Journal of Infectious Diseases and Immunity Vol. 3(8), pp. 134-139, August, 2011
Available online at http://www.academicjournals.org/JIDI
ISSN 2141-2375 ©2011 Academic Journals

Full Length Research Paper

Antibiotic susceptibility patterns of *Salmonella* and *Shigella* isolates in Harar, Eastern Ethiopia

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Accepted 24 June, 2011

Antimicrobial resistance of *Salmonella* and *Shigella* are emerging global challenges. There are no studies conducted on the antimicrobial susceptibility patterns of *Salmonella* and *Shigella* in eastern Ethiopia. This study aims to investigate antimicrobial susceptibility patterns of these microbes. A total of 244 diarrheic stool specimens were collected and cultured for screening of microbes using standard methods. Afterwards, the isolates were confirmed as *Salmonella* or *Shigella* by using a battery of biochemical reactions. Antibiotic susceptibility testing was performed using six selected drugs (ampicillin, amoxicillin, tetracycline, chloramphenicol, norfloxacin, and gentamicin). 28 (11.5%) *Salmonella* and 17 (6.7%) *Shigella* organisms were isolated from 244 stool samples. Sensitivity of the *Salmonella* isolates were 0.0% to ampicillin; 0.0% to amoxicillin; 14.2% to tetracycline; 28.6% to chloramphenicol; 89.3% to norfloxacin; and 92.8% to gentamicin. *Shigella* had sensitivities of 0.0% to ampicillin; 0.0% to amoxicillin; 11.8% to tetracycline; 41.2% to chloramphenicol; 88.2% to norfloxacin; and 94.1% to gentamicin. A high level of antimicrobial resistance was detected in both *Salmonella* and *Shigella* isolates. The organisms developed complete resistance to ampicillin and amoxicillin. The results of the study demonstrated that gentamicin and norfloxacin are drugs of choice for treating diarrhea caused by these microbes.

Key words: Infection, *shigella*, *salmonella*, diarrhea, drug resistance.

INTRODUCTION

Salmonella and *Shigella* infections are major global public health problems. Two hundred million to more than one billion cases of diarrhea result worldwide due to non-typhoidal *Salmonella* every year leading to 3 million deaths (Goburn et al., 2007). Ninety-nine percent of the 200 million cases and more than 650,000 deaths per year that result from infection with *Shigella* commonly occur in developing countries, primarily among children and young adults (Kasper et al., 2005; Niyogi. 2005; Khatun et al., 2011). *Salmonella* and *Shigella* cause mild to severe forms of intestinal tract infection. *Salmonella* cause self-limited gastro-enteritis and the more severe forms of systemic typhoid fever (Kasper et al., 2005; Goburn et al., 2007). *Shigella* species are limited to the intestinal tract of humans and cause bacillary dysentery leading to watery or bloody diarrhea. They are transmitted through ingestion of contaminated food and water. These infections are prevalent in developing countries where lack of clean water supply, lack of proper sewage disposal system and flies aggravate the spread of the diseases (Kasper et al., 2005). Epidemiological surveillance is an essential component in controlling *Salmonella* and *Shigella* infections (Sharma et al., 2005).

Drug resistance is the decreased sensitivity or the complete insensitivity of microbes to drugs that cause cell death or inhibition of growth (Sharma et al., 2005). Anti-

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microbial resistance of *Salmonella* and *Shigella* are emerging global challenges, especially in developing countries where there is an increased misuse of antimicrobial agents in humans and animals (Kasper et al., 2005). In most developing countries, laboratory investigations of *Shigella* and *Salmonella* are diagnostic challenges due to lack of adequate facilities that enable culture and antimicrobial susceptibility testing (Collee et al., 1999). As a result, there is limited awareness of the prevalence of infections and antimicrobial resistance (Cook et al., 2003; Sharma et al., 2005). Also, the injudicious use of antibiotics by patients and physicians alike in many developing countries such as Ethiopia has lead to an increased antibiotic resistance and in turn reduced therapeutic efficacy in these countries (Okeke et al., 2007; Asrat, 2008). Examining the antibiotic susceptibility patterns of pathogens is important toward tailoring treatment to the ever changing resistance patterns and distribution of pathogenic bacteria. In Ethiopia, *Salmonella* and *Shigella* have been reported to be resistant to first line antibiotics such as ampicillin, tetracycline and chloramphenicol (Assefa et al., 1997; Mache et al., 1997; Roma et al., 2000; Mache, 2002; Yismaw et al., 2006; Asrat, 2008; Tiruneh, 2009; Beyene et al., 2011). To date, there are no studies examining the prevalence and antibiotic susceptibility patterns of *Salmonella* and *Shigella* pathogens in eastern Ethiopia. Therefore, the aim of this study was to examine antibiotic susceptibility of these pathogens among diarrheal patients in the city of Harar in eastern Ethiopia.

MATERIALS AND METHODS

Study design

The study was conducted in two of the four referral hospitals located in Harar, eastern Ethiopia. A convenience sample of patients who visited the adult outpatient departments of Hiwot Fana and Misrak Arbegnoch teaching hospitals from January 2007 to February 2007 for complaints of diarrheal disease was included in the study. These hospitals were selected for the study because of their close affiliation to the Faculty of Health Sciences of Haramaya University where the laboratory used for this study is located. The patient flow of the hospitals ranges from rural and urban dwellers to clients of diverse socioeconomic and ethnic backgrounds.

Specimen collection

244 stool specimens were collected from all patients that presented with diarrhea (defined three or more loose stools per day) using dry, clean, leak proof, and wide mouth stool containers. Participants who took antibiotics for the diarrheal attack were excluded from the study.

Isolation and identification of bacteria

For detection of *Salmonella* and *Shigella* isolates, about 1 to 2 g of feces was collected. Subsequently, the specimens were transported to the laboratory using Cary Blair transport media for further processing and analysis (National Committee for Clinical Laboratory Standards, 2004). Using sterile swabs, the samples were directly inoculated onto plates of deoxycholate citrate (DCA) agar (Oxoid CM 35; Oxoid Ltd, UK) and xylose lysine deoxycholate (XLD) agar (Oxoid CM 469; Oxoid Ltd, UK) and the plates were incubated aerobically at 37°C for 24 h.

The same samples were then plated onto Selenite F broth (Mast Diagnostics DM 210, Mast Diagnostics, UK) and incubated as aforestated for enrichment. Following the incubation of Selenite F broth, a loopful was streaked onto both DCA and XLD pates and incubated at 37°C for 24 h. One to three colonies suspected to be *Salmonella* or *Shigella* (small red colonies on XLD and colorless colonies sometimes with black spot on DCA) were selected, purified by streaking on to nutrient agar plates and characterized biochemically using Klingler Iron Agar (KIA), Urease tests, motility and indole test (Biomerieux, France) (National Committee for Clinical Laboratory Standards, 2004).

Antimicrobial susceptibility testing

The disc diffusion was performed to test susceptibility of *Salmonella* and *Shigella* isolates using standard procedures (Bauer et al., 1966). In brief, a MacFarland 0.5 standardized suspension of the bacteria in 0.8% sterile saline was prepared and swabbed over the entire surface of Mueller Hinton agar (Oxoid) with a sterile cotton swab. A ring of disks of each (Mast Diagnostics, UK) containing single concentrations of each antimicrobial agent was then placed onto the inoculated surface. After overnight incubation at 37°C, clear zones produced by antimicrobial inhibition of bacterial growth were measured in mm using a straight line ruler. The diameter of the zone was read using an interpreting chart for zone sizes (NCCLS, 1998). For the susceptibility testing, the following six antimicrobial drugs and concentrations were used: ampicillin 10 µg, chloramphenicol 30 µg, gentamycin 30 µg, norfloxacin 10 µg, amoxicillin 20 µg and tetracycline 30 µg (Oxoid Ltd, UK). Findings of antibiotic resistance testing were recorded as susceptible, intermediate, and resistant (National Committee for Clinical Laboratory Standards, 2004).

Quality control

Quality control was set up using an *Escherichia coli* strain (ATCC 25922) which was susceptible to all the tested drugs (ampicillin, 10 µg; chloramphenicol, 30 µg; gentamycin, 30 µg; norfloxacin, 10 µg; amoxicillin, 20 µg; and tetracycline, 30 µg).

Data analysis

Tables and percentages were used to the describe findings. Cross tabulations were used to examine the levels of drug resistance to the respective antibiotics. Data analysis was performed with SPSS version 15.

Ethical clearance

Ethical clearance was obtained from the Research Ethics Committee of the Faculty of Health Sciences of Haramaya University. Informed consent was requested from patients. Anonymous forms were used to collect data required for the study to secure confidentiality.

RESULTS

In the study period, a total of 244 diarrheal stool samples were collected for culture and antibiotic susceptibility testing. Twenty-eight (11.5%) *Salmonella* and 17 (6.7%) *Shigella* were isolated. The mean age of the patients from whom either *Salmonella* or *Shigella* microbes were isolated was 26 years (SD ±15.4; Range = 11 months to 70 years). There was one infant of 11 months with a *Shigella* infection. Twenty-two (48.9%) were males while 23 were (51.1%) females. Nine incomplete or empty forms were discarded. The most common appearance of diarrhea was mucoid for both *Salmonella* (12, 42.8%) and *Shigella* (9, 52.9%) as displayed in Table 1. The highest level of resistance was detected for ampicillin and amoxicillin in which all (100%) *Salmonella* and *Shigella* isolates were found to be resistant. The highest level of susceptibility was detected for gentamicin and norfloxacin, where, respectively, 92.8 (26) and 89.3% (25); and 94.1% (16) and 88.2% (15) of the *Salmonella* and *Shigella* isolates were susceptible (Table 2).

DISCUSSION

Our findings show that except for gentamicin and norfloxacin, the organisms in this study have a high level of resistance to ampicillin, amoxicillin, tetracycline and chloramphenicol. In Tables 3 and 4, the findings of this study are displayed against findings from other parts of the country and neighboring Kenya (Brooks et al., 2006) and Eritrea (Naik, 2006). The fact that diarrheal patients with Salmonellosis or Shigellosis present with mucoid and bloody diarrhea is an interesting finding that may have an impact on the way practitioners treat diarrhea in the study area. It is reported (Roma et al., 2000) that physicians in Ethiopia wrongly treat bloody diarrhea as an amoebiasis attack. However, our findings are in sharp contrast to a study in Addis Ababa by Asrat who reported that in addition to mucoid (8.4%), and bloody (6.8%) diarrhea, the majority (82.4%) of the diarrhea samples in which *Salmonella* and *Shigella* were isolated, had a watery nature (Asrat, 2008). This may reflect underlying variations in strain patterns from place to place. In developing countries (Kasper et al., 2005) *Shigella dysenteriae* and *Shigella flexneri* are prevalent species causing mucoid to bloody diarrhea, while in the United States where *Shigella sonei* and *Shigella boydi* species predominate, Shigellosis is commonly associated with mild watery diarrhea. While *S. dysentriae* are consistently associated with dysentery, it is less common for *S. flexneri* to cause bloody diarrhea (Kasper et al., 2005). The fact that *S. flexneri* made up 54% of the isolates in Asrat (Asrat, 2008) may explain the watery diarrhea in his sample. These findings may indicate the need for strain identification in our study area, in order to better understand the prevalent strains and their clinical manifestations.

There seems to be complete resistance to ampicillin and amoxicillin by *Salmonella* organisms in the study which is in disagreement with reports from other parts of the country (Mache et al., 1997; Asrat, 2008). This is a sharp increase from earlier reports indicating the aggravating problem of drug resistance by these microbes over the years. Unlike our findings, a high level of resistance to gentamicin (75.6%) was reported from Gondar, northwest Ethiopia by Asrat (2008), whereas the same author reports absence of resistance to norfloxacin. In rural western Kenya, Brooks et al. (2006) found a lower level of resistance to the antibiotics used in this study as well as elsewhere in the country (Ashenafi et al., 1985; Mache et al., 1997; Asrat, 2008). The only exception was gentamicin to which 13.0% of *Salmonella* isolates in Kenya were resistant (Brooks et al., 2006).

Compared to studies reported in other parts of the country (Assefa et al., 1997; Mache et al., 1997; Roma et al., 2000; Yismaw et al., 2006; Asrat, 2008; Tiruneh, 2009), *Shigella* isolates had a lower level of resistance to chloramphenicol (29.4%). We detected a high level of susceptibility to gentamicin. Excepting reports by Tiruneh (2009) and Gedebou et al. (1982) from Gondar who reported resistance levels of 12.2 and 25% respectively. *Shigella* organisms in this study seem to be highly susceptible to gentamicin. This is similar to reports from other parts of the country (Ashenafi et al., 1985; Assefa et al., 1997; Roma et al., 2000) including a report from Kenya (Brooks et al., 2006). Unlike studies in other places (Assefa et al., 1997; Mache et al., 1997; Roma et al., 2000; Yismaw et al., 2006; Asrat, 2008; Tiruneh, 2009) including neighboring Eritrea (Naik, 2006) and Kenya (Brooks et al., 2006), *Shigella* isolates are completely resistant to ampicillin and amoxicillin.

However, there seems to be a similar pattern of high resistance to these drugs in the studies in the rest of the country, even though lower in extent than our findings (Table 4). This could be due to the fact that ampicillin and amoxicillin have been used in the country for a long time and because of their easy availability and potential for misuse. Even though a reduced level of resistance was detected for tetracycline (70.6%), compared to ampicillin and amoxicillin, a relatively similar pattern of resistance (74 to 97.3%) was reported from other parts of the country (Assefa et al., 1997; Roma et al., 2000; Yismaw et al., 2006; Asrat, 2008) and outside (Karuiki et al., 2001). The organisms seem to also have increased their resistance to the drugs from lower levels reported earlier (Gedebou et al., 1982; Mache et al., 1997) to levels of more than 90% in reports by Asrat (2008) and Truneh et al. (Truneh. 2009) in 2009. This is similar to the pattern across the globe where the organisms are consistently increasing their resistance to these commonly used first line drugs (Karuiki et al., 2001; Sharma et al., 2005; Okeke et al., 2007). Resistance is a natural biological response of microbes to antimicrobials and is currently a

Table 1. Appearance of diarrhea in patients with *Salmonella* and *Shigella* infection in Harar, eastern Ethiopia, between January to February 2007.

Appearance of diarrhea	Organism isolated		Total
	***Salmonella* (n, %)**	***Shigella* (n, %)**	
Bloody	7 (25)	1 (5.9)	8 (17.8)
Mucoid	12 (42.8)	9 (52.9)	21 (46.8)
Watery	0 (0)	1 (5.9)	1 (2.2)
Mucoid and Bloody	9 (32.1)	3 (17.6)	12 (42.8)
Other	0 (0)	3 (17.6)	3 (6.7)
Total	28 (100)	17 (100)	45 (100)

Table 2. Antibiotic susceptibility of *Shigella* and *Salmonella* isolates among diarrheic patients in Harar, eastern Ethiopia, between January to February 2007.

Organism isolated	Outcome of susceptibility test		
	Susceptible (n, %)	Intermediate (n, %)	Resistant (n, %)
***Salmonella* (N=28)**			
Chloramphenicol 30 µg	8 (28.6)	2 (7.1)	18 (62.3)
Gentamicin 30 µg	26 (92.8)	1 (3.6)	1 (3.6)
Ampicillin 10 µg	0 (0.0)	0 (0.0)	28 (100.0)
Norfloxacin 10 µg	25 (89.3)	1 (3.6)	2 (7.1)
Amoxicillin 20 µg	0 (0.0)	0 (0.0)	28 (100.0)
Tetracycline 30 µg	4 (14.2)	3 (10.7)	20 (71.4)
***Shigella* (N=17)**			
Chloramphenicol 30 µg	7 (41.2)	5 (29.4)	5 (29.4)
Gentamicin 30 µg	16 (94.1)	1 (5.9)	0 (0.0)
Ampicillin 10 µg	0 (0.0)	0 (0.0)	17 (100.0)
Norfloxacin 10 µg	15 (88.2)	1 (5.9)	1 (5.9)
Amoxicillin 20 µg	0 (0.0)	0 (0.0)	17(100.0)
Tetracycline 30 µg	2 (11.8)	3 (17.6)	12 (70.6)

Table 3. Comparison of antimicrobial resistance by *Salmonella* isolates in the current study with reports from the previous literatures.

Antibiotics	Addis Ababa, Ethiopia Ashenafi et al., 1985 (Ashenafi et al., 1985)	Gondar, Ethiopia Mache et al., 1997 (Mache et al., 1997)	Rural western, Kenya Brooks et al., 2001 (Brooks et al., 2006)	This study Harar, Ethiopia (2007)	Gondar, Ethiopia (Asrat, 2008)
Chloramphenicol	-	46.7	36.0	62.3	83.7
Gentamicin	0.0	-	13.0	3.6	75.6
Ampicillin	-	68.9	53.0	100.0	81.2
Norfloxacin	-	-	-	7.1	0.0
Amoxicillin	-	-	35.0	100.0	-
Tetracycline	-	71.1	44.0	71.4	94.5

worrisome scenario affecting many parts of the world (Sharma et al., 2005; Khatun et al., 2011). Apart from intrinsic resistance, gene transfer and mutation are among the underlying mechanisms involved in the development of antimicrobial resistance by microbes (Sharma et al., 2005). Several factors contribute to resistance by pathogens causing gastroenteritis in the setting of a developing country like Ethiopia. These include frequent overuse, misuse and factors related to the potency and quality of antimicrobials and the distribution of resistant strains (Sharma et al., 2005; Asrat, 2008). In addition, syndromic diagnosis and

Table 4. Comparison of antimicrobial resistance by *Shigella* isolates in this study with reports from the previous literatures.

Antibiotics	Addis Ababa, Ethiopia Gedebou et al., 1982 (Gedebou et al., 1982)	Addis Ababa, Ethiopia Ashenafi et al., 1985 (Ashenafi et al., 1985)	Gondar, Ethiopia Assefa et al., 1997 (Assefa et al., 1997)	Jimma, Ethiopia Mache et al., 1997 (Mache et al., 1997)	Awassa, Ethiopia Roma et al., 1998 (Roma et al., 2000)	Rural western Kenya Brooks et al., 2001 (Brooks et al., 2006)	Gondar, Ethiopia Yismaw et al., 2005 (Yismaw et al., 2006)	Asmara, Eritrea Naik, 2006 (Naik, 2006)	Harar, Ethiopia This study (2007)	Gondar, Ethiopia Asrat, 2008 (Asrat, 2008)	Gondar, Ethiopia Tiruneh, 2008 (Tiruneh, 2009)
Chloramphenicol	25.0	-	-	50.0	63.0	77.0	52.8	72.9	29.4	74.7	67.8
Gentamicin	-	0.0	2.0	0.0	2.0	1.0	7.9	-	0.0	0.0	12.2
Ampicillin	21.0	-	90.0	70.0	93.0	53.0	79.9	82.4	100.0	78.7	78.9
Norfloxacin	-	-	-	-	-	-	-	-	5.9	0.0	1.1
Amoxicillin	-	-	-	-	-	43.0	-	-	100.0	-	-
Tetracycline	42.0	-	91.2	74.0	90.0	97.0	86.0	-	70.6	97.3	90.0

diagnostic imprecision usually force physicians to opt for broad spectrum antibiotics such as amoxicillin and tetracycline, over prescribing; and less antibiotic diversity which lead to the emergence and spread of antimicrobial resistance (Okeke et al., 2007). For instance, since non-typhoidal gastro-enteritis is usually self-resolving, antibiotic treatment is not commonly recommended (Kasper et al., 2005; Okeke et al., 2007). However, physicians in our study area are observed to prescribe medication on an empirical basis (Yismaw et al., 2006). Fortunately, there seems to be limited resistance to the drugs gentamicine and norfloxacine. However, given current trends in the country, unless concerted efforts are made to stem the unrestricted use of antimicrobials in the area, it will not probably be long before the microbes develop resistance to these expensive drugs and complicate effective treatment of gastroenteritis.

One of the limitations of this study was that due to lack of facilities, it was not possible to conduct identification of strain groups and susceptibility to multiple antibiotics which would have provided us with further insight into the distribution of strains and the extent of antibiotic susceptibility patterns in the area. However, this study is a pragmatic one, given that in the study area in particular, and in Ethiopia in general, antibiotics are prescribed on empirical bases without implementing the commonly recommended strain isolation and susceptibility testing procedures (Kasper et al., 2005). The small sample size of the current study may also be a limitation. However, given the lack of previous studies in the area, it could provide valuable information to clinicians and researchers. As the study is an *in vitro* one, it may not necessarily reflect *in vivo* resistance patterns and patient outcomes. Unlike previous studies in other parts of the country that assessed *Salmonella* or *Shigella* only (Gedebou et al., 1982; Mache et al., 1997; Mache et al., 1997; Roma et al., 2000; Yismaw et al., 2006; Tiruneh, 2009), the fact that the current study examined antimicrobial susceptibility tests for both *Salmonella* and *Shigella* is one of its strengths.

Conclusion

In conclusion, except for gentamicin and norfloxacin for which both *Salmonella* and *Shigella* isolates were highly susceptible, a high level of antimicrobial resistance was detected. Notably, the organisms seem to have developed complete resistance to ampicillin and amoxicillin. We assert that gentamicin and norfloxacin may be drugs of choice for treating diarrheal attacks by these organisms in the study area. It is recommended that a more rigorous study of the prevalence, antimicrobial susceptibility pattern and underlying mechanisms of drug resistance by *Salmonella* and *Shigella* isolates be conducted. In addition, treatment needs to be based on species identification and susceptibility testing rather than the currently practiced empirical treatment.

ACKNOWLEDGMENT

We thank Haramay University for funding the study.

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