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LABORATORY-BASED SURVEILLANCE OF SHIGELLA, ITS SEROTYPE AND RESISTANCE PATTERNS IN JOHANNESBURG, SOUTH AFRICA

Monde A. Nyila[1] and Lawrence J. Zulu [1, 2]

Addresses:

[1] Department of Life and Consumer Sciences, College of Agriculture and Environmental Sciences, P/Bag X6, University of South Africa, Florida 1710, Johannesburg, Republic of South Africa

[2] Department of Medical Microbiology Richmond, Lancet Laboratories, Cnr Menton & Stanley Road P.O. Box 8475 Johannesburg, 2000 Republic of South Africa

Corresponding author: Dr Monde A Nyila, PhD

E-mail: nyilama@unisa.ac.za

Abstract

Infectious diarrhoea such as shigellosis causes considerable morbidity and mortality, especially in infants, immune-compromised individuals and those living with HIV/AIDS. It is endemic in developing countries and in Sub-Saharan Africa, including South Africa, where diarrhoeal disease remains a leading cause of morbidity and mortality. This study was undertaken to establish incidences of *Shigell*a, its serotype and resistant pattern of isolates from human faeces from residence of Johannesburg, South Africa. All stools received between January to April from the private healthcare system were cultured on Xylose Lysine Deoxycholate and MacConkey Agar and Shigella was confirmed by standard biochemical reactions and a serological method. An antimicrobial sensitivity test was used. A total of 11 009 samples from patients between 22 days to 94 years old yielded 110 Shigella isolates, of which 47 (43%) were *S. flexneri*, 61 (55%) were *S. sonnei*, 1 (1%) was *S. dysenteriae* and 1 (1 %) was *S. boydii*. The majority of patients were children between < 1 to 5 years, 76 (69%), followed by those between 6 to 10 years 13

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(12%). In children up to 10 years, *S. sonnei* was confirmed in 52 cases (59%) and *S. flexneri* in 36 cases (41%). Overall, 53 (48%) males and 57 (52%) females were infected.

Key words: antibiotic, diarrhoea, prevalence, *Shigella*, Shigellosis,

1. Introduction

Infectious diarrhoea, such as shigellosis, is a major cause of disease and death that remains a significant human problem globally[1] particularly in children, the elderly, immune-compromised individuals and/or those living with HIV/AIDS[2]. South Africa faces its unique economic, political, socio-cultural and personal factors that interact to create distinctive continuous challenges that impede control and prevention of shigellosis. Laboratory-confirmed isolates have been reported in outbreaks in Delmas, a town 40 kilometres east of Johannesburg, in 1993, 2005 and 2007[3,4], on 25 April 2012, at the Paul Jungnickel Rehabilitation Centre in Pretoria, South Africa where three deaths from 140 habitants was reported[5], and in 1994 in KwaZulu-Natal[6]. *Shigella* has also been isolated from surface waters, sewage, food, kitchen towels and on crops contaminated by human faeces at varying temperatures[7,8]. South African studies have confirmed *Shigella* isolates from water[9,10] and from human faeces and blood samples[10,11]. Johannesburg, South Africa's largest city is situated in Gauteng, the country's most populous province. As the financial and manufacturing centre of South Africa, the city attracts workers from within South Africa, southern Africa, the rest of Africa, as well as from overseas. The migration of people into Johannesburg may pose major public health challenges; therefore, laboratory-based surveillance from assessing food, water, human and environmental specimens can provide crucial information about pathogen trends and aid in pathogen management. After a literature search, we found no published data regarding *Shigella* isolated from stools from the private healthcare system.

MATERIALS AND METHODS

Study design

The study was conducted from January to April 2013, a period when our laboratory receives the highest number of specimens. All stool specimens submitted for culture were included in the study. The specimens were received from paying patients, mostly with medical aid facility, of all ethnic backgrounds.

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Confirmation of shigellosis was based on laboratory culture and isolated of *Shigella* organism from faeces and antimicrobial susceptibility by the disc diffusion method[12].

Specimen collection

Specimens were collected in dry, clean, leak-proof stool containers. Total stool and/or rectal swabs specimens (n=11009) were cultured from all patients that presented with gastroenteritis. This study included all stools irrespective of participants being on antibiotic therapy.

Isolation of *Shigella*

Rectal swab and/or stool specimens were cultured in two ways according to Koneman *et al*. [13] In brief, the first was by inoculating a gram of stool into enrichment media, Selenite-F broth (Selecta Media, South Africa) followed by incubation for 18 to 24 hours after which a loopful of bacteria were streaked onto Xylose Lysine Deoxycholate (XLD) and MacConkey (MAC) (Selecta Media, South Africa) agar plates and incubated at 35-37 °C for 18 to 24 hours.
The second was by directly inoculating stool onto XLD and MacConkey agar plates. All media were aerobically incubated at 35-37 °C for 18 to 24 hours.

Characterisation of *Shigella*

Shigella-suspicious colonies were picked for characterisation on Vitek 2 (BioMérieux) according to the manufacturer's instructions. Where the Vitek 2 system was inconclusive, API 20E strips (BioMérieux) were used to characterise according to BioMérieux[14].
All *Shigella*-positive isolates were confirmed by slide agglutination assay by using commercially available polyvalent antiserum (Pro Lab Diagnostics, UK) against O for groups A, B, C, and D.

Antimicrobial-susceptibility testing

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The disc diffusion method was performed on *Shigella* isolates and interpreted according to the Clinical and Laboratory Standards Institute[12]. On Mueller–Hinton agar (Selecta, South Africa) the following commercially available antimicrobial discs were tested: ampicillin10µg, amoxicillin/clavulanic acid 30µg, azithromycin 10µg, ceftriaxone 30µg, ciprofloxacin 5µg, co-trimoxazole 25µg and tetracycline 30µg (Davies Diagnostics, South Africa). The "keyhole" effect, due to double disk synergism produced by ESBL strains, was examined for according to CLSI guidelines. A fully susceptible *Escherichia coli* strain (ATCC 25922) was used as quality control in each batch of assay.

Ethical clearance

This study has received the approval from the Lancet Publications Committee based on a review of its scientific content and data interpretation and the Research Ethics Committee of the College of Agriculture and Environmental Sciences, University of South Africa, ethics number 2013/CAES/039.

Results

Total isolates and species distribution

During the study period, from a total of 11 009 stool specimens examined, 110 *Shigella* isolates were recovered. The monthly distribution of stool specimens examined, and the organisms isolated are shown in Table 1. The *Shigella* incidences were essentially driven by *S. sonnei* accounting for 59% in January, 38% in February, 59% in March and 65% in April as shown in Table 1 and overall accounted for 55% (61/110). This was followed by *S. flexneri* (43% (47/110)). Single cases each of *S. boydii* and *S. dysenteriae* (1% (1/110) were recovered.

The infection age distribution ranged from 22 days to 94 years with the median being three years old from children below 10 years old. In this study there were 69% (76/110) cases followed by the 6 to 10 years age group 13% (3/110) as shown in Table 2. Despite the difference in gender behaviour and responsibilities such as changing diapers, mostly done by females, the difference between male (n=53) and female (n=57) infection frequency was negligible.

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The disc diffusion results of 110 isolates tested for antibiotic susceptibility (in Table 3) show that 13% (14/110) of strains were fully susceptible to all antibiotics. The monthly distribution of resistant and susceptible strains is shown in Table 4. Of the 96 strains, the following resistance patterns frequency was exhibited towards co-trimoxazole (83%), tetracycline (71%), ampicillin (27%), 5% towards augmentin (amoxicillin/ clavulanic acid) and 2% to azithromycin. Towards third-generation cephalosporin viz. ceftriaxone and fluoroquinolone viz. ciprofloxacin we observed a nil resistance. Strains resistant to two, three and or four antibiotics are shown in Table 3. A significant number of strains were resistant to single drugs of co-trimoxazole (n=91) and tetracycline (n=77). Likewise, 68% (n=75) of strains were resistant to both co-trimoxazole and tetracycline, 26% (n=29) to ampicillin, co-trimoxazole and tetracycline, while only two strains showed resistance to four drugs viz ampicillin, azithromycin, co-trimoxazole and tetracycline.

The single *Shigella boydii* isolated in this study demonstrated a full susceptibility to all antibiotics, while *Shigella dysenteriae* demonstrated resistance toward ampicillin, co-trimoxazole and tetracycline.

Resistance to ampicillin, augmentin, azithromycin, co-trimoxazole and tetracycline over the study period was largely by *S. sonnei* followed by *S. flexneri* as shown in Table 4.

Discussion

The prevalence rate of isolating *Shigella* 1% (110/11 009) observed in the study maybe due, in part, to continuing educational programmes especially at elementary schools, aggressive infection-control measures and possibly patients being on antibiotic before submitting stools for culture. Our study group involved people that paid for medical care and hence the head of household is assumed to be educated. The challenges of overcrowding, poor sanitation, malnutrition and lack of clean water are, therefore, assumed not to be synonymous with an educated population. No outbreak was reported during the study period. The highest *Shigella* incidence was noticed in March when there was a shift from estimated average of 21 isolates per month to more than double (n=46). The shift may be influenced by

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an increase in stools examined from children below 10 years [Table 1], which contributed 80% of the study, endorses that Shigellosis is mainly a childhood disease[1]. Children's susceptibility may be attributed to poor resistance, lack of previous exposure and exposure to contaminated environment due to play-related activities.

Findings of predominant species have important implications for treatment and prevention strategies. In most developing countries, *S. flexneri* is the predominant *Shigella* serogroup isolated from stool of patients with infectious diarrhoea[15]. Our findings agree with reports that state that the predominant serogroup is *S. sonnei* (n=61), followed by *S. flexneri* (n=47). All four serogroups co-exist in different proportions in our study region [Table 1]. In contrast to global prevalence of untypical isolates of Shigella[16,17], all our isolates agglutinated with specific antisera. Dominancy of *S. sonnei* has been noted in other cities globally, in the USA[18] and Asian countries[19]. Nevertheless, some studies suggest S. *flexneri* is more prevalent in tropical countries. Serogroup A (*S. dysenteriae*) (n=1) and serogroup C (*S. boydii*) (n=1) isolation frequency was low in this study. Not surprisingly, this is consistent with previous reports[11,20] of isolates from the public healthcare system in South African. The low isolation rate could be because serogroup A is commonly associated with and cause large dysentery outbreaks[21]; however, *S. boydii* (serogroup C) is generally the least frequently isolated of *Shigella* strains. It is typically associated with individuals who have travelled to endemic areas as equally observed in other studies[20,22]. However, due to low isolation rate in this study, incidence of these serotypes in the private healthcare system can be ascertained by longer surveillance studies. However, it is not unusual for one serogroup to replace another in the community from time to time. The comparative dominancy of serogroups fluctuates with time, hygienic conditions and difference in population. The serogroup dominancy in Ethiopia[23] and some other parts of the world seem to alternate mainly between *S. flexneri*, and *S. sonnei* as the most active agents of shigellosis.

Antimicrobial therapy is the cornerstone for treatment of shigellosis for reasons of shortening severity and duration of illness, reduce shedding of the organisms and prevent subsequent infection by household contacts, development of secondary complications and death. The guiding principles for the choice of antimicrobial in developing countries such as South Africa are cost and availability of drugs. The USA[18] and studies of the public healthcare system in South Africa [19] agree with our findings of ceftriaxone and ciprofloxacin being 100% potent over four months. Our study shows an increase in strains resistant to ampicillin, augmentin and co-trimoxazole, while random resistance to azithromycin was observed. Both *S. flexneri* and *S. sonnei* demonstrated resistance to

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azithromycin unlike23. In Bangladesh studies, 100% susceptibility was demonstrated. Tetracycline resistance pattern was constant for January, February and April while an increase in resistance for a corresponding increase in isolates was observed in March.

ESBL-producing *Shigella* pose a distinctive test to clinical microbiologists, clinicians, infection control professionals and scientists tasked with finding new drugs. ESBL-producing strains are usually found in hospitals where antibiotic is used frequently and the patients are critical. Despite high prevalence of resistant strains, ESBL production associated with *S. flexneri* and *S. sonnei* previously seen in South Africa between 2003 to 2009[24] was not observed in our study. However, ESBL-producing isolates have been observed elsewhere, like Nigeria (Asrat, 2008). Observed in our studies, though, is the growing number [n=7(6%)] of strains resistant to beta-lactam/beta-lactamase inhibitor and augmentin (amoxacillin-Clavulanic acid) that could eventually pose a threat in therapy programmes.

We noticed a portion of stool where *Shigella* species was isolated and were not bloody diarrhoea stools. This could then mean therapy based on bloody stool for shigellosis could miss some cases.

These findings confirm the need to formulate long-term surveillance programmes that would identify changes in antimicrobial susceptibility patterns and the dissemination of such formation to clinicians, scientists and all involved with human healthcare.

Conclusion and recommendations

Despite pivotal in confirming and identifying shifts in trends, laboratory-based study depends only on healthcare-seeking behaviour of individual patients. However, the following can be drawn from this study: all four *Shigella* strains are present in Johannesburg residences and all ages are vulnerable. *S. flexneri* and *S. sonnei* are clearly the leading causes of shigellosis isolated from human stool in this region. Since there was no *Shigella* outbreak during the study, we can statistical assume a possibility of every 2752.25 (11009/4) stools assayed to yield 27.5 *Shigella* of which 15.25 would be *S. Sonnei,* 11.75 *S. flexneri* and 0.5 shared between *S. boydii* and *S. dysenteriae.* This assumption represents a significant presence of Shigellosis in this society; however, only long time and/or continuous surveillance would probability give precise prevalence with the change of climatic seasons.

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TABLES

Table 1: Monthly and gender infection distribution of *Shigella* serogroups isolated from human stool specimens submitted for culture between January to April.

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Months	Total stools cultured	Total isolates	*Shigella* serogroups				Total infected	
			S. Boydii	*S. dysenteriae*	*S. Flexneri*	*S. Sonnei*	male	female
January	2 518	17			4 3	6 4	10	7
February	2 753	24	1		9 5	4 5	14	10
March	2 588	46		1	9 9	11 16	20	26
April	3 150	23			3 5	6 9	9	14
Total	11 009	110	1	1	47	61	53	57

Table 2: Distribution of Shigellosis cases according to the age group of patients with diarrhoea.

Age groups	*S. boydii*	*S. dysenteriae*	*S. flexneri*	*S. sonnei*	Total
<1-5			29	47	76
6-10		1	7	5	13
11-20			2	2	4
21-30			2	2	4
31-40				2	2
41-60			7	2	9
+61	1			1	2
total	1	1	47	61	110

Table 3: Antimicrobial resistance test results for *Shigella* isolates.

Antibiotic agent	Study months

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	Disc potency (μg)	January (n=17)	February (n=24)	March (n=46)	April (n=23)	Total resistance (n=110)
AM	10	4(24)	5(19)	14(30)	7(30)	30(27)
AUG	20/10	1(6)	2(8)	3(7)	1(4)	7(6)
AZM	10	1(6)	0	1(2)	0	2(2)
CRO	30	0	0	0	0	0
CIP	5	0	0	0	0	0
SXT	25	14(82)	19(79)	40(87)	18(78)	91(83)
TE	30	15(88)	15(63)	32(70)	15(65)	77(70)
SXT, TE		14(82)	15(63)	32(70)	14(61)	75(68)
AM,SXT,TE		4(24)	6(25)	14(30)	5(22)	29(26)
AM, AUG,SXT, TE		1(6)	1(4)	3(7)	1(4)	6(5)
AZM, AM, SXT, TE		1(6)	0	1(2)	0	2(2)
Fully susceptible		2	2	6	4	14(13)

Note: Numbers in the parenthesis show percentage
Where: AM, ampicillin; AUG, augmentin, AZM, azithromycin; CIP, ciprofloxacin; CRO, ceftriaxone; SXT co-trimoxazole; TE, tetracycline

Table 4: Monthly distributions of antibiotic resistant *Shigella* isolates.

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Months	Organisms	Antibiotic agents				
		SXT	TE	AUG	AZM	AM
		(n=13)	(n=15)	(n=1)	(n=1)	(n=4)
January	*S. flexneri*	3	5	0	1	3
	S. sonnei	10	10	1	0	1
February		(n=19)	(n=15)	(n=2)	(n=0)	(n=5)
	S. flexneri	10	8	2		3
	S. sonnei	9	7	0		2
March		(n=41)	(n=32)	(n=3)	(n=1)	(n=14)
	S. flexneri	14	11	2	0	8
	S. sonnei	26	20	1	1	5
	S. dysenteriae	1	1			1
April		(n=18)	(n=15)	(n=1)	(n=0)	(n=7)
	S. flexneri	5	3			4
	S. sonnei	13	12	1		3
	Total	91(83)	77(70)	7(6)	2(2)	30(27)

Note: Numbers in the parenthesis show percentage.