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

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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, millk, 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* sps, *Salmonella* sps, 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 [15]. 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) [30]. 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 diarrhoea 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 37^0C for 24 hrs for resuscitation. Buffered peptone water was also used for enrichment of milk and farm water samples and incubated at 37^0C for 24 hrs. A loopful of the enriched samples was directly inoculated onto MacConkey agar and *Salmonella-Shigella* agar and was incubated at 37^0C 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- silfamethoxazole) (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* sps by culture method (Table.1 & Fig. 1)

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

S. No	Samples	No. collected	Positive by Culture method
1	Farm water	40	6 (15.0%)
2	Milk	40	3 (7.5%)
3	Chicken	40	3 (7.5%)
4	Fish	40	5 (12.5%)
TOTAL		160	17 (10.63%)

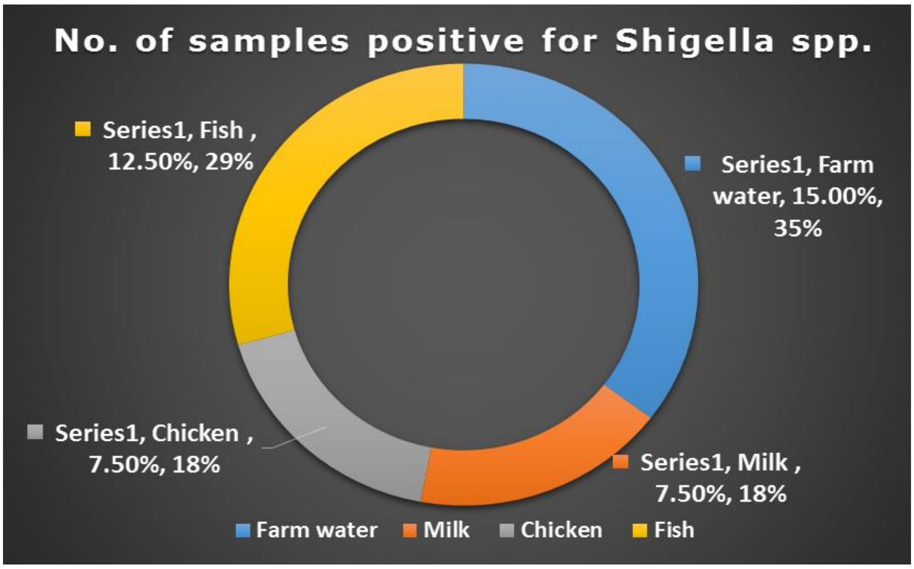

Fig 1: Samples positive for *Shigella* spp.

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-trimaxazole (17.7%) and ceftriaxone and gentamicin (11.8%), as shown in Table:2 & fig.2.

Table.2: Antimicrobial sensitivity/ Intermediate/ Resistance patterns of *Shigella* isolates from different sources

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

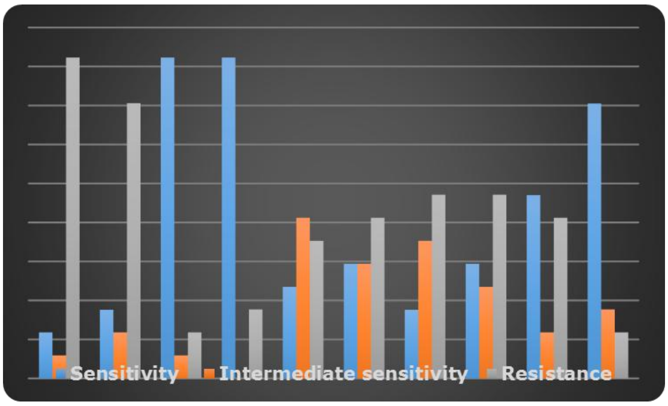

Fig 2: Antimicrobial sensitivity/ Intermediate/ Resistance patterns of *Shigella* isolates from different sources

Discussion

Among enteric pathogens, *Shigella* should be emphasized because of its prevalence and the severity of the associated disease, accounting for 140 million cases globally per year and 60,000 deaths annually of which 60% occur in children below 5 years of age. The geographical distribution, frequency of occurrence and the pathogenicity of the four *Shigella* spp. are different by country and also different among populations within a country [21, 17]. Many studies have demonstrated the prevalence of *Shigella* in different parts of the world. In our present study, the incidence of shigellosis was 10.63%, which is higher than the findings of Mamuye *et al*. [32], Ashenafi [3], Mulatu *et al*. [35], Reda *et al* [43], Jesudason [25], Nath *et al*[36], Opintan and Newman [39], Tesfaye *et al* [46], Beyene and Tasew [8] who reported 9.1%, 9.0%, 7.0%, 6.7%, 5.4%, 5.03%, 5.0%, 4.57%, 2.3% respectively. No *Shigella* spp. was isolated from stool samples by Aklilu *et al* [1], whereas higher incidence rates (12.1%, 11.7% and 11.0%) were reported by Bhattacharya *et al* [10], Asrat *et al* [4]., and Gunasekaran *et al* [22].

Makintubee *et al*. [32] reported 62% of incidence in the livestock farm water, which is higher incidence than the present investigation (15.0%) from farm water samples. In the present investigation, 40 milk samples were screened for *Shigella* spp. and three (7.5%) could be identified as *Shigella*. The higher incidence of *Shigella* (20%) in the raw milk than the present study was reported by Yagoub *et al*, [52] and Tadesse and Dabassa [46], whereas Jeyakumar and Lawrence [26] reported 14% of S.flexneri from raw milk samples. Out of 40 chicken samples, three (7.5%) were positive for *Shigella* spp. in the present study whereas higher incidence (80%) was reported by Bhatia and Patak [9]. Cardoso *et al* [13] reported 5.5% and 14.3% from refrigerated chicken and freshly dressed chicken respectively. The incidence of *Shigella* in the fish samples in the present investigation was 12.5%. Higher incidence (44%, 39.7%) than the present findings was recorded from fish samples by Floyd and Jones [18], Onyango *et al* [40] respectively whereas low incidence (2.2%) reported by Yagoub [53].

Changes in the antibiotic resistance patterns among *Shigella* isolates, pose major difficulties in selecting an appropriate antibiotic for the treatment of shigellosis (Niyogi *et al*., [39]). Indiscriminate usage of antibiotics in the treatment and as growth promoters are considered the most important issues that promotes the emergence, selection and spreading of antibiotic resistant microorganisms in both veterinary and human medicine.

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* [19], Assefa *et al* [6], 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* [2], 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* [6], who recorded 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 flexeneri* whereas 14% for *Shigella* dyssenteriae 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% oof 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-trimaxazole by *Shigella* isolates was 17.7% in the present study. Very high resistance to co-trimaxazole 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-trimaxazole 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 [50] 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* [54] who reported 7.9% resistance, Roma *et al* [44] 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 [5].

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.

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