Increasing single and multi–antibiotic resistance in *Shigella species* isolated from shigellosis patients in Sana'a, Yemen

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ABSTRACT

Objectives: The epidemiology and antibiotic susceptibility of *Shigella species* changes over time. Updated susceptibility knowledge is necessary for appropriate empirical antibiotic treatment. Thus, this research aimed to study these changes in 2 time periods with an interval of 10 years.

Methods: Two hundreds and three *Shigella* strains, isolated from stool samples of diarrheic patients at the Central Health Laboratory in Sana'a, Yemen in 2 time periods (1993 and 2003) with a 10-year interval, were examined for serotyping and drug resistance pattern. Resistance patterns of the strains to 12 commonly used antimicrobial agents and minimum inhibitory concentrations of the antibiotics were tested.

Results: *Shigella flexneri* (60%) was found to be the most common isolate of the total *Shigella species*, followed by *Shigella dysenteriae* (28.6%) and *Shigella boydii* (11.3%). In *Shigella flexneri* strains, *Shigella flexneri* 3 (30.5%) was the most prevalent serotype, followed by *Shigella flexneri* 6 (17.2%), and *Shigella flexneri* 1 (12.3%). All strains were found equally susceptible to cefotaxime, ceftriaxone, ciprofloxacin, and gentamicin, but more than 80% of the strains of 2003 were resistant to tetracycline, co-trimoxazole, and 52% of the same strains were resistant to ampicillin. Resistance to chloramphenicol was found in 61%, cefuroxime in 56.2%, and cephradine, 52% of the strains. Overall, *Shigella species* showed statistically significant increase in resistance against tetracycline, cephradine, trimethoprim/sulfamethoxazole, nalidixic acid, and aztreonam ($p<0.05$) over the 10 years period. This indicates decreased efficacy of co-trimoxazole and nalidixic acid for the empirical treatment of shigellosis in Sana'a, Yemen. Almost 55.2% of the strains were resistant to 4 drugs.

Conclusion: This is one of the first studies reporting epidemiological pattern of *Shigella species* in Sana'a, Yemen with regard to serotypes and antibiotic resistance patterns. Based on these antibiotic resistance pattern findings, it is suggested that the commonly in use antibiotics including ampicillin, trimethoprim/sulfamethoxazole, tetracycline, and chloramphenicol should not be used for empirical treatment of shigellosis in Yemen.

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Shigellosis is a global human health problem.[1,2] Four *Shigella species (Sh. species)*, *Shigella dysenteriae (Sh. dysenteriae)*, *Shigella flexneri (Sh. flexneri)*, *Shigella boydii* (*Sh. boydii),* and *Shigella sonnei* (*Sh. sonnei)* are able to cause the disease. These species are subdivided into serotypes based on O-specific polysaccharide of the lipopolysaccharides (LPS).[3] *Shigella dysenteriae* type 1 produces severe

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disease and may be associated with life-threatening complications. The symptoms of shigellosis include; diarrhea and dysentery with frequent mucoid bloody stools, abdominal cramps and tenesmus. *Shigella spp*. causes dysentery by invading the colonic mucosa. *Shigella* bacteria multiply within colonic epithelial cells, cause cell death and spread laterally to infect and kill adjacent epithelial cells, causing mucosal ulceration, inflammation and bleeding.[2-5] Transmission usually occurs via contaminated food and water or through person-to-person contact.[3-5] Antimicrobial agents are the mainstay of therapy of all cases of shigellosis.[6,7] Due to the global emergence of drug resistance, the choice of antimicrobial agents for treating shigellosis is limited.[6-8] Although single dose of norfloxacin and ciprofloxacin has been shown to be effective, they are currently less effective against *Sh. dysenteriae* type 1 infection.[6] Newer quinolones, cephalosporin derivatives, and azithromycin are the drugs of choice.[3,6,7] However, fluoroquinolone-resistant *Sh. dysenteriae* type 1 infection have been reported.[7] In Yemen, the global emergence of multiple drug resistance in *Sh. species* is a serious public health concern and is posing a severe problem in treatment of intestinal and extra-intestinal infections due to these organisms. Currently, no vaccines against *Shigella* infection exist. Both live and subunit parenteral vaccine candidates are under development. Because immunity to *Shigella* is serotype-specific, the priority is to develop a vaccine against *Sh. dysenteriae* type 1 and *Sh. flexneri* type 2a.[8] *Shigella species* are important pathogens responsible for diarrheal diseases and dysentery occurring all over the world.[1,2] The morbidity and mortality due to shigellosis are especially high among children in developing countries.[1-3,9-11] A recent review of literature by Niyogi in 2005[5] concluded that, of the estimated 165 million cases of *Shigella diarrhea* that occur annually, 99% occur in developing countries, and in developing countries 69% of episodes occur in children <5 years of age. Moreover, of the case per million deaths attributed to *Shigella* infections in developing countries, 60% of deaths occur in the <5 age group. The present study describes the prevalence of different serotypes of *Shigella spp* and the prevalence of antibiotic resistant *Sh. species* isolated in 2 time periods from Sana'a, Yemen.

Methods. The *Sh. species* were isolated from fecal samples of patients who were attending to the Central Health Laboratory in Sana'a, Yemen in 2 time periods (1993 and 2003). Fecal samples were cultured directly on Xylose Lysine Deoxycholate agar (XLD), and inoculated in selenite F broth (Oxoid Ltd. UK), which was then sub-cultured on XLD agar after 24 hours of incubation at 35-37^{O}C. *Shigella spp* were biochemically identified by the API-20E system (BioMerioux SA, France) and sero-grouped using somatic group *Shigella antisera* (Murex Biotech Ltd, UK). Antibiotic susceptibility was performed by the disc diffusion technique according to the criteria of National Committee for Clinical Laboratory Standards (NCCLS).[3] Susceptibility tests were carried out on Mueller Hiton agar (Oxoid Ltd. UK) using the following concentrations (μg/ disc) of antibiotics discs (Becton Dickinson Co, Maryland USA); ampicillin (AMP) 20 μg, trimethoprim/sulfamethoxazole (SXT) 25 μg, tetracycline (TCY) 30 μg, cephradine (RAD) 30 μg, cefuroxime (CFX) 30 μg, chloramphenicol (CHL) 30 μg, ceftriaxone (CRO) 30 μg, nalidixic acid (NAL) 30 μg, gentamicin (GEN) 10 μg, aztreonam (AZM) 30 μg, cefotaxime (CTX) 30 μg, and ciprofloxacin (CIP) 5 μg.

Statistical analysis for comparison of data on resistance between the 2 time periods was carried out by the chi-square and fisher's exact tests. Statistical significance was set at the probability value (p value) 0.05 level or less.

Results. *Shigella spp* strains belonging to *Sh. flexneri* 1, 3 and 6 were the more common (60%), followed by *Sh. dysenteriae* 2 and 3, and *Sh. boydii* 2 (28.6% and 11.3%) (**Table 1**). Resistance to TCY and SXT was observed in most strains 94% and 79% of 2003 isolates, while in 1993 isolates strains, the resistance to TCY and SXT was lower (62.2% and 41%). Resistance to AMP and CHL was occurred in 46% and 43.8% of 1993 strains, on the other hand, only small percentage increases in the resistance among the 2003 strains towards these 2 antibiotics (49.5% and 58%). Resistance rate to CFX for 1993 strains was 31.2% and NAL 2%, and increased to 49.5% and 13.7% for 2003 strains. All the isolated strains of both 1993 and 2003 were sensitive to CTX, CRO, CIP and GEN (**Table 2**). Resistance to SXT significantly increased from 41% in 1993 to 79% in 2003 (p=0.001) and TCY and RAD resistance significantly increased from 62.2% to 94% (p=0.001) and 32-50% (p<0.04) (**Table 2**). In addition, resistance to NAL and RAD significantly increase from 2% and 31.2% to 13.7% and 49.5% (p<0.0001). Resistance against AMP and CHL was slightly increased, but this increase was not significant.

Discussion. Antimicrobial resistance in *Sh. species* has increased worldwide due to excessive use of antimicrobial agents.[12-14] Antimicrobial susceptibility to AMP, SXT and quinolones for

Table 1 - The prevalence of the different *Shigella* serotypes isolated.

***Shigella* serotypes**	**Frequency n (%)**
Shigella boydii 2	23 (11.3)
Shigella dysenteriae	58 (28.6)
Shigella dysenteriae 2	38 (18.7)
Shigella dysenteriae 3	20 (9.8)
Shigella flexneri	122 (60)
Shigella flexneri 1	25 (12.3)
Shigella flexneri 3	62 (30.5)
Shigella flexneri 6	35 (17.2)
Total	**203 100**

Table 3 - Antibiotic multiresistance patterns of *Shigella* isolated in 2003 comparing with 1993 isolates.

Resistance pattern	**Isolates of 1993 n = 98 n (%)**	**Isolates of 2003 n = 105 n (%)**
AMP, SXT, TCY, CHL, NAL	2 (2)	11 (10.5)
AMP, SXT, TCY, CHL	19 (19.4)	58 (55.2)
AMP, SXT, TCY	26 (26.5)	65 (62)
AMP, TCY, CHL	27 (27.6)	65 (62)
SXT, TCY, CHL	37 (37.8)	71 (67.6)
No resistance to all tested antibiotics	27 (27.4)	0 (0)

AMP - ampicillin, CHL - chloramphenicol, NAL - nalidixic acid, TCY - tetracycline, SXT - trimethoprim/sulfamethoxazole

Table 2 - Antibiotics susceptibility of *Shigella species i*solated in 2003 comparing with 1993 isolates.

Antibiotics	***Shigella resistance***		χ^2	***P*-value**
	2003 n = 105 N (%)	**1993 n = 98 N (%)**		
Trimethoprim/sulfamethoxazole	83 (79)	40 (41)	38	>0.001
Tetracycline	99 (94)	61 (62.2)	33	>0.001
Cephradine	52 (49.5)	31 (31.2)	6.7	0.009
Ampicillin	52 (49.5)	45 (46)	0.26	0.6
Chloramphenicol	61 (58)	43 (43.8)	4.1	0.04
Cefuroxime	59 (56.2)	8 (8.2)	53	>0.001
Nalidixic acid	14 (13.7)	2 (2)	14	>0.001
Aztreonam	4 (3.8)	0 (0)	3.8	0.05
Cefotaxime	0 (0)	0 (0)	-	-
Ceftriaxone	0 (0)	0 (0)	-	-
Ciprofloxacin	0 (0)	0 (0)	-	-
Gentamicin	0 (0)	0 (0)	-	-

Shigella isolated from fecal samples should be routinely tested and reported by clinical laboratories as the recommendations of NCCLS. The susceptibility to antimicrobial agents in intestinal isolates of *Shigella* has epidemiological importance.[6,7] AMP, SXT, NAL and cephalosporin are established as standard first line therapy for shigellosis.[6,7,9] The appearance of resistance to these antibiotics in *Sh. species* is posing a serious problem in the treatment of infections due to these organisms.[6,7,9,13-15] Resistant *Shigella* has a selective advantage in the environment, where excessive antibiotics are used and the antibiotic treatment itself is a major risk factor for infection with resistant bacteria.[6-9,13] The high level of resistance to TCY, SXT, AMP and CHL antibiotics might be due to the widespread using of these antibiotics as an empirical treatment of inflammatory diarrhea in Yemen (**Table 3**). This result is also supported by the fact that resistance to these antibiotics among enteric bacterial pathogens has increased dramatically over the last 30 years in developing countries.[6,7,16,17] In addition, one of the main factors of the widespread use of these antibiotics are the cheap price and broad-spectrum activities, they are thereby ensuring strong selection pressure for the maintenance of resistance to these antibiotics.[5-9] The cephalosporin is the largest and most diverse family of antimicrobial agents available. Although, they are rarely considered as drugs of first choice for therapy of bacterial infections, they are one of the most commonly prescribed agents for both ambulatory and hospitalized patients.[3-9] The resistance level to cephalosporin in this study varies from 52% for the first generations and 59% for the second generations and zero for the third generation. The absent of resistance to the third generation of cephalosporin might be due to the limitation use of these antibiotics due to the absence of oral formulation of these antibiotics and the high cost. Nalidixic acid was specifically tested in the present study as it is still used for dysenteric illness in Yemen. Only 13.7% of 2003 isolated strains were resistant to NAL and this result is lower than that reported in Israel,[9] India,[10,18]

and Nigeria[14] In those countries, the higher resistance level might be referred to the high rate of isolation of *Sh. dysenteriae* type 1, which developed more resistance to antibiotics than the other *Sh. species*.[6-9,12,15] Multi resistance patterns to several antibiotics are documented for *Shigella* isolates in the present study. All the isolates of 2003 were resistant to at least one antibiotic, with over than 58% of the isolates were resistant to ≥4 antibiotics. This result is similar to that reported in other developing countries in the same period.[9-14]

In conclusion, *Shigella* strains isolated in 2003 showed a significant increase resistance to the SXT, TCY ($p<0.01$) and RAD ($p<0.04$) and high increasing in the resistance rate to AMP in comparing with those isolated strains in 1993. Based on these antibiotic resistance pattern findings, it is suggested that the commonly in use available antibiotics including AMP, SXT, TCY, and CHL should not be used for empirical treatment of shigellosis in Yemen.

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