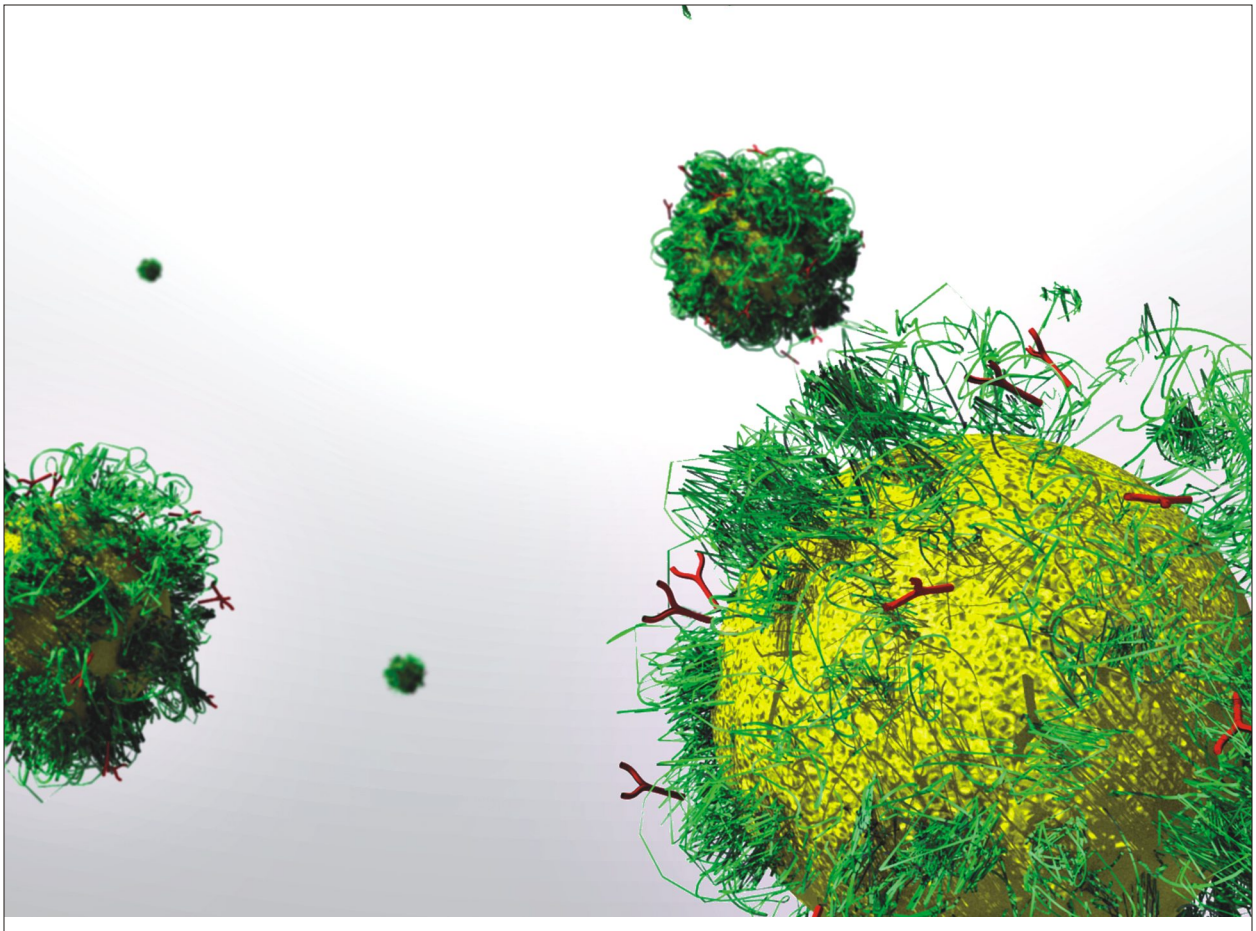

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Antibiotic Susceptibility Pattern of *Salmonella* and *Shiegella* Isolates Among Diarrheal Patients in Gedo Hospital, West Shoa Zone, Oromia State, Ethiopia

[1]Thirumurugan Gunasekaran, [2]Yonas Haileselassie and [3]Befikadu Asefa
[1]Department of Pharmacy,
[2]Department of Medicine,
[3]Departments of Nursing and Midwifery, College of Medicine and Health Sciences, Ambo University, Ambo, Ethiopia

Corresponding Author: Thirumurugan Gunasekaran, Department of Pharmacy, College of Medicine and Health Sciences, Ambo University, Ambo, Ethiopia Tel: 011-2365356

ABSTRACT

To evaluate the antimicrobial resistance profiling of bacterial strains from clinical isolates in Gedo Hospital, West Shoa Region, Oromia State, Ethiopia to create awareness of periodic antibiotic susceptibility testing, antimicrobial resistance among the health care providers. A convenience sample of patients who visited the adult outpatient department of Gedo hospital for diarrheal complaint was incorporated into the study. Isolation, identification of bacterial strains and antibiotic susceptibility tests were performed by the disk diffusion method recommended by the Clinical and Laboratory Standards Institute using commercial antibiotic discs. Out of 200 samples, 40 diarrheagenic bacteria were isolated and identified as *Shiegella* (22 = N) and *Salmonella* strains (18 = N). *Shiegella* and *Salmonella* strains were susceptible to gentamicin and ciprofloxacin, respectively but a high rate of resistance were observed to amoxicillin, chloramphenicol and tetracycline for both *Shiegella* and *Salmonella* strains. Notably, both bacteria have developed complete resistance to amoxicillin. Gentamicin may be the drug choice for *Shiegella* stains caused diarrhea and ciprofloxacin for the diarrhea caused by *Salmonella* in the study area. Antibiotics selection and treatment of infections based on bacterial strains identification and *in vitro* susceptibility testing rather than current empirical treatment.

Key words: Antibiotic resistance, Gedo hospital, diarrhea, *Shiegella*, *Salmonella*

INTRODUCTION

Diarrheal disease amounts to an estimated 4.1% of the total global burden of disease and is responsible for the deaths of 1.7 million people every year. Among the microbial pathogens *Vibrio cholera*, *Salmonella* and *Shiegella* strain are the most serious as they cause severe illness and as they are associated with outbreaks. Among the enteric bacterial pathogens *Salmonella* and *Shiegella* are of particular concern as causes of enteric fevers, food poisoning and gastroenteritis. The retrospective studies carried out in Ethiopia showed the prevalence of *Salmonella* (5.3-15.4%) and *Shiegella* (5-7.5%) (Mengistu *et al*., 2014). Antibiotic resistance is a drug resistance where a microorganism has developed the ability to survive exposure to an antibiotic. The volume of antibiotic prescribed is the major cause in increasing rates of microbial resistance rather than

compliance with antibiotics (Thirumurugan and Dhanaraju, 2011). Antimicrobial resistance is a global concern causes infections by resistant microorganism resulting in prolonged illness and death and reduces effectiveness of treatment, thus patient's remains infectious, increasing risk of spreading resistant to others pathogens and many infectious diseases become untreatable and increases the cost of therapy. Also the achievements of modern medicine are put at risk by antimicrobial resistance because lack of effective antimicrobials the successive treatments such as organ transplantation, cancer chemotherapy, major surgery would be compromised and possibilities of spreading antimicrobial resistance rapidly from countries to countries and continents through humans and food. Resistance has spread worldwide, antimicrobial resistance gonorrhoea emerged in Vietnam in 1967 and then spread to the Philippines and finally the USA (Thirumurugan *et al*., 2009; Holmes *et al*., 1967).

Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public often utilize these agents inappropriately. Inappropriate use results from physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for diagnosis of infections that potentially have a microbial etiology, unnecessarily prescribing expensive, broad-spectrum agents and not following established recommendations for using common prophylaxis. The availability of antibiotics over the counter, despite regulations to the contrary, also fuel inappropriate use of antimicrobial drugs in developing countries. The epidemiological and prescriptions pattern studies conducted in Ethiopia showed that a higher percentage and irrational use of antibiotics. Antibiotic prescribing pattern at Bishoftu hospital shows 28.3% of polypharmacy (Feleke *et al*., 2013), Hawassa University Referral hospital 58% (Desalegn, 2013) and average number of antibiotics per patient was 2.17 for prophylaxis and 2.18 for treatment at northwest Ethiopia (Abula and Kedir, 2004).

In most developing countries, laboratory investigations of resistant microbes are diagnostic challenges due to lack of adequate facilities that enable culture and antimicrobial susceptibility testing (Collee *et al*., 1996). As a result, there is restricted awareness of the prevalence of infections and antimicrobial resistance (Cook and Zumla, 2003; Sharma *et al*., 2005). Also, the injudicious use of antibiotics by patients and physicians alike in many developing countries such as Ethiopia has led to an increased antimicrobial resistance and in turn reduced therapeutic efficacy in these countries (Asrat, 2008). In Harar, Eastern Ethiopia, *Salmonella* and *Shiegella* have been reported to be resistant to first line antibiotics such as ampicillin, tetracycline and chloramphenicol and also resistant *Shiegella* serogroups identified from adult diarrhoeal out patients in Addis Ababa, Ethiopia (Aseffa *et al*., 1997; Mache *et al*., 1997).

The objective of this study is to evaluate the antibiogram and antibiotic resistance of bacterial strains from clinical isolates in Gedo Hospital, West Shoa Region, Oromia State, Ethiopia to create the awareness of periodic antibiotic susceptibility testing, antimicrobial resistance among the health care providers.

MATERIALS AND METHODS

Study design and specimen collection: The study was conducted in Gedo Hospital, West Shoa, Oromia state, Ethiopia. A convenience sample of patients who visited the adult outpatient department of Gedo hospital for diarrheal complaint was incorporated into the study. The convenient sampling technique employed to collect 200 samples and the patients who took any antibiotics for treatment was not included in the study. About 1.5 g of diarrheal stool was collected randomly using clean, dry, non-leak wide mouth stool containers from patients with different socio-demographic status.

Bacteriological investigation: The specimens collected were precisely transported to the Ambo University Microbiology Laboratory using Cary Blair transport media for isolation and identification of bacteria (NCCLS, 2004). Using sterile swabs, the samples were directly inoculated onto plates of Deoxycholate Citrate Agar and Xylose Lysine Deoxycholate Agar and the plates were incubated aerobically at 37°C for 24 h. The same samples were plated onto Selenite F broth and incubated as a fore stated for enrichment. Following the incubation of Selenite F broth, a loop (0.001 mL) were streaked onto both Deoxycholate Citrate Agar and Xylose Lysine Deoxycholate plates and incubated at 37°C for 24 h. Two to three colonies suspected were selected, purified by streaking on to nutrient agar plates and characterized biochemically using Klingler Iron Agar, Urease tests, motility and indole test (NCCLS, 2004).

Antibiotic susceptibility test: Antibiotic susceptibility tests were performed by disk diffusion method using guidelines established by Bauer *et al.* (1966) and recommended by the Clinical and Laboratory Standards Institute utilizing commercial antibiotic discs. Characterization of the resistance or susceptibility profile of the isolates were determined by measuring the inhibitory zone and then compared with the interpretative chart to determine the susceptibility of the isolates to the antibiotics (Thirumurugan *et al.*, 2009). The antibiotics tested include Amoxicillin 20 μg (AMX), Chloramphenicol 30 μg (CHP), Tetracycline 30 μg (TEC), Gentamicin 30 μg (GEM), Co-trimaxazole 10 μg (COT) and Ciprofloxacin 30 μg (CIF) were purchased local distributers (Liverpool, UK).

Quality control: Culture media were checked for sterility and performance. Standard strains of *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were employed for culture and antimicrobial susceptibility testing.

Data analysis: Tables and percentages were used to describe findings. Cross tabulations were utilized to examine the levels of antimicrobial resistance to the respective antibiotics.

Ethical considerations: Written consent was obtained from every study participant. The study will not cause any harm to the respondent, sample collectors and supervisors. The privacy was maintained for each procedure and confidentiality has also been kept. Ethical clearance was sought from the College of Medicine and Health Sciences and Ethical Review Committee, Ambo University, Ambo, Ethiopia.

RESULTS

In this study, 40 stool samples were detected with the diarrheagenic enterobacteriaceae from 200 diarrheal samples. Of 200 participants, 93 were male and 107 were female. Eighteen of the patients who became positive were male and 22 positive patients were female. Age distribution of diarrheagenic enterobacteriaceae positive patients ranged from 0-64 years. Nine (22.5%) diarrheagenic enterobacteriaceae positive patients were pediatric age group (up to 16 years) and out of nine pediatric patients, 4 was male and 5 were female. Fourteen (35%) positive samples were detected from the age group of 17-32 years and out of 14, 4 was male and 10 were female. From the age group 33-48, 12(30%) stool samples detected were diarrhegenic and 7 were male, 5 were female. Only 5 positive samples with 3 males and 2 females were detected in the age group of 49-64 years (Table 1).

Table 1: Distribution of *Shiegella* and *Salmonella* strains isolated from diarrhea stool by age and sex in Gedo hospital, Western shoa, Oromia state

	Male				Female				Total	
Age group (years)	*Sal.*	*Shi.*	No.	%	*Sal.*	*Shi.*	No.	%	No.	%
0-16	0	4	4	22.2	3	2	5	22.7	9	22.5
17-32	2	2	4	22.2	6	4	10	45.5	14	35
33-48	3	4	7	38.9	3	2	5	22.7	12	30
49-64	1	2	3	16.7	1	1	2	9.1	5	12.5
Total	6	12	18	100	13	9	22	100	40	100

Table 2: Percentage of stool consistency in patients with *Shiegella* and *Salmonella* infection in Gedo hospital, Western shoa, Oromia state

	Pathogenic bacteria isolated					
	Shiegella		*Salmonella*		Total	
Stool consistency	No.	%	No.	%	No.	%
Watery diarrhea	3	13.6	1	5.6	4	10.0
Mucoid diarrhea	12	54.6	6	33.3	18	45.0
Bloody diarrhea	2	9.1	5	27.8	7	17.5
Bloody mucoid diarrhea	5	22.7	6	33.3	11	27.5
Total	22	100.0	18	100.0	40	100.0

Table 3: Comparison of stool consistency results for age and sex groups of patients in Gedo hospital

	Age groups (years)								
	Male				Female				
Stool consistency	0-16	17-32	33-48	49-64	0-16	17-32	33-48	49-64	Total
Watery diarrhea	9	10	29	9	7	21	26	1	112
Mucoid diarrhea	0	8	9	0	5	7	8	2	39
Bloody diarrhea	2	2	5	2	2	8	8	2	31
Bloody mucoid diarrhea	1	1	1	2	2	7	2	2	18
Total	12	21	44	13	16	43	44	7	200

Of a 40 enterobacteriaceae infected stool with various consistencies, 22 were *Shiegella* strains and 18 were *Salmonella* strains. Diarrheal stool samples were classified as watery diarrhea, mucoid diarrhea, bloody diarrhea and bloody mucoid diarrhea. Totally 4(10%) has been identified as watery diarrhea, *Shiegella* sp. were detected from 3(13.6) samples and *Salmonella* sp. were detected from one (5.6%) sample. Mucoid stools were 18(45%) in number, 12(54.6%) samples showed the presence of *Shiegella* sp. and 6(33.3%) showed the presence of *Salmonella*. Seven (17.5%) samples were bloody diarrhea and *Shiegella*, *Salmonella* detected were 2(9.1%), 5(27.8%), respectively. Among 11(27.5%) bloody mucoid diarrhea samples, 5(22.7%) were *Shiegella* and 6(33.3%) were *Salmonella* (Table 2 and 3).

Antimicrobial susceptibility test was carried out using the disc diffusion method against the *Shiegella* and *Salmonella* strains. Antimicrobial resistance pattern results were categorized as

Table 4: Antimicrobial susceptibility profiling of *Shiegella* and *Salmonella* isolates among patients with diarrhea stool in Gedo hospital, Western shoa, Oromia state

		Antimicrobial susceptibility test result					
		Resistance		Intermediate		Susceptible	
Pathogenic bacteria isolated	Antibiotics tested	No.	%	No.	%	No.	%
Shiegella (N = 22)	AMX	21	95.5	1	4.5	0	0
	CHP	8	36.4	9	40.9	5	22.7
	TEC	17	77.3	5	22.7	0	0
	GEM	0	0	6	27.3	16	72.7
	COT	3	13.6	9	40.9	10	45.5
	CIF	2	9.1	9	40.9	11	50
Salmonella (N = 18)	AMX	18	100	0	0	0	0
	CHP	13	72.2	2	11.1	3	16.7
	TEC	15	83.3	2	11.1	1	5.6
	GEM	13	72.2	3	16.7	2	11.1
	COT	5	27.8	4	22.2	9	50
	CIF	0	0	1	5.6	17	94.4

resistance, intermediate and susceptible for all antibiotics. Among all antibiotics tested, of a 22 *Shiegella*, 21(95.5%) *Shiegella* strain showed the resistance, 1(4.5%) intermediate to Amoxicillin (AMX) and 17(77.3%) *Shiegella* strain showed resistance, 5(22.7%) intermediate against TEC. Gentamicin (GEM) showed activity against *Shiegella* strain compared with other antibiotics tested, 16(72.7%) were susceptible and 6(27.3%) were intermediate. The CIF 11(50%) and COT 10(45.5%) showed more or less equal susceptibility pattern, 2(9.1%) were resistance, 9(40.9%) were intermediate for CIF and 3(13.6%) were resistance, 9(40.9%) were intermediate for COT. CHP showed 8(36.4%) resistance, 9(40.9%) intermediate and 5(22.7%) were susceptible against *Shiegella*.

AMX showed poor antibacterial activity against *Salmonella* compared with the *Shiegella* strain. Of 18 diarrheagenic *Salmonella* isolates, total 18(100%) were resistance for AMX but CIF showed good antibacterial spectrum, 17(94.4%) were susceptible and 1(5.6%) were intermediate. After CIF, COT showed 9(50%) susceptibility, 5(27.8%) were resistance and 4(22.2%) were intermediate. CHP, TEC and GEM showed more or less equal resistance pattern against *Salmonella* strain. CHP showed 13(72.2%) resistance, 2(11.1%) intermediate and 3(16.7%) were susceptible and GEM showed 13(72.2%) resistance, 3(16.7%) intermediate and 2(11.1%) was susceptible. Also TEC showed weak antibacterial activity, 15(83.3%) were resistance, 2(11.1%) were intermediate and 1(5.6%) were susceptible (Table 4).

DISCUSSION

The present study provides results of antibiotics resistance pattern of diarrheagenic *Shiegella* and *Salmonella* strains isolates conducted in Gedo hospital, West Shoa, Oromia, Ethiopia. Out of 200 samples, 40 diarrheagenic bacteria were isolated and identified as *Shiegella* (22 = N) and *Salmonella* strains (18 = n) (Table 1). Similarly, the study conducted at Harar, Estern Ethiopia reveals the presence of *Salmonella* (28 = n) and *Shiegella* (17 = n) isolates from 244 stool samples Reda *et al.* (2011), Asrat (2008) and Aseffa *et al.* (1997) also reported the similar percentage of presence of *Salmonella* and *Shiegella* strains in the diarrheal stool, but Roma *et al.* (2000) reported

34.6% prevalence of *Shiegella* isolated from a study done in Awassa, Southern Ethiopia. Out of 40 diarrheagenic isolates, 18(45%) were isolated from the mucoid diahrrea, 7(17.5%) from bloody diarrhea and 11(27.5%) from the bloody mucoid diarrhea sample (Table 2 and 3). The previous literature showed the many controversy in the presence of *Shiegella* and *Salmonella* strains, Asrat (2008) reported that 82.4% of *Shiegella* and *Salmonella* strains were isolated from watery diarrhea stool but Reda *et al.* (2011) reported that mucoid were 46.8%, mucoid and bloody were 42.8%. This may be because of variations in the strain from place to place and our findings were similar compared to the study done in Harar, Eastern Ethiopia (Reda *et al.*, 2011).

Antimicrobial resistance was high among the *Shiegella* strains isolated in the study were resistant to AMX (95.5%), TEC (77.3%) and CHP (36.4%) and these findings were comparable with previous studies carried out in Ethiopia (Asrat, 2008; Mache *et al.*, 1997; Roma *et al.*, 2000). The *Shiegella* strains showed no resistance to GEM, but six had intermediary susceptibility and 72.7% were susceptible to this antibiotic tested. Similarly, the study done in harar, Estern Ethiopia showed that *Shiegella* strains were 94.1% of susceptibility, 0% of resistance to GEM but the study conducted in Gondar Teaching hospital, Northwest Ethiopia showed that *Shiegella* strains were 10% resistant to GEM (Huruy *et al.*, 2011) and similar data has been reported in other African countries, such as Nigeria (Iwalokun *et al.*, 2001) (Table 4). Resistance to CIF (9.1%) was observed in our study comparable with previous study in which 3.1% of were resistant against CIF (MoezArdalan *et al.*, 2003) but 8.3% of resistance pattern of *Shiegella* strains to CIF were reported in the study conducted in the Gondar Teaching hospital, Northwest Ethiopia (Huruy *et al.*, 2011). COT showed less susceptible (45.5%), 13.6% resistances compared with the CIF but the previous study showed 91.7% of resistance to COT (Jafari *et al.*, 2008). The pattern of resistance to *Salmonella* strains isolates were 100% resistant to AMX, 83.3% to TEC followed by CHP (72.2%) and GEM (72.2%). The CIF showed 94.4% susceptibility, 0% resistance and 5.6% intermediate against *Salmonella* strains. COT showed 50% susceptibility and 27.8% resistance against *Salmonella* strains isolated in our study. The similar results were observed in the previous studies conducted in Ethiopia (Asrat, 2008; Huruy *et al.*, 2011; Amabile-Cuevas, 2010). The high resistant to AMX might be due to high rate of AMX prescription in the study area and CHP, TEC resistance might reflect the indiscriminate and widespread uses of antibiotics in public health practices since the society in the setting have easy access to different antibiotics without prescription. Also, an antibiotic has been prescribed irrationally without susceptibility test.

CONCLUSION AND RECOMMENDATION

Shiegella and *Salmonella* strains were susceptible to gentamicin and ciprofloxaxin, respectively but a high rate of resistance were observed to AMX, CHP, TEC for both *Shiegella* and *Salmonella* strains. Notably, both bacteria have developed complete resistance to AMX. We suggest that gentamicin may be the drug choice for *Shiegella* strains caused diarrhea and ciprofloxacin for the diarrhea caused by *Salmonella* strains in the study area. However, there is required of extensive prevalence, antibacterial susceptibility profiling and underlying mechanisms of antimicrobial resistance by *Shiegella* and *Salmonella* isolates to be conducted. Moreover, selection of antibiotics and treatment of infections based on bacterial species identification and *in vitro* susceptibility testing should be implemented rather than current empirical treatment.

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