Shigella are the most common cause of bacillary dysentery, this term has become synonymous with all clinical presentations of shigellosis, although these presentations range from asymptomatic carriage to mild, watery diarrhea to overt dysentery [30], also including an acute rectocolitis associated with nausea, fever, anorexia, dehydration, mucopurulent and bloody diarrhoea, tenesmus [16].

Shigellosis is difficult to be prevented because only a small number of bacteria are required to cause infection and it has exhibited steady trends towards multiple drug resistance, is most probably by acquisition of extra-chromosomal DNA called he R’ plasmids or transposons, cellular mutation and efflux, alternation of the target cell structure such that the antibiotics no longer affects it, prevention of antibiotics from reaching its target cell structure and antibiotics inactivation by microbial enzyme [45, 29].

Keeping in view of the public health significance of Shigella, this study was designed to isolate and identify Shigella from farm water, milk, chicken and fish and also to study the antimicrobial resistance profiles of Shigella.

Materials and methods
Collection of specimens
A total of 160 samples, of which 40 were farm water samples from local animal farms, 40 were milk samples from local vendors, 40 were chicken samples and 40 were fish samples from local markets, were collected for bacteriological study.
All the samples from selected critical control points that were considered to be associated with contamination were aseptically collected using separate sterile plastic bags for chicken and fish samples, and sterile test tubes for farm water and milk samples.

**Isolation and identification of Shigella sps.**
25 gms of chicken and fish samples were homogenized in 225 ml of 0.1% buffered peptone water by shaking for 5 min in a sterile stomacher bag and incubating at 37°C for 24 hrs for resuscitation. Buffered peptone water was also used for enrichment of milk and farm water samples and incubated at 37°C for 24 hrs. A loopful of the enriched samples was directly inoculated onto MacConkey agar and Salmonella-Shigella agar and was incubated at 37°C for 24 - 48 hrs following previously described protocols [14].

Presumptive Shigella colonies were identified using a series of biochemical tests including triple sugar iron agar (TSI), urea, motility, indole production, methyl red (MR), Voges-Proskauer (VP), citrate utilization and oxidase test.

**The antimicrobial susceptibility test**
The antimicrobial susceptibility test of the isolates was performed according to Bauer and his colleagues method on Mueller-Hinton agar [28]. The antibiotics tested include ampicillin (10µg), tetracycline (30 µg), gentamicin (10 µg), cotrimoxazole (trimethoprim- sulfamethoxazole) (25 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), ciprofloxacin (05 µg), erythromycin (15 µg), ofloxacin (05 µg), azithromycin (15 µg) and ceftriaxone (30 µg). All discs were purchased from Hi-Media laboratories, Mumbai. Disc readings were performed 24 hrs after incubation and the diameter of inhibition halos was measured with the aid of a ruler. The interpretation was made as per the zone size interpretation chart provided by manufacturer of discs.

**Results and Discussion**

**The prevalence of Shigella isolates**
From the total samples analysed, Shigella was isolated from 17 (10.63%) samples. Six farm water samples (15.0%), three milk samples (7.5%), three chicken samples (7.5%) and five fish samples (12.5%) were positive for Shigella spp by culture method (Table.1 & Fig. 1)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Samples</th>
<th>No. collected</th>
<th>Positive by Culture method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Farm water</td>
<td>40</td>
<td>6 (15.0%)</td>
</tr>
<tr>
<td>2</td>
<td>Milk</td>
<td>40</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>3</td>
<td>Chicken</td>
<td>40</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>4</td>
<td>Fish</td>
<td>40</td>
<td>5 (12.5%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>160</td>
<td>17 (10.63%)</td>
</tr>
</tbody>
</table>

Table 1: Source and Number of samples collected, and number of samples positive to Shigella spp.

The antimicrobial susceptibility profile of the isolates revealed a higher rate resistance against ampicillin (82.3%), followed by erythromycin and ofloxacin (47.1%), ciprofloxacin (41.2%), azithromycin and chloramphenicol (35.3%), tetracycline and co-trimoxazole (17.7%) and ceftriaxone and gentamicin (11.8%), as shown in Table:2 & fig.2.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Antibiotic</th>
<th>No. of isolates</th>
<th>Sensitivity</th>
<th>Intermediate sensitivity</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ampicillin</td>
<td>17</td>
<td>2 (11.8%)</td>
<td>1 (5.9%)</td>
<td>14 (82.3%)</td>
</tr>
<tr>
<td>2</td>
<td>Tetracycline</td>
<td>17</td>
<td>3 (17.6%)</td>
<td>2 (11.8%)</td>
<td>12 (70.6%)</td>
</tr>
<tr>
<td>3</td>
<td>Gentamicin</td>
<td>17</td>
<td>14 (82.3%)</td>
<td>1 (5.9%)</td>
<td>2 (11.8%)</td>
</tr>
<tr>
<td>4</td>
<td>Co-trimoxazole</td>
<td>17</td>
<td>14 (82.3%)</td>
<td>0 (0.00%)</td>
<td>3 (17.7%)</td>
</tr>
<tr>
<td>5</td>
<td>Chloramphenicol</td>
<td>17</td>
<td>4 (23.5%)</td>
<td>7 (41.2%)</td>
<td>6 (35.3%)</td>
</tr>
<tr>
<td>6</td>
<td>Ciprofloxacin</td>
<td>17</td>
<td>5 (29.4%)</td>
<td>5 (29.4%)</td>
<td>7 (41.2%)</td>
</tr>
<tr>
<td>7</td>
<td>Erythromycin</td>
<td>17</td>
<td>3 (17.6%)</td>
<td>6 (35.3%)</td>
<td>8 (47.1%)</td>
</tr>
<tr>
<td>8</td>
<td>Ofloxacin</td>
<td>17</td>
<td>5 (29.4%)</td>
<td>4 (23.5%)</td>
<td>8 (47.1%)</td>
</tr>
<tr>
<td>9</td>
<td>Azithromycin</td>
<td>17</td>
<td>8 (47.0%)</td>
<td>2 (11.8%)</td>
<td>7 (41.2%)</td>
</tr>
<tr>
<td>10</td>
<td>Ceftriaxone</td>
<td>17</td>
<td>12 (70.5%)</td>
<td>3 (17.7%)</td>
<td>2 (11.8%)</td>
</tr>
</tbody>
</table>

Table 2: Antimicrobial sensitivity/ Intermediate/ Resistance patterns of Shigella isolates from different sources
The antimicrobial resistance patterns of *Shigella* isolates identified during the current study were random among the samples. The *Shigella* isolates cultivated in this study were highly resistant to ampicillin (82.3%), which was similar to the results reported by Naik [37]. Higher resistance (100%, 93%, 90.6%, 90%, 89.15% and 86.8%) than the present study was reported by Reda et al [43], Roma et al [44], Garedew et al [21], Assefa et al [39], Bhattacharya et al [10], and Bhattacharya et al [11] respectively to the ampicillin, whereas lower resistance of 79.9%, 78.9%, 78.7%, 72%, 70%, 65.6%, 63.8%, 56% and 53% to ampicillin than the present study was reported by Yismaw et al [54], Tiruneh [50], Asrat [5], Ali et al [2], Mache et al [31], Mardaneh et al [35], Jomezadeh et al [27], Mandomando et al [34] and Brooks et al [12]. Gedebou et al [20], who observed very low resistance (21%) of *Shigella* isolates to ampicillin. After ampicillin, the highest rate of resistance in our study was against tetracycline (70.6%), which was similar to the results (70.6%) recorded by Reda et al [43]. The results are comparable with Ali et al [5], who observed 100% resistance to tetracycline by *Shigella* isolates. Asrat [5] observed 97.3% of resistance, Brooks et al [12] reported 97% of resistance, Assefa et al [45], who reported 91.2% of resistance, Tiruneh [50] and Roma et al [44] also observed antimicrobial susceptibility of *Shigella* and reported that 90% isolates were resistance to tetracycline. Yismaw et al [54] observed 86% of resistance. A little lesser resistance to tetracycline than in the present investigation was observed by Mandomando et al [34] and Mardaneh et al [35], who reported 66% and 65.6% respectively and in contrast to the results obtained in this study, Gedebou et al [20] and Garedew et al [19], reported 42% and 9.4% of resistance among the *Shigella* isolates to tetracycline respectively.

*Shigella* isolated in this study was 47.1% resistance to erythromycin and ofloxacin. Temu et al [48], who observed 19% of resistance for *Shigella flexneri* whereas 14% for *Shigella dysenteriae* in stool samples of diarrheic patients to erythromycin. Higher resistance (80%) to ofloxacin than the present study was observed by Bhattacharya et al [10]. In the present study, 41.2% resistance was observed for ciprofloxacin and higher resistance of 82% than the present study was reported by Bhattacharya et al [10], whereas Mandomando et al [34] and Mardaneh et al [35], who reported zero per cent (0.0%) resistance to ciprofloxacin. The resistance to azithromycin was 41.2% and sensitivity was 47.1%. The resistance to azithromycin in the present study was lower than the resistance of 50%, was reported by Bhattacharya et al [10]. Higher sensitivity by *Shigella* isolates for this antibiotic was observed by Hoge et al [23], who observed 100% of sensitivity. Basualdo and Arbo [7], who reported clinical success rate of 98% was observed for azithromycin.

The *Shigella* isolates in this study exhibited 35.3% resistance to chloramphenicol. Almost similar to the present investigation, Jomezadeh et al [27] reported 33.3% resistance to chloramphenicol. Higher resistance by *Shigella* isolates for this antibiotic was observed by Brooks et al [12], who observed 77% of resistance. Asrat [5] reported 74.7% of resistance, Naik [37] observed 72.9% of resistance, Tiruneh et al [50] observed 67.8% of resistance and Roma et al [44] reported 63% of resistance to chloramphenicol by *Shigella* isolates, whereas Reda et al [43] and Gedebou et al [20] analysed the antibiotic resistance pattern of *Shigella* and reported that only 29.4% and 25% of isolates were resistant to chloramphenicol respectively.
The resistance to co-trimoxazole by Shigella isolates was 17.7% in the present study. Very high resistance to co-trimoxazole was observed by Mardaneh et al [35] who reported 92.2% of resistance, Mandomando et al [34] who found 84% of resistance and Jomezadeh et al [27] reported 80.5% of resistance, when compared to the results obtained in the present investigation. On contrary, Garedew et al [19] who reported 100% sensitivity to the co-trimazoxale by the Shigella isolates. Shigella isolates in the present study have shown 11.8% of resistance to ceftriaxone. Similar to the present investigation Bhattacharya et al [10] reported 12% of resistance to ceftriaxone. Garedew et al [19] observed 18.8% of resistance, which is higher than the present study, on contrary Mardaneh et al [35] and Mandomando et al [34] reported zero (0.0%) per cent of resistance by the Shigella isolates towards ceftriaxone. In the present study 11.8% of resistance was observed for gentamicin by the Shigella isolates. Higher resistance to gentamicin than in the present investigation was observed by Jomezadeh et al [27], who observed 36% of resistance and Bhattacharya et al [10] reported 26% of resistance among the Shigella isolates, whereas Tiruneh [9] found 12.2% of resistance which is almost similar to the present study. Comparatively lower resistance to gentamicin than in the present study was observed by Yismaw et al [34] who reported 7.9% resistance, Roma et al [34] and Assefa et al [6] found two per cent (2%) of resistance and Brooks et al [12] observed one per cent (1%) of resistance among the Shigella isolates to gentamicin, whereas zero (0%) per cent resistance to gentamicin was observed by Garedew et al [19], Reda et al. [43] and Asrat [8]. Although it is extremely difficult to explain these conflicting data with regards to both time and place of study, the variation is probably due to differential clonal expression and drug pressure in community.

Bibliography


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