doi.org/10.36721/PJPS.2022.35.1.REG.041-048.1

Prevalence of antibiotic resistance pattern in *shigella* isolates procured from pediatric patients at Faisalabad - Pakistan

Sami Ullah Khan[1]*, Rizwan Aslam[1], Muhammad Ashraf[1], Sultan Ali[1], Muhammad Saqib[2], Muhammad Arshad Khattak[3], Umer Sadique Khattak[4], Haq Amanullah[4], Hastari Wuryastuty[5], R. Wasito[6], Aris Haryanto[7], Farman Ullah[8], Menggen Ma[9] and Sardar Ali[9]

[1]Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan
[2]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan
[3]Department of Mathematics and Statistics, University of Agriculture, Faisalabad, Pakistan
[4]College of Veterinary Sciences, Faculty of Animal Husbandry & Veterinary Sciences (FAHVS) The University of Agriculture, Peshawar, Pakistan
[5]Department of Veterinary Internal Medicine, Faculty of Veterinary Medicine, Universitas Gadjah Mada,Yogyakarta, Indonesia
[6]Department of Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada Jl.Yogyakarta, Indonesia
[7]Department of Biochemistry and Molecular Biologi, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[8]Faculty of Veterinary and Animal Sciences, Department of Animal Breeding and Genetics, National Center for Livestock Breeding Genetics and Genomics LUAWMS, Uthal, Pakistan
[9]Department of Applied Microbiology, College of Resources, Sichuan Agricultural University, Sichuan, PR China

Abstract: Shigella infection (shigellosis) is an intestinal disease caused by a *shigella* isolates belongs to a family Enterobacteriacea. Watery diarrhea, abdominal pain and tenesmus are the prominent symptoms of shigella infection. The present study was designed to determine period prevalence and antimicrobial susceptibility of *Shigella* species recovered from stool specimens obtained from diarrheal paediatric patients under 5 years of age. This cross-sectional study was carried out for a period of six months (Jan to June, 2016). All *Shigella* isolates were identified based on colony morphology, microscopic characteristics, and biochemical characteristics. After applying Kirby Baur disc diffusion method only 22 (18.96%) stool specimens were found positive for *Shigella* isolates *among the 116 stool specimens*. The isolates were also found susceptible to Levofloxacin (72.72%), Azithromycin (59.09%), and Cefotaxime (40.90%). However, the said isolates were resistant to Lincomycin (100%) and Penicillin-G (100%), followed by Amoxicillin (95.45%) and Oxacillin (95.45%). The chi-square test was used to check the close association among antimicrobial agents used and as highly significant (p-value < 2.2e-16). Based on antimicrobial susceptibility findings, Levofloxacin, Azithromycin and Cefotoxime were found effective for the control of shigellosis.

Keywords: Shigella bacteria, shigellosis, diarrhea, pediatrics, period prevalence, antibiotic resistance, antibiotic susceptibility

INTRODUCTION

Bacillary dysentery or Shigellosis is triggered by several species of *Shigella*, which are classified into four sero-groups, *S. boydii, S. sonnei, S. flexneri, S. dysenteriae.* The *S. dysenteriae* type-1, *S. flexneri*-2a and *S. sonnei* are the three major strains that are accountable for Shigellosis (Anand *et al*., 1986).

The *S. sonnei* is responsible for the commencement of outbreaks, which are detected in industrialized countries, while *S. dysenteriae* type-1 is a single pandemic and epidemic strain and *S. flexneri*-2a in the developing countries (Mathan and Mathan, 1986). Shiga toxins are cytotoxic as well as neurotoxic in nature that is responsible for watery diarrhea. The factor which is accountable for invasion of different tissues is lipopolysaccharide cell wall antigen. In rare cases, *Shigella* enters and travels in blood stream but in most of the cases they divide and stay within the epithelial cell lining of colon.

A Japanese Scientist, Dr. Kiyoshi Shiga worked for first time and discovered bacteria named as *Shigella* in 1896 as causative agent of dysentery in primates and human beings (Niyogi, 2005). Pathogenicity of *S. flexneri* was recognized in 1900 by Dr. Simon Flexner, while Dr. Carl Olaf Sonne identified the disease emergence potential of *S.sonnei* in 1915 (Cheesbrough, 2006). Mark Frederick Boyd, an American Bacteriologist and Epidemiologist, identified and recovered *S. boydii* during 1931 in India. Based on antigenic characteristics, 46 *Shigella* serotypes have been identified. Based on 'O' antigens, serotyping of *Shigella* was established (Tao *et al.*, 2004).

Clinically, the disease begins within 24 to 48 hours of ingestion of the food and water containing *Shigella*. Shigellosis is characterized by frequent small volume

**Corresponding author:* e-mail: marwatsuk@gmail.com

passage of stools that consist largely of blood, mucus and pus, accompanied by fever and stomach cramps. Blood, mucus and pus cells in the stools are the signs of colorectal inflammation (Tao *et al*., 2004; Niyogi, 2005). Apart from bloody stools, patients with dysentery often have rectal pain, fatigue, malaise and anorexia.

Different *Shigella* species causing dysentery, becomes fatal, if not treated during initial phase of infection. Biochemical and serological differences are used as a hallmark to classify *Shigella* into different species. The lipopolysaccharide in the cell wall of *Shigella* species is consisting of 'O' antigen provided the basis for identification of four serogroups of *Shigella* (Niyogi, 2005).

All species of *Shigella* are capable of attacking and disturbing the immune system of human being of almost every age group but usually the children's less than 5 years of age are highly susceptible and sensitive, because of moderately established immunological status, lack of proper personal hygiene and no evidence of previous contact to *Shigella* in life. Biochemical tests, antibiotic disk diffusion technique and Slide agglutination test followed by PCR assays, conducted to isolate *Shigella* spp., measurement of antibiotic susceptibility testing and identify *ipaH* gene; respectively. Peak antibiotic resistance percentage of 82% and 77% was noted against ampicillin and trimethoprim-sulfamethoxazole. *Shigella* spp. were found highly susceptible to ciprofloxacin and ceftriaxone (Sheikh *et al*., 2019).

Prospective studies were conducted to determine antibiotic resistance and frequency of recovered isolates of *Shigella*, and overall, 4.8% prevalence of *Shigella* species was recorded in Karachi, Pakistan (Zafar *et al*., 2009). The percent prevalence of the *Shigella* species was as follows; *S. flexneri* (62%), followed by *S. sonnei* (18%), *S. boydii* (11%) and *S. dysenteriae* (9%.). The magnitudes of isolates of *S. dysenteriae* resistance towards Co-trimoxazole and Ampicillin were 81% and 68%, respectively (Zafar *et al*., 2009).

The most predominant reasons of diarrheal diseases are *Shigella* isolates that become a prominent communal health overload throughout the world. According to latest study conducted in Shanxi province of China in which they collected a total of 474 *Shigella* isolates and investigated the antimicrobial profiles. Besides they illuminated the molecular structures of *Shigella* species expressing multidrug resistance (MDR) to two major groups of antibiotics including cephalosporins and fluoroquinolones in diarrheal patients. The mutual localization of antibiotic resistant genes of *Shigella* species to several antibiotics that make the overall treatment and prevention of bacillary dysentery much more complicated (Wang *et al*., 2019).

Broad analysis of prevalence and antibiotic resistance profiles of *Shigella* serotypes were carried out in Xinjiang province, China. The complete Screening of 458 *Shigella* isolates was conducted, among which the major prevalent specie is *Shigella flexneri.* Later *on the* rare serotypes including 1c, 2c and 4s and predominant serotypes 2a, 1b, 2b, and Xv of *Shigella* were identified. Three extended spectrum β- lactamase (ESBL) genes (*bla* CTX-M, *bla* OXA and *bla* TEM) as well as integrons carrying antibiotic resistant trait transmitted horizontally, eventually resulting in emergence of *Shigella* subtypes that are resistant to cephalosporin (Liu *et al*., 2018).

The existence of bacterial pathogens in gastro-intestinal tract (G.I.T.) including *Shigella* in tap water and there accessibility towards drinking water due to availability of toilets near wells or sewage pipes are broken, is the usual way of dissemination of these microbes in pediatric population especially in developing countries A total of 69% of all incidents and 61% of all deaths assigned to shigellosis involved in children under five years of age. Shigellosis is often associated with significant morbidity and mortality, especially among children. During the period of 1969 to 1973, the *S. dysenteriae* type-1 was in epidemic form and caused a total of 20,000 deaths and 500,000 shigellosis cases in Central America (WHO, 2005). Due to lack of cleaning practices particularly in day care centers, military barracks and primary schools in the developing countries, also facilitate the emergence of food borne illness especially Shigellosis. Flies is an important biological vector involved in the transmission of food borne illness in hygiene deprived surroundings and adulteration of food products with contaminated tape water (Ugbogu *et al*., 2006).

A recent epidemiological report was published which described that around 164.7 million children and young adults suffered worldwide from shigellosis annually, out of these 163.2 cases were observed in developing countries and 1.5 million shigellosis episodes in industrialized countries (Kotloff *et al*., 1999).

Comprehensive molecular study about determination of antibiotic resistance due to *Shigella* species, was conducted for the first time at Faisalabad region, Pakistan and found the highest resistance towards Ampicillin (96.84%), Tetracycline (93.68%), Streptomycin (77.89%), and Chloramphenicol (72.63%) (Tariq *et al*., 2012). Therefore, the current study was designed to investigate the overall prevalence and antimicrobial resistance of *S. dysenteriae* associated with dysentery cases in rural areas of Faisalabad, Pakistan.

MATERIALS AND METHODS

Study design and site

The cross-sectional study was designed and carried out during January to June, 2016 at the Chemotherapeutics laboratory, Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan, with following criteria.

Inclusion criteria

An inclusion criterion was formulated to include patients in current study, which was as follows:

- Children (1-5 years of age) belonging to district Faisalabad, Pakistan.
- Children with complaints of diarrhea / dysentery.
- Children who have not already taken antibiotic therapy.

Exclusion criteria

- Children above 5 years of age due to difficulty in sample collection.
- Children having persistent diarrhea.
- Children, who have already taken antibiotic therapy.

Sample collection

The stool samples were collected from 116 children (under 5 years) who attended emergency or Pediatrics patients with complaints of diarrhea / dysentery at various health care units i.e., Civil Hospital and Allied Hospital, Faisalabad, Pakistan. Cases were selected by purposive random method. From each diarrheal child patient, hygienic measures were maintained which involved collection of stool specimens in hygienic plastic stool collection tubes, followed by keeping in phosphate buffered saline (Sigma Aldrich) and immediately transported to Chemotherapeutics laboratory, Institute of Microbiology, University of Agriculture Faisalabad, Pakistan under refrigerated conditions.

Isolation and identification of isolates

Three different selective agar mediums including, MacConkey (Oxoid Ltd. Basinstoke, Hampshire, England), Salmonella-Shigella (SS - Oxoid Ltd. Basinstoke, Hampshire, England), and Xylene lysine deoxycholate (XLD - Remil Ltd., Lenexa, USA) were used for the isolation of *Shigella*. Each specimen was inoculated on MacConkey agar (low selective medium), Salmonella Shigella agar (high selective medium), and XLD (high selective medium) agar media, respectively upon arrival to the laboratory. The inoculated Petri plates were incubated at 37°C for 18 to 24 hours. The suspected colonies including colorless to slight pale colonies, colorless transparent colonies, and small reddish translucent colorless, respectively produced on the MacConkey agar, SS agar and the XLD agar, were subjected to be studied by Gram's staining method. The further identification of these colonies was carried out by using different biochemical tests i.e., triple sugar iron, sucrose fermentation, glucose fermentation, lactose fermentation, mannitol fermentation, indole production, methyl red, Vogues Prausker's, urease, nitrate reduction, citrate utilization and motility tests (Awan and Rahman, 2002).

Antimicrobial sensitivity test

Isolated strains of the *S. dysenteriae* were further analyzed for the antimicrobial resistance against following nine antibiotics including Azithromycin, Amoxicillin, Tetracycline, Ciprofloxacin, Penicillin-G, Levofloxacin, Cefotaxime, Oxacillin, and Lincomycin. For antibiotic resistance evaluation, Kirby-Bauer disk diffusion method was used according to the guidelines of Clinical Laboratory Standard Institute (Wayne, 2012). Briefly, 3 to 4 pure colonies of the *S. dysenteriae* were mixed in 300 microliter of sterile Mueller Hinton broth, turbidity was adjusted to 0.5 McFarland standards. Then culture suspension was spread over Mueller Hinton agar (Oxoid Ltd. Basinstoke, Hampshire, England). By using forceps antibiotic disks were placed over MH plates and inoculated plates were incubated at 37^{o}C for 18 to 24 hours. Similarly, the forceps was used to pick up blank paper discs (control) to be fixed over Muller Hinton agar plates. After the incubation, zone of inhibition was measured from the center of antibiotic disc, while the blank paper disc has not shown the zone of inhibition (Baur *et al*., 1966). The diameter of complete inhibition zones (including the diameter of the disk) was measured with Vernier Caliper after incubation and recorded in millimeters.

Ethical approval

Ethical endorsement of this research was offered by ORIC University of Agriculture (UAF), Faisalabad – Pakistan.

STATISTICAL ANALYSIS

Chi-Square test for independence

The degree of association among variables (table 2) was evaluated via Chi-square test.

Testing of hypothesis

H_0: $\mu 1 = \mu 2 = \mu 3 \ldots . \mu 8 = 0$ etc. All the antibiotics against *S. dysentriae* have same effect at all levels.

H_1: $\mu 1 \neq \mu 2 \neq \mu 3 \ldots . . \mu 8 \neq$ All the antibiotics against *S. dysentriae* have different effect at all levels (claim).

Level of significance

$\alpha = 5\%$ ($\alpha = 0.05$, Confidence Interval =0.95)

Test Statistic

$$X^2 = \sum \frac{(\text{Observed Value} - \text{Expected Value})^2}{\text{Expected Value}} \quad \text{Or}$$

$$X^2 = \sum \frac{(O - E)^2}{}$$

E

Chi-squared test for given probabilities
data: c (susceptible, intermediate, resistance)
X-squared = 887.18, degree of freedom (d.f.) = 23, p-value < 2.2e-16

Decision Rule
Reject H_0 if p-value < 0.05

Result
We reject H_0 for p-value = 2 X 10^{-16} which is highly significant and accept the claim H_1 alternative hypothesis that all antibiotics are significantly different.

All the recorded data were analyzed using latest statistical software M-Language to display recorded information about antimicrobial susceptibility with the help of different graphs (fig. 1). The Chi-Square independence test was used to check association between two variables that is there any association between variables including different antibiotics (table 2) and their effect on *Shigella* species, (susceptible, intermediate, and resistant). In this test we were interested to check the effects (3 levels) for all the eight antibiotics against the bacteria. Test for independence is displayed in the result section.

RESULTS

The cross-sectional study was conducted for six months (Jan to June, 2016), and a total of 116 stool samples were collected from Emergency and Pediatric patients of two hospitals i.e., District Head Quarter (DHQ) Hospital, and Allied Hospital, Faisalabad, Pakistan, and transported immediately to Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan. Samples were swab cultured on MacConkey agar and subsequently streaked over Salmonella-Shigella (SS) and XLD agars. All the cultured strains were analyzed based on their colony characteristics and later on their microscopic characteristics. After 24 hours of incubation at 37°C, out of 116 samples, the 22 isolates were corresponding to gram negative rods and produced colorless to slight pale colonies on the MacConkey agar, colorless transparent colonies on the SS agar and small reddish translucent colonies on XLD agar. Microscopic and colony characteristics initially identified the *Shigella* species. These isolates were further confirmed for *Shigella* species based on the biochemical characteristics during later phases of the study.

Biochemical characterization of S. dysenteriae
Non lactose fermenting, transparent and colorless, 2 to 3 mm convex and round colonies were appeared on the MacConkey and Salmonella-Shigella agars, and were analyzed for various biochemical characteristics including various tests i.e., indole, Voges-Proskauer, Nitrate reduction and methyl red tests which produced positive reaction, while the citrate, triple sugar iron and urease tests showed negative reaction.

After initial screening via biochemical profiling, carbohydrate fermentation tests were isolates of *S. dysenteriae* undergoes delayed fermentation of glucose, lactose and sucrose with only acid production but no gas, while majority of them were mannitol fermenters. The XLD plates were inoculated and streaked via non lactose fermenting colonies recovered from MacConkey agar plates. Small red-pink and round colonies of 1 to 2 mm in diameter were appeared on XLD plates. These biochemical reactions accompanied by sugar fermentation tests and colony characteristics confirmed that these isolates correspond to *S. dysenteriae*. In this study, the *Shigella* species as pathogen were not identified to the species level using biochemical tests and hence biochemical tests should be substantiated by serological and molecular identification for better taxonomy of the pathogens to species and strain level.

Overall period prevalence of S. dysenteriae
Among 116 stool specimens collected from the pediatric patients, 22 samples were found positive. Overall, the percent prevalence of *S. dysenteriae* isolated from diarrheal patients was 18.96% (table 1). The collected samples were divided into two sub-groups based on the age of patients i.e., 0 to 2 years and 2 to 5 years of age. The percent prevalence's of both groups were 22.5% and 11.12%, respectively. In the new born infants group, the high prevalence was might be due to low immunity.

Antibiotic sensitivity analysis
Kirby Baur disc diffusion method was used to determine the antimicrobial resistance and sensitivity all the isolates. A total of nine antimicrobial agents including azithromycin, amoxicillin, tetracycline, ciprofloxacin, penicillin-g, levofloxacin, cefotaxime, oxacillin and lincomycin were used. After spreading suspension of *Shigella* species via L-shaped glass spreader over Muller Hinton agar plates, Lawn culture of *Shigella* species were obtained. Finally the antibiotic coated discs were applied and fixed on Muller Hinton agar plates (150 mm) followed by overnight incubation period. The zone of inhibition (ZOI) was measured in millimeter (fig. 2).

Antibiotic resistance and susceptibility were formulated by comparing the zone of inhibition with the standard values described by the clinical laboratory standard institute (CLSI). A brief comparison of resistance and susceptibility percentage is presented in table 2.

Table 1: Prevalence and overall prevalence of *S. dysenteriae* in two different age groups

Age groups	Samples (#)	*S. dysenteriae* Isolates (#)	Prevalence (%)	Overall Prevalence (%)
0-2 years	80	18	22.5	18.97%
>2-5 years	36	4	11.12	

Table 2: Antibiotic susceptibility, intermediate resistance and complete resistance as per CLSI guidelines

Antibiotics	Concentration	Susceptible Level (%)	Intermediate Level (%)	Resistant Level (%)
Azithromycin	15 µg	59.09	22.72	18.18
Amoxicillin	10 µg	4.54	0.00	95.45
Tetracycline	30 µg	27.27	50	22.72
Ciprofloxacin	05 µg	31.81	54.54	13.63
Penicillin-G	10 units	0.00	0.00	100
Levofloxacin	5 µg	72.72	18.18	9.09
Cefotaxime	30 µg	40.90	45.45	13.63
Oxacillin	1 µg	4.54	0.00	95.45
Lincomycin	2 µg	0.00	0.00	100

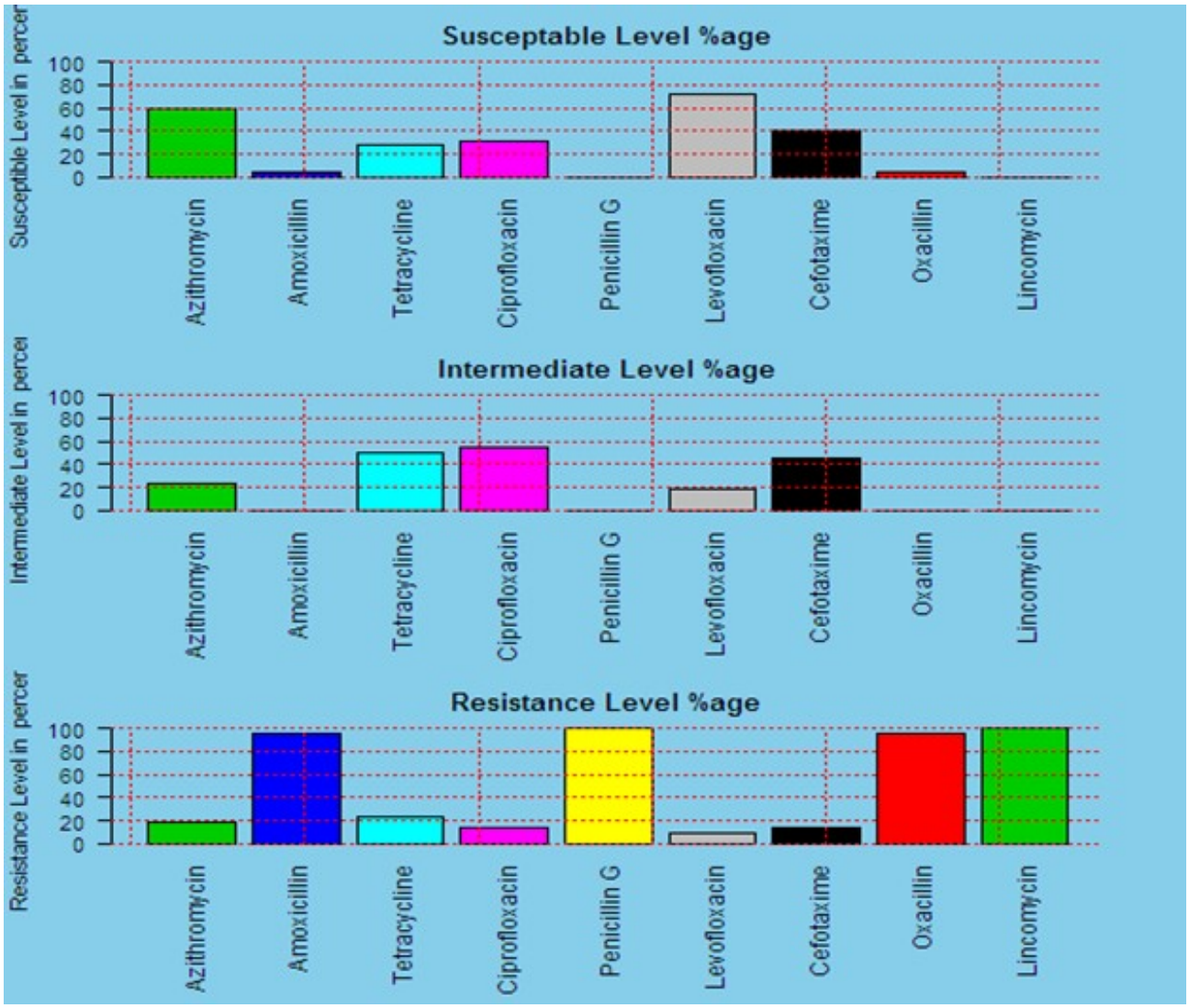

Fig. 1: Comparison of overall susceptibility, intermediate resistance and complete resistance of *S. dysenteriae* against various antibiotics

However, all the 22 isolates were found resistant to four out of nine antibiotics tested including Amoxicillin (95%), Penicillin-G (100%), Oxacillin (95%) and Lincomycin (100%) (table 2, fig. 1). Results further revealed that most of the isolates were susceptible to Azithromycin (59%), Levofloxacin (72%) and Cefotaxime (40%) (fig. 2). However, isolates were slightly susceptible to the drugs i.e., Tetracycline (27%), and Ciprofloxacin (31%) (fig. 2).

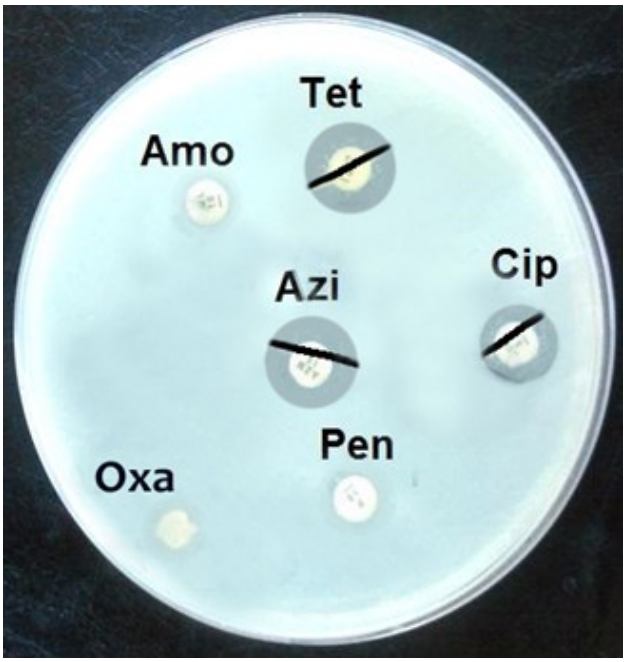

Fig. 2: Kirby Baur disc diffusion test conducted on Muller Hinton agar to measure antimicrobial susceptibility of *Shigella dysenteriae.* Arrows indicating zone of inhibition in mm, azithromycin, amoxicillin, ciprofloxacin, penicillin, tetracycline and oxacillin were screened

The antibiotics were allotted as treatment regimes to culture sensitivity plates and were replicated 22 times for the recovered isolates of *S. dysenteriae*. Results further confirmed the large differences among the percent susceptibility and percent resistant levels of *Shigella,* which revealed that there was a close association among the eight antimicrobial agents.

DISCUSSION

Present study revealed that overall percent prevalence was about 19% among the children up to five years of age. The said prevalence rate was much comparable with few developing countries. In past studies, the *Shigella* prevalence was increased and reported up to 16.9% (Huruy *et al*., 2008). Similarly, in present studies the frequency of recovered isolates of *S. dysenteriae* was correspondingly much higher than other developed countries where these values were 7.1% and 5.8% (Mache *et al*., 1997; Andualem and Geyid, 2003). In Pakistan, this much higher prevalence might be due to poor hygienic conditions and food security issues. Moreover, the scarcity of pure drinking water is also accountable for the high infection rate of *Shigella*.

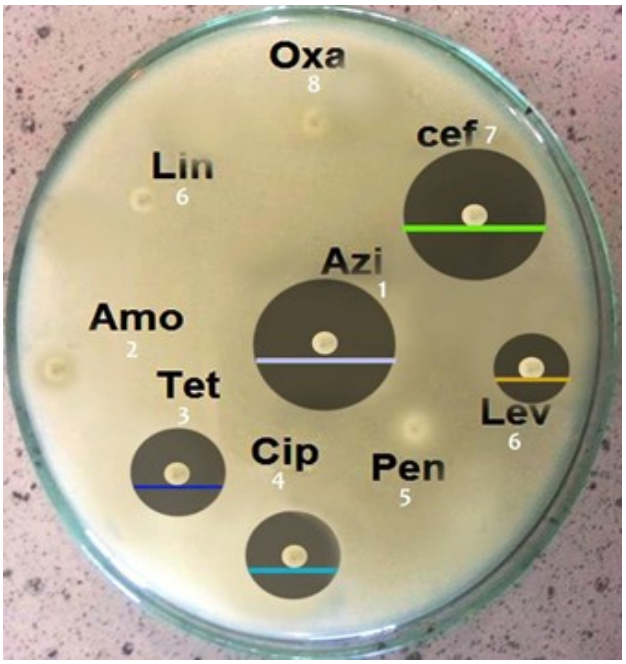

Fig. 3: Kirby Baur disc diffusion test conducted on Muller Hinton agar to measure antimicrobial susceptibility of *Shigella dysenteria. Lines in center (*Green, orange, steel gray, cyan and royal blue) indicating zone of inhibition in mm, azithromycin, amoxicillin, tetracycline, ciprofloxacin, penicillin G, levofloxacin, cefotaxime, oxacillin, lincomycin were screened.

In the present study, greater proportion of *S. dysenteriae* confirmed isolates were found resistant to Amoxicillin (95.45%) which might be due to its mode of action. Mode of action of amoxicillin is to inhibit cell wall synthesis in gram positive bacterial species but unable to restrict the cell wall development of the recovered isolates of *Shigella*. Present observations were also comparable with the past findings who reported 100% resistance of *Shigella* species against two antibiotics including Amoxicillin and Cefotaxime (Patil and Lava, 2012).

Present results further revealed that antimicrobial resistance of *S. dysenteriae* to tetracycline was 18.97%. Past studies revealed that *Shigella* isolates exhibited 23% resistance to tetracycline and were confirmed at Rabin Medical Centre, Central Israel (Askenazi, 2004). Some other previous findings documented that 60% resistance was found in *Shigella* isolates to Penicillin-G at Azad Kashmir, Pakistan (Ahmad *et al*., 2010). However, in a recent study about antimicrobial susceptibility, the 56.3% resistance was observed in *Shigella* species to Penicillin-G.[26] In present study, the *Shigella* confirmed isolates were completely (100%) resistant to Penicillin-G. The *Shigella* isolates resistance to antibiotic is rapidly increasing by

comparing with previous studies as discussed (Ahmad *et al*., 2014).

Present results further authenticated that confirmed isolates of *S. dysenteriae* exhibited moderate to low resistance of 18.18% to Azithromycin. The present observations were also comparable with the past findings as reported 16.0% resistance of *Shigella* to Azithromycin in Bangladesh (Rahman *et al*., 2007). In our study, the resistance of recovered isolates of *S. dysenteriae* towards Levofloxacin was 9.09%. Another study on identification of 92 distinct strains of *Shigella* in Beijing, China, detected 41% antimicrobial sensitivity of *S. dysenteriae* isolates to Cefotaxime and antimicrobial resistance towards Levofloxacin was 21.9% (Qiu *et al*., 2013). In other past studies, the 1411 stool specimens were screened, and reported 95.1% antimicrobial susceptibility of *Shigella* species to Cefotaxime (Nath *et al*., 2013).

The *S. dysenteriae* confirmed isolates resistance was 95.45% to antibiotic Oxacillin as detected in this study. In Nigeria, the studies revealed 90.0% antimicrobial resistance in *Shigella* spp towards oxacillin (Bolaji *et al*., 2011). According to present studies, the antibiotic resistance of *Shigella* isolates is slightly increased as compared to past findings. Recent antimicrobial resistance of *S. dysenteriae* affirmed isolates were completely resistant to Lincomycin. However, there is no available data about the findings based on the zone of inhibition (ZOI) for Lincomycin according to CLSI guidelines. Contemporary findings can be correlated with past findings as they reported that *Shigella* spp SS10 was found highly resistant to Lincomycin in Ibadan, Nigeria (Ayeni and Ayeni, 2016). They also investigated the antagonistic effects of lactic and acetic acid bacteria including *lactobacillus,* and *Acetobacter plantarum,* respectively on the *Shigella* species.

Present study also enunciated that the *Shigella* isolates were significantly susceptible to ciprofloxacin (100%). High sensitivity of *Shigella* isolates (88.89%) was reported to ciprofloxacin in Ethiopia (Terfassa and Jida, 2018). Present findings agreed with the previous results as determined the highest antimicrobial susceptibility of *Shigella* isolates (98.7%) towards Ciprofloxacin in Ethiopia (Mache, 2001).

CONCLUSION

Present results revealed 9.2% prevalence of *Shigella* species among the diarrheal patients. Since *Shigella* species were found highly resistant to most common drugs including amoxicillin, penicillin-g, oxacillin, lincomycin and care should be taken in selecting antimicrobials in treating the disease caused by *Shigella*. By intensive health education together with more careful use of antimicrobials could conserve antimicrobial efficacy and significantly reduce diarrheal illness. *Shigella* isolates were also found highly sensitive to azithromycin, tetracycline, ciprofloxacin, levofloxacin. Using biochemical tests, the *Shigella* species as pathogens were not identified to the species level, and hence it should be substantiated by serological and molecular identification for better taxonomy. Based on antibiotic susceptibility pattern, it is recommended to use azithromycin, ciprofloxacin, and levofloxacin against *Shigella* for better control. The study also verified that further epidemiological study should be conducted on patients attending different health centers.

REFERENCES

Ahmed B, Al-ghanim K, Mahboob S, Shakoori FR and Shakoori AR (2014). Plasmid profile analysis and antimicrobial resistance pattern of *Shigella flexneri* strains isolated from Azad Kashmir, Pakistan. *Pak. J. Zool*., **46**(6): 1605-1616.

Ahmed B, Farrah RS, Ali SS and Shakoori AR (2010). Characterization of multiple antimicrobial resistant *Shigella sonnei*, isolated from diarrheal patients in Azad Kashmir, Pakistan. *Pak. J. Zool*., **42**(5): 587-595.

Anand BS, Malhotra V, Bhattacharya SK, Datta P, Datta D, Sen D, Bhattacharya MK, Mukherjee PP and Pal SC (1986). Rectal histology in acute bacillary dysentery. *Gastroenterology*, **90**(3): 654-660.

Andualem B and Geyid A (2003). The prevalence of *Yersinia entrocolitica* isolates in comparison to those of the commonly encountered entero-pathogens causing diarrhea among Ethiopian patients in Addis Ababa. *Ethiop. Med. J*., **41**(3): 257-266.

Awan JA and Rahman SU (2002). Microbiology manual. 2nd ed., Unitech communications, Faisalabad, pp.1-63.

Ayeni AO and Ayeni FA (2016). Antagonistic effects of lactic and acetic acid bacteria on *Shigella* sp. SS10 in co-culture. *TAF Prev. Med. Bull*. **15**(1): 27-31.

Baur AW, Kirby WM, Sherris JC and Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*., **45**(4): 493-496.

Bolaji AS, Akande IO, Iromini FA, Adewoye SO and Opasola OA (2011). Antibiotic resistance pattern of bacteria spp isolated from hospital waste water in Ede South Western, Nigeria. *Eur. J. Exp. Biol*., **1**(4): 66-71.

Cheesbrough M (2006). District Laboratory Practice in Tropical Countries. 2nd ed., Cambridge University Press, New York, pp.01-434.

Huruy K, Kassu A, Mulu A, Gebretsadi S, Andargie G, Tadesse T, Birhan W, Worku N, Ghebreselassie D, Belyhun Y, Yifru S, Adugna S and Tiruneh M (2008). High Level of antimicrobial resistance in *Shigella* species isolated from diarrheal patients in university of Gondar teaching hospital, Gondar, Ethiopia. *Pharmacologyonline*, **2**(29): 328-340.

Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ, Adak GK and Levine

MM (1999). Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. *Bull. World Health Organ*., **77**(8): 651-666.

Liu H, Zhu B, Qiu S, Xia Y, Liang B, Yang C, Dong N, Li Y, Xiang Y, Wang S, Xie J, Mahe M, Sun Y, Song H (2018). Dominant serotype distribution and antimicrobial resistance profile of Shigella spp. in Xinjiang, China. PLoS ONE **13**(4): 1-16.

Mache A (2001). Antimicrobial resistance and sero-group of *Shigella* among pediatric out patients in southwest Ethiopia. *East Afr*. *Med. J*., **78**(6): 296-299.

Mache A, Mengistu Y and Cowley S (1997). *Shigella* serogroups identified from adult diarrhea outpatients in Addis Ababa, Ethiopia: Antibiotic resistance and plasmid profile analysis. *East Afr. Med. J*., **74**(3): 179-182.

Mathan MM and Mathan VI (1986). Ultrastructural pathology of the rectal mucosa in *Shigella* dysentery. *Am. J. Pathol*., **123**(1): 25-38.

Nath R, Saikia L, Choudhury G and Sharma D (2013). Drug resistant *Shigella flexneri* in and around Dibrugarh, North-East India. *Indian J. Med*. *Res*., **137**: 183-186.

Niyogi SK (2005). Shigellosis. *J. Microbiol*., **43**(2): 133-143.

Patil DP and Lava R (2012). Identification, characterization and antibiotic susceptibility of *Shigella* species isolated from stool samples in children. *Int. J. Biol. Med. Res*., 3(2): 1640-1643.

Qiu S, Wang Y, Xu X, Li P, Hao R, Yang C, Liu N, Li Z, Wang Z, Wang J, Zhihao Wu Z, Su W, Yang G, Jin H, Wang L, Sun Y, Yuan Z, Huang L and Song H (2013). Multidrug-Resistant A typical variants of *Shigella flexneri* in China. *Emerg*. *Infect*. *Dis*., **19**(7): 1147-1150.

Rahman M, Shoma S, Rashid H, Arifeen SE, Baqui AH, Siddique AK, Nair GB and Sack DA (2007). Increasing spectrum in antimicrobial resistance of *Shigella* isolates in Bangladesh: Resistance to azithromycin and ceftriaxone and decreased susceptibility to ciprofloxacin. *J. Health Popul. Nutr.*, **25**(2): 158-167.

Sheikh AF, Moosavian M, Abdi M, Heidary M, Shahi F, Jomehzadeh N, Mohammadi SS, Saki M, Khoshnood S (2019). Prevalence and antimicrobial resistance of Shigella species isolated from diarrheal patients in Ahvaz, southwest Iran. *Infect. Drug. Resist*. **12**: 249-253.

Tao J, Feng L, Guo H, Li Y and Wang L (2004). The O-antigen gene cluster of *Shigella boydii* O11 and functional identification of its '*wzy*' gene. *FEMS Microbiol. Lett*., **234**: 125-132.

Tariq A, Haque A, Ali A, Bashir S, Habeeb MA, Salman M and Sarvar Y (2012). Molecular profiling of antimicrobial resistance and integron association of multidrug-resistant clinical isolates of *Shigella* species from Faisalabad, Pakistan. *Can*. *J. Microbiol*., **58**(9): 1047-1054.

Terfassa A and Jida M (2018). Prevalence and antibiotics susceptibility pattern of *Salmonella* and *Shigella* species among diarrheal patients attending Nekemte Referral Hospital, Oromia, Ethiopia. *Int*. *J. Microbiol*., 9214689: 1-6.

Ugbogu OC, Nwachukwu NC and Ogbuagu UN (2006). Isolation of *Salmonella* and *Shigella* species from house flies (*Musca domestica* L.) in Uturu, Nigeria. *Afr. J. Biotechnol*., **5**(11): 1090-1091.

Wang Y, Ma Q, Hao R, Zhang Q, Yao S, Han J, Ren B, Fan T, Chen L, Xu X, Qiu S and Yang H (2019). Antimicrobial resistance and genetic characterization of Shigella spp. in Shanxi Province, China, during 2006-2016. *BMC Microbiol*., **116**(19): 1-11.

Wayne P (2012), Performance standards for antimicrobial testing twenty second informational supplement. Clinical laboratory and standards institute (CLSI), **32**(3): 1-179

World Health Organization (2005). Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type-1. *World Health Organization(WHO)*, 1-62.

Zafar A, Hasan R, Nizami SQ, Seidlein LV, Soofi S, Ahsan T, Chandio S, Habib A, Bhutto N, Siddiqui FJ, Rizvi A, Clemens JD and Bhutta ZA (2009). Frequency of isolation of various subtypes and antimicrobial resistance of *Shigella* from urban slums of Karachi, Pakistan. *Int. J. Infect. Dis*., **13**(6): 668-672.