DISTRIBUTION OF SEROGROUPS AND SEROTYPES OF MULTIPLE DRUG RESISTANT SHIGELLA ISOLATES

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SUMMARY

Background: The distribution of *Shigella* serotypes is of epidemiological importance and antimicrobial therapy for shigellosis can prevent potential complications of shigellosis. Studies done fifty years ago in Ghana indicated the predominance of *Shigella flexneri.*

Objectives: To describe the distribution of *Shigella* serogroups and serotypes and their antibiogram profiles.

Study design: A prospective descriptive study.

Setting: The Microbiology Department of the Korle Bu Teaching Hospital.

Methods: Consecutive stool specimens from patients with diarrhoea submitted between February 2004 and June 2005 were cultured for *Shigella* and the isolates typed with commercial anti-sera. The susceptibilities of the isolates were also tested against eleven antimicrobial agents by the disc diffusion method. Minimum inhibitory concentrations (MIC) of isolates to ciprofloxacin were also determined by the E-test.

Results: Five hundred and ninety four diarrhoea stool specimens yielded 24 *Shigella* isolates with the following serogroup distribution: *S. flexneri* 70.8%, *S. dysenteriae* 16.7%, *S. sonnei* 8.3% and *S. boydii* 4.2%. Approximately 96% of the isolates were multi-drug resistant but all twenty four were susceptible to nalidixic acid and the fluoroquinolones (ofloxacin and ciprofloxacin). The MICs of twenty one of the isolates to ciprofloxacin were ≤ 0.064 μg ml^{-1}.

Conclusions: The predominance of *S. flexneri* was confirmed and *Shigella* isolates from Accra are susceptible to nalidixic acid and the fluoroquinolones. Surveillance of antimicrobial resistance particularly to monitor the emergence of *Shigella* strains resistant to nalidixic acid and the fluoroquiolones is important.

Keywords: Shigella, serogroups, serotypes, multidrug resistant, MIC

INTRODUCTION

The four species of the genus *Shigella*; *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* cause a wide spectrum of illness from watery diarrhoea to fulminant dysentery. The low infectious inoculum, (as few as 10 organisms) render *Shigella* highly contagious. Shigellosis therefore occurs as an endemic disease in populations characterized by over-crowding, poor housing, poor sanitation and inadequate water supply. The predominant serogroups of *Shigella* occurring in a region also appears to be related to the socioeconomic development; and evidence also indicates that the severity of shigellosis is related to the infecting serogroup[1]. For example, *S dysenteriae* type 1, also known as Shiga bacillus, has been recognized as the major cause of epidemic dysentery for nearly 100 years. Pandemics of Shiga dysentery have spread across Central America, Bangladesh, South Asia and Central and East Africa[2-4].

Antimicrobial therapy for shigellosis reduces the duration and severity of the disease and can also prevent potentially lethal complications. However, over the past few decades most bacteria have become progressively resistant to most of the first-line drugs used and the prevalence of multi-drug resistant strains is an important concern of treatment. It is also well documented that pandemic strains often exhibit multiple antibiotic resistance and induce severe illness with high case fatality in all age groups[5]. The current recommended antimicrobial of choice for the treatment of shigellosis is ciprofloxacin because of its efficacy, safety and reduced cost[6].

In the tropics, most infections are due to *S. flexneri*, and infections primarily due to *S. sonnei* are less common[7]. Studies done in Ghana over a fifty year period indicate the predominance of *S. flexneri*[8-10], but only Agbodaze et al[8] reported on the sensitivity of isolates to antimicrobial drugs.

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Knowledge of the distribution of *Shigella* serotypes among clinical isolates is of epidemiological importance. Six years ago, the World Health Organisation (WHO) emphasized the need to understand the burden of shigellosis in developing countries[11].

The purpose of this study was to determine the distribution of current *Shigella* serogroups and serotypes from Accra and to compare data obtained with earlier studies in Accra and the Central region of Ghana . Current antibiogram profiles and the MICs of isolates to ciprofloxacin are also reported.

METHODS

Consecutive diarrhoea stool specimens submitted for culture and sensitivity tests at the Microbiology Department, Korle Bu Teaching Hospital (KBTH), between February 2004 and June 2005 were cultured for *Shigella* using standard bacteriological methods[12]. Non-diarrhoea stool specimens were excluded and basic demographic data (age and sex) were obtained from request cards which accompanied the specimens.

Stool specimens were plated onto full plate deoxycholate citrate agar and *Salmonella Shigella* agar (Oxoid, Maryland, USA) as primary plates and a loopfull also inoculated into selenite F broth. Suspicious pale colonies on solid plates, which were Gram negative and oxidase test negative were biochemically characterized. Presumptive *Shigella* isolates on Triple Sugar Iron agar (Oxoid, Maryland, USA) were typed by slide agglutination tests with *Shigella* polyvalent grouping (Mast Group Ltd., Merseyside, U.K.) and monovalent antisera (Denka Seiken Co. Ltd., Tokyo, Japan).

Shigella isolates were tested for their susceptibility to 11 antimicrobials by the Kirby-Bauer method[13]. A control was set up using *Escherichia coli*, ATCC 25922, which was susceptible to all the tested drugs (ampicillin, 10µg; chloramphenicol, 30µg; cotrimoxazole, 25µg; tetracycline, 30µg; gentamicin 10µg; amikacin, 30µg; nalidixic acid, 30µg; cefuroxime 30µg; cefotaxime, 30µg; ofloxacin, 5µg; ciprofloxacin 5µg). The commercial antimicrobial discs were purchased from Oxoid Ltd., Basingstoke, United Kingdom. Zone sizes were measured in milliliters and compared to standards for interpretation. The MICs of twenty three of the isolates to ciprofloxacin were determined by the E-test (AB Biodisk, Solna, Sweden). The E-test consists of a concentration gradient of ciprofloxacin (range of 0.002-32 µg ml^{-1}) immobilized on one side of a strip which is placed on a seeded agar plate. After overnight incubation at 37^0C, the intersection of the growth ellipse and the strip gives the MIC by direct reading.

RESULTS

A total of 594 diarrhoea stool specimens were bacteriologically cultured and 24 *Shigella* isolates were obtained. This represents an isolation rate of 4.04%. All the 24 isolates were obtained from the primary plates and no mixed infection was observed. The age distribution of patients who were positive for *Shigella* ranged from 8months to 62 years with a mean age of 16.7 years. The age distribution and clinical isolates are shown in Table 1. Approximately 38% (9) of the patients who cultured positive for shigellosis were five years and below and 41.7% (10) were 20 years and above (Table 1). The ratio of females to males was 2.4:1.

Table 1 Age group distribution of patients from whom *Shigella* isolates were obtained

Age group/years	No. of Isolates	Shigella serogroups isolates
<1	2	*S.flexneri*(1), *S.dysenteriae* (1)
1-5	7	*S.flexneri*(5), *S.dysenteriae* (1) *S. sonnei* (1)
6-10	2	*S.flexneri* (1), *S.sonnei*(1)
11-15	3	*S.flexneri*(3)
16-20	1	*S.flexneri*(1)
21-25	-	-
26-30	3	*S.flexneri*(2), *S.dysenteriae*(1)
31-35	2	*S.flexneri*(1), *S.dysenteriae*(1)
36-40	1	*S.flexneri* (1)
41-45	2	*S.flexneri* (1), *S.boydii* (1)
>45	1	*S.flexneri* (1)
Total	**24**	

All the four serogroups of *Shigella*; Groups A (*S. dysenteriae*), B (*S. flexneri*), C (*S. boydii*) and D (*S. sonnei*) were isolated. *Shigella flexneri* formed 70.8% (17) followed by *S.dysenteriae* at 16.7% (4), *S. sonnei* at 8.3% (2) and *S. boydii* 4.2% (1). Stereotyping for *S.flexneri* gave the following distribution; four types 2, three type 1, three type 6, two type 3, two group 7 (8), and one type 4. Two of the isolates which agglutinated with the group anti-sera for *S.flexneri* were however non-reactive for any of the tested anti-sera for serotyping supplied by the manufacturer (Denka Seiken, Tokyo, Japan). All the *S.dysenteriae* were reactive to poly A and types 1-7 (Mast Group Ltd., U.K.). Typing with monovalent antisera from Denka Seiken, Japan showed that all the *S.dysenteriae* obtained were not the Shiga bacillus (epidemic strain). The

isolated *S.boydii* was reactive to poly C2 and types 12, 13, 14 and 15 (Mast Group Ltd., U.K.).

The only *S. boydii* isolate encountered in the study was isolated from a Ghanaian who showed symptoms of shigellosis upon his return from the United Kingdom.

The overall percentage resistance in the various serogroups is as follows: ampicillin, 95.8%; tetracycline, 91.7%; cotrimoxazole, 91.7%; chloramphenical, 83.3%; amikacin, 37.5%; gentamicin, 37.5%; cefuroxime, 16.7%; cefotaxime, 12.5%; nalidixic acid, ofloxacin and ciprofloxacin, 0% each. A total of 12 distinct resistance patterns were encountered in all the *Shigella* strains tested (Table 2).

Table 2 Distinct antibiograms of the 24 *Shigella* isolates from Accra

Resistance Pattern	Number of Isolates
Am, Ch, Co, Te, Ak, Gn, Cu, Ct	2
Am, Ch, Co, Ak, Gn, Cu, Ct	1
Am, Ch, Co, Te, Ak, Gn	4
Am, Co, Te, Ak, Gn	1
Am, Ch, Co, Te, Gn	1
Am, Ch, Co, Te, Ak	1
Am, Ch, Co, Te, Ct	1
Am, Ch, Co, Te	8
Am, Ch, Te	1
Am, Co, Te	2
Am, Co, Gn	1
Co, Te	1

Am=ampicillin; Ch=chloramphenicol; Co=cotrimoxazole; Te=tetracycline; Ak=amikacin; Gn=gentamicin; Cu=cefuroxime; Ct=cefotaxime

Twenty-three of the isolates (96%) were multiple drug resistant (MDR). Two of the strains were however resistant to 8 of the 11 antimicrobials tested whilst all 24 were susceptible to nalidixic acid and the fluoroquinolones (ofloxacin and ciprofloxacin). The MICs of twenty three of the isolates to ciprofloxacin were tested. Twenty one of the strains had MICs of ≤ 0.064 µg ml^{-1} and the other two were 0.125 µg ml^{-1} and 1.0 µg ml^{-1}.

DISCUSSION

In our present study, the isolation rate of 4.04% compares with a figure of 4.9% reported for work done in Calabar, Nigeria[14]. The two studies used similar cultural methods and media, and also sampled all age groups. Work done in Gomoa Fetteh[8], a rural community in Ghana, which sampled children between 0-6 years gave an isolation rate of 14.5%. Using similar cultural methods and media, isolation rates of 34.8% was achieved in Uganda[15], 2.2% in Northern Greece[16] and 7.1% in Ethiopia[17]. Modern techniques like the polymerase chain reaction (PCR) could detect *Shigella* infection more rapidly than the conventional culture methods[18]. In developing countries, cost implication however prohibits the use of PCR for routine laboratory work. Other limitations of the PCR methods also include the inability to conduct sensitivity tests which are vital in directing the choice of antimicrobials for treatment.

The order of serogroups distribution observed in the present study had been reported earlier in several studies done in Ghana over a 50 year period (Table 3). The predominance of *S. flexneri* had also been reported elsewhere, especially in Africa. In Calabar, Nigeria, it was 55% between 1986 and 1988[14] and in Kolkata, India it was 54.4%[19]. In Ethiopia, Mache et al[17] had *S. flexneri* distribution rate of 44% and in Northern Greece Kavaliotis et al[16], recorded 55%.

Any anti-*Shigella* vaccine in any particular setting will depend in part on the representation of serotypes in the vaccine and on the relative epidemiological importance of the different serotypes in that setting[11]. For *S. flexneri*, several authors in the past have reported the predominance of serotype 2 in Africa. In a three year study, Afoakwa[9] observed that the predominant serotype of *S. flexneri* varied from year to year and was shared among types 2, 3

Table 3 Serogroups distribution of *Shigella* over a 50 year period in Ghana

Studies in Ghana	*S.flexneri* n (%)	*S.dysenteriae* n (%)	*S. boydii* n(%)	*S. sonnei* n(%)	Total
Present study	17(70.8)	4(16.7)	1(4.2)	2(8.3)	24
[8]Agbodaze et al, 1989	38(80.9	7(14.9)	1(2.1)	1(2.1)	47
[9]Afoakwa, 1973	690(82.1)	94(11.4)	30(3.6)	23(2.9)	827
[10]Hughes, 1955	159(76.3)	22(10.7)	2(1.0)	25(12.0)	208

and 4. In the present study, out of the seventeen *S. flexneri* isolates, the distribution was as follows: four type 2, three type 1, three type 6, two type 3, two groups 7(8), one type 4 and two isolates were non-reactive with the specific anti-sera supplied by the manufacturer (Denka Seiken, Tokyo, Japan). A study with a larger sample size is required to make a firm conclusion on the predominance of *S. flexneri* type 2 in Ghana. Several studies suggest that the epidemic strain, *Shigella dysenteriae* type 1 is not frequently encountered in Ghana[8-10].

The changing pattern in antimicrobial susceptibilities among *Shigella* isolated poses a major difficulty in the determination of an appropriate drug for the treatment of shigellosis[19]. Approximately 96% of the isolates in the study were classified as MDR based on their resistance to three or more of the tested drugs. Fifteen to twenty years ago, shigellosis was treated successfully with inexpensive drugs like chloramphenicol, cotrimoxazole, and tetracycline. Nineteen of the isolates were resistant to all these 'first line' drugs of choice according to the Standard Treatment Guidelines, Ghana[20]; indicating that resistance to these antibiotics may be more problematic than once thought. Our *Shigella* isolates were all susceptible to the fluoroquinolone antibiotics tested. Earlier studies in Ghana by Afoakwa[9] and Hughes[10] did not report on the antibiogram of the isolates. However, Agbodaze et al[8] reported that approximately 94% of the *Shigella* strains they studied were sensitive to nalidixic acid. In Saudi Arabia, *Shigella* strains which became resistant to 'first line' drugs were successfully treated with nalidixic acid until emergence of resistance to the drug was detected[21]. The fluoroquinolones have been shown to be useful in the treatment of shigellosis[21] and the current recommended treatment of choice for multiple drug resistant *Shigella* is ciprofloxacin[6]. Though the MICs of 91% (21) of the isolates to ciprofloxacin showed relatively low values (≤ 0.064 μg ml^{-1}), its prescription and use must be monitored to prolong its effectiveness. Shigella isolates resistant to the fluoroquinolones has been reported in Japan[22].

The high frequency of multiple antibiotic resistant *Shigella* isolates observed in this study most probably reflect the ease of access and the extensive use of these antibiotics in Accra and probably across the entire country. Laws regulating the sale and use of antimicrobial agents must be strictly enforced to prolong the effectiveness of the newer drugs. Continual surveillance of the use of these antimicrobials and the dissemination of such information is also required.

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