Shigella and *Salmonella* serogroups and their antibiotic susceptibility patterns in Ethiopia

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المجموعات المصلية من الشيغيلات والسالمونيلات ونماذج حساسيتها للمضادات الحيوية في إثيوبيا

دانيال أسرات

الخلاصة: استفرد الباحث في هذه الدراسة المجموعات المصلية من أنواع الشيغيلات والسالمونيلات من مزارع البراز ودرس أنماط حساسيتها للمضادات الحيوية باستخدام الإجراءات المختبرية المعيارية. ومن بين 76 مستفردة من أنواع الشيغيلات كانت المجموعة المصلية "بي" (الشيغيلة الفلكسنرية) أكثر الأنواع السائدة (54.0%). ومن بين 37 مستفردة من ذراري السالمونيلات كانت المجموعة المصلية "بي" هي الأكثر مصادفة (81.1%). وقد أظهرت مخططات الحساسية للمضادات الحيوية لأنواع الشيغيلات والسالمونيلات مقاومة مطلقة (100%) للإريثروميسين، ومقاومة مرتفعة تزيد معدلاتها على 75% للأمبيسيلين والسيفالوتين والكلورامفينيكول والتتراسكلين. وكانت أنواع السلمونيلات مرتفعة المقاومة للجنتاميسين والسلفوناميد والتريميثوبريم – سلفاميتوكسازول. وكانت الشيغيلات حساسة للجنتاميسين (100%) ولحمض الناليديكسيك (97.3%)، في حين كانت الشيغيلات والسالمونيلات حسّاسة بنسبة (100%) للنورفلوكساسين.

ABSTRACT In this study, the serogroup and susceptibility patterns of *Shigella* and *Salmonella* spp. isolated from stool cultures were determined using standard laboratory procedures. Among the 76 *Shigella* isolates serogroup B (*Sh. flexeneri*) was the most prevalent species (54.0%) and among the 37 *Salmonella* strains serogroup B was also the most prevalent (81.1%). Antibiograms of *Shigella* and *Salmonella* spp. showed 100% resistance to erythromycin and high resistance rates (≥ 75%) to ampicillin, cephalothin, chloramphenicol and tetracycline. *Salmonella* spp. also had high resistance to gentamicin, sulphonamide, and trimethoprim–sulfamethoxazole. *Shigella* were susceptible to gentamicin (100%) and nalidixic acid (97.3%) and *Shigella* and *Salmonella* were 100.0% susceptible to norfloxacin.

Les sérogroupes de *Shigella* et de *Salmonella* et leur profil de sensibilité aux antibiotiques en Éthiopie

RÉSUMÉ Dans cette étude, le sérogroupe et le profil de sensibilité de bactéries *Shigella* et de *Salmonella* spp. isolées à partir de coprocultures ont été déterminés grâce à des procédures de laboratoire normalisées. Parmi les 76 isolats de *Shigella*, le sérogroupe B (*Sh. flexeneri*) était l'espèce la plus fréquemment retrouvée (54,0 %) et parmi les 37 souches de *Salmonella*, le sérogroupe B était également le plus représenté (81,1 %). Les antibiogrammes réalisés sur les espèces *Shigella* et *Salmonella* ont montré une résistance de 100 % à l'érythromycine et des taux de résistance élevés (≥ 75 %) à l'ampicilline, à la céfalotine, au chloramphénicol et à la tétracycline. *Salmonella* spp. présentait également une résistance élevée à la gentamicine, au sulfamide et au triméthoprime-sulfaméthoxazole. *Shigella* était sensible à la gentamicine (100 %) et à l'acide nalidixique (97,3 %) et *Shigella* et *Salmonella* étaient sensibles à 100 % à la norfloxacine.

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Introduction

Gastroenteritis-causing pathogens are the second leading cause of morbidity and mortality worldwide; it is mainly children under the age of 5 years who are at risk. The organisms responsible are rotaviruses, Norwalk-like viruses, enterotoxigenic *Escherichia coli* (ETEC), *Campylobacter jejuni* and *Clostridium difficile*, *Shigella* spp., *Salmonella* spp., *Cryptosporidium* spp. and *Giardia lamblia*. These organisms are readily transmitted via food, water, environmental contacts, pets and from person to person, with morbidity rates in developing countries 3-to-6-fold higher than in developed countries [*1*].

Antimicrobial resistance has complicated the selection of antibiotics for the treatment of enteric bacterial pathogens, particularly to commonly used antimicrobial agents such as ampicillin, tetracycline and trimethoprim–sulfamethoxazole [*2*].

In Ethiopia there is a great need to establish the identity and antibiotic susceptibility patterns of different bacterial agents which cause enteric infections in order to introduce effective treatment for diarrhoeal illness. This paper reports the results of the serogroups and antimicrobial susceptibility patterns of 76 *Shigella* and 37 *Salmonella* strains isolated from the stool cultures of patients and controls, with and without diarrhoea illnesses respectively, in Addis Ababa, Ethiopia.

Methods

Source of bacterial strains

The source of *Shigella* and *Salmonella* strains were stool specimens obtained from patients with diarrhoeal disease and controls without symptoms of diarrhoeal disease who were diagnosed with other illnesses. The nature of the diarrhoeal stool specimens was watery (82.4%), bloody (6.8%), mucoid (8.4%) and mixed (2.4%). From February 1992 to January 1993, a total of 76 *Shigella* and 37 *Salmonella* strains were isolated from Tikur Anbassa and Ethio-Swedish Children's Hospital, Addis Ababa, Ethiopia. All the isolated strains were kept frozen at –20 ºC in 15% tryptone soya broth (Oxoid Ltd., Basingstoke, Hampshire, England) containing 15% (v/v) glycerol.

Culture and identification of strains

Frozen *Shigella* and *Salmonella* strains were subcultured on MacConkey agar no.2 (Oxoid) and incubated at 37 ºC for 24 hours. These bacteria were identified by their characteristic appearance on the media and further confirmed by the pattern of biochemical reactions using a standard bacterial identification system (API 20E, bioMérieux, Marcy-l'Etoile, France). From a pure culture serogrouping and antimicrobial susceptibility testing were done.

Serogrouping of *Shigella* and *Salmonella* species

Shigella strains were serogrouped by slide agglutination tests using A1, A2, A3, B, C1, C2, C3 and D antisera (National Bacteriological Laboratory, Stockholm, Sweden). For *Salmonella* strains serogrouping was done by slide agglutination tests using poly O and groups A, B, C, D, E antisera (NBL, Stockholm, Sweden). These strains were further tested against poly H antisera. Those strains identified biochemically as *Salmonella typhi* were tested against Vi antisera.

Antimicrobial susceptibility testing

All *Shigella* and *Salmonella* strains were tested for their susceptibility to different antibiotics using the agar diffusion method according the methodology described by the National Committee for Clinical Laboratory Standards [*3*]. A McFarland 0.5 standard suspension of the bacteria in 5 mL of phosphate-buffered saline (Oxoid) was then prepared and swabbed over the entire surface of Petragnani culture medium (PDM Antibiotic Sensitivity Medium II, AB Biodisk, Solna, Sweden) with a sterile cotton swab. The inoculated plates were left at room temperature to dry for 3–5 minutes. With the aid of an automatic dispenser (Oxoid) a set of 10 antibiotic disks (Oxoid) with the following concentrations were then delivered to the surface of the PDM II plate: ampicillin 10 µg; cephalothin 30 µg; chloramphenicol 30 µg; erythromycin 15 µg; gentamicin 30 µg; nalidixic acid 30µg; norfloxacin 10 µg; sulfonamide 300 µg; tetracycline 30 µg and trimethoprim–sulfamethoxazole (TMP–SXT) 25 µg. The disks were gently pressed onto the medium with sterile forceps to ensure firm contact. Following overnight incubation at 37 ºC, clear zones produced by antimicrobial inhibition of bacterial growth were measured to the nearest millimetre using metal callipers. The zone diameter was interpreted using an interpretive chart defined by the Clinical and Laboratory Standards Institute [*4*].

A reference strain of *E. coli* (ATCC 25922) was used as a quality control for culture and susceptibility testing.

The criteria used to select the antimicrobial agents tested were based on availability and frequency of prescriptions for the management of enteric bacterial infections in Ethiopia (personal communication).

Results

Serogrouping

The results of serogrouping of the 76 *Shigella* isolates are presented in Table 1. Serogroup B (*Sh. flexneri*) was the most commonly isolated species (54.0%), followed by group A (*Sh. dysenteriae*) (22.4%), group D (*Sh. sonnei*) (15.8%) and group C (*Sh. boydii*) (7.8%). Of the serogroup *Sh. dysenteriae*, 82.4% were serotypes A1 and 17.6% were type A2. Among serogroup *Sh. boydii* the prevalence of serotypes were C1 (33.3%), C2 (50.0%) and C3 (16.7%). Of the 76 *Shigella* isolates, 74 were recovered from patients and 2 from controls (1 *Sh. dysenteriae* and 1 *Sh. flexneri*).

Among the 37 *Salmonella* strains, the most commonly isolated serogroup was group B (81.1%), followed by group D (*S. typhi*) (10.8%) and group C (8.1%) (Table 2). All *S. typhi* isolates were recovered from patients. Of the 37 *Salmonella* isolates, 24 were recovered from patients and 13 from controls.

Table 1 **Serogroups of 76 *Shigella* strains isolated from patients and controls**

Serogroups	No.	%
Sh. dysenteriae (A)	17	22.4
Type A1	14[a]	18.4
Type A2	3	4.0
Type A3	0	0.0
Sh. flexneri (B)[a]	41	54.0
Sh. boydii (C)	6	7.8
Type C1	2	2.6
Type C2	3	3.9
Type C3	1	1.3
Sh. sonnei (D)	12	15.8
Total	76	100.0

[a] *1* Sh. dysenteriae *and 1* Sh. flexneri *were recovered from controls.*

Table 2 **Serogroups of 37 *Salmonella* strains isolated from patients and controls**

Serogroups	No.	%
A	0	0.0
B	30	81.1
C	3	8.1
D *(S. typhi)*	4	10.8
E	0	0.0
Total[a]	37	100.0

[a] *24 recovered from patients and 13 from controls.*

Antimicrobial susceptibility testing

The results of antimicrobial susceptibility patterns of the *Shigella* and *Salmonella* isolates are shown in Table 3. Antibiograms of *Shigella* species showed that most strains were resistant to ampicillin (78.7%), cephalothin (86.7%), chloramphenicol (74.7%), erythromycin (100.0%), sulfonamide (54.7%), tetracycline (97.3%) and TMP–SXT (45.3%), but susceptible to gentamicin (100%), nalidixic acid (97.3%) and norfloxacin (100.0%). The *Salmonella* species were resistant to ampicillin (81.2%), cephalothin (86.4%), chloramphenicol (83.7%), erythromycin (100.0%), gentamicin (75.6%), nalidixic acid (37.8%), sulfonamide (81.1%), tetracycline (94.5%) and TMP–SXT (75.7%). All strains were susceptible to norfloxacin (100.0%). Among *Salmonella* spp. a comparatively low level of resistance (20%–25%) was detected in *S. typhi* to all antimicrobial agents tested except for erythromycin. Multidrug resistance (2 or more antibiotics) was noted in 80%–90% of both isolates (data not shown).

Table 3 **Antimicrobial susceptibility patterns of *Shigella* and Salmonella isolates as a whole**

Antimicrobial agent	Resistance (%)	
	***Shigella* spp. (*n* = 76)**	***Salmonella* spp. (*n* = 37)**
Ampicillin	78.7	81.2
Cephalothin	86.7	86.4
Chloramphenicol	74.7	83.7
Erythromycin	100.0	100.0
Gentamicin	0.0	75.6
Nalidixic acid	2.7	37.8
Norfloxacin	0.0	0.0
Sulfonamide	54.7	81.1
Tetracycline	97.3	94.5
Trimethoprim–sulfamethoxazole	45.3	75.7

n = *total number of isolates.*

Discussion

In this study, serogroup B (*Sh. flexeneri*) was the dominant *Shigella* serogroup, followed by group A (*Sh. dysenteriae*), group D (*Sh. sonnei*) and group C (*Sh. boydii*). These findings are in accordance with previous Ethiopian studies, except that in those studies *Sh. boydii* was the 3rd most commonly isolated species [*5–8*]. It is not unusual for one serogroup to replace another in the community from time to time. The comparative frequencies of *Shigella* serogroups vary with time, hygienic conditions and among different populations. In the early 1900s *Sh. dysenteriae* type 1 was the most common strain, whereas *Sh. flexneri* and *Sh. sonnei* are currently isolated most often, except for certain epidemics in which *Sh. dysenteriae* has been identified as the causative organism [*9,10*]. In developed countries, higher frequencies of *Sh. sonnei* have been reported, but these frequencies are gradually decreasing [*11*]. Epidemics of dysentery with frequent passage of blood and mucus, high fever, cramps and tenesmus are mainly caused by *Sh. dysenteriae* type 1 and *Sh. flexenri*, while *Sh. boydii* and

Sh. sonnei often causing non-watery (often bloody) diarrhoea during non-epidemic episodes [*12*]. Bennish and Wojtyniak [*13*] reported most fatal cases of shigellosis occur in developing countries as a result of severe dysentery and in rare cases, bacteraemia, especially that caused by *Sh. flexneri*.

The susceptibility of *Shigella* spp. to antibiotics has changed considerably over time. In the 1940s bacillary dysentery was treated successfully with sulfa-drugs and in the 1950s with tetracycline [*14*]. In the 1970s, resistance to one or more of the antimicrobial agents then in use began to emerge [*15*], but ampicillin was available and was used successfully to treat shigellosis by that time. When *Shigella* spp. began to develop resistance to ampicillin, TMP–SXT became the drug of choice [*16*]. Since 1980, however *Shigella* spp. have demonstrated a frequent and alarming resistance to TMP–SXT [*17*]. With the usefulness of these antimicrobials curtailed by the emergence of resistant strains, investigators are challenged to find new alternative drugs.

In this study the *Shigella* isolates were more susceptible to gentamicin (100%), nalidixic acid (97.3%) and norfloxacin (100%) than to drugs commonly used to treat shigellosis including ampicillin (21.3%) and TMP–SXT (54.7%). In the early 1980s, studies done in Addis Ababa, Ethiopia, indicated that all or most *Shigella* spp. were susceptible to TMP–SXT (98.0%–100%) and ampicillin (52%–79.0%) [*5*,*6*]. Furthermore, O'Brien reported in 1987 that in many areas of the world the susceptibility of *Shigella* spp. to nalidixic acid and aminoglycosides remains constant, whereas their susceptibility to ampicillin and TMP–SXT has decreased considerably [*18*]. The present study also revealed that a high level of resistance to cephalothin (86.7%), chloramphenicol (74.7%), erythromycin (100.0%), sulfonamide (54.7%) and tetracycline (97.3%). These findings are in agreement with the previous data obtained from Ethiopia [*7,8*] and other developing countries such as Bangladesh [*19*], and eastern Africa [*20*]. Similar patterns of antimicrobial susceptibility have been observed in the United States of America [*21*], Europe and Latin America [*22*].

Among the *Salmonella* strains, the most commonly isolated serogroup was group B, followed by group D (*S. typhi*) and group C. This is an agreement with some previous studies in Ethiopia [*6,8*], but in contrast to the earlier studies which showed that *S. typhi* was the dominant species [*23,24*]. All serogroups of *Salmonella* isolated in this study are known to cause gastrointestinal infections.

Among all antibiotics tested for *Salmonella* spp., the highest resistance was observed with ampicillin (81.2%), cephalothin (86.4%), chloramphenicol (83.7%), erythromycin (100.0%), gentamicin (75.6%), sulfonamide (81.1%), tetracycline (94.5%) and TMP–SXT (75.7%). These findings are in contrast with those studies done in Ethiopia in the 1980s that showed that most *Salmonella* spp. were sensitive to the majority of drugs tested (77.8%–98.4%) [*6,23–25*], but in agreement with those studies done in the 1990s [*8,26*]. The marked resistance pattern observed in this study also agrees with reports from other parts of the world [*22,27,28*]. Reports of antimicrobial resistance trends in *Salmonella* isolates by these investigators show that *Salmonella* has developed resistance to the above antimicrobial agent over the years. It was not possible to include 3rd-generation cephalosporins for susceptibility testing in this study. In Ethiopia, these drugs are not widely used for treatment of salmonellosis/shigellosis. In the near future there is a need to determine the susceptibility pattern for cephalosporins because resistance to these

drugs has been increasing, as documented elsewhere [*22*].

Many factors have contributed to the development of resistance in gastrointestinal pathogens, including misuse, overuse, quality and potency of the antimicrobial agents [*29*]. According to Salyers and Amábile-Cuevas [*30*], acquiring resistant genes, even from distantly related genera, is what accounts for the development and spread of drug resistance in bacteria. These authors further explained that the ability of resistance genes to adapt rapidly to new hosts so that they are not readily lost even in the absence of antibiotic selection might be the reason why increases in resistance can be so hard to reverse.

In conclusion, periodic evaluation of the susceptibility pattern of *Shigella* and *Salmonella* spp. would be particularly useful. In addition, controlled clinical trial studies are needed to verify the demonstrated efficacy of alternative drugs in treating shigellosis and salmonellosis. Furthermore, developing a broadly protective vaccine may be a more effective approach to curbing morbidity and mortality against these enteric pathogens.

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Climate and health: issues of concern in the Eastern Mediterranean Region

Findings of the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) indicate that the Eastern Mediterranean Region is one of those that will be worst affected. Climate change will aggravate current water scarcity to unprecedented levels that will seriously challenge water security for people and for food production. A rise in endemic morbidity and mortality due to diarrhoeal disease is expected, and malnutrition due to reduced food production will be exacerbated. A general rise in temperature and an increase in the number, intensity and duration of heatwaves and dust storms are expected, with potential for adverse health impacts. Natural disasters such as flooding and drought are projected to increase, with corresponding injuries and death. Changes in the distribution of vector-borne diseases such as malaria and dengue are also expected as a result of the changing environment.

Source: Fact sheets (http://www.emro.who.int/whd2008/factsheets.htm)