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Epidemiology of Shigellosis in Lagos, Nigeria: Trends in Antimicrobial Resistance

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ABSTRACT

Antimicrobial susceptibility of *Shigella* spp. and *Escherichia coli*, isolated from diarrhoeal patients in Lagos, was studied from March 1999 to February 2000. Four hundred fifty-nine isolates were identified as shigellae (62) and *E. coli* (397). *Shigella flexneri, S. dysenteriae, S. boydii*, and *S. sonnei* accounted, respectively, for 51.6%, 17.7%, 17.7%, and 13% of the total number of shigellae isolated. Eleven cases of shigellosis occurred in the age group of 0–9 years, 22 cases in the age group of 10–19 years, and 29 cases in the age group of ≥20 years. Of the 397 *E. coli* isolates, 11 were enteropathogenic *E. coli* (EPEC), and 7 of these strains were isolated with shigellae from stools of patients aged 0–9 year(s) (71.4%) and 10–19 years (28.6%). Over 70% of the *Shigella* isolates were resistant to two or more drugs, including ampicillin and tetracycline. Twenty-one distinct multidrug resistance patterns were observed in these isolates. During 1990–2000, resistance to ampicillin increased from 70% to 90%, co-trimoxazole from 77% to 85%, chloramphenicol from 71% to 77%, streptomycin from 71% to 79%, and nalidixic acid from 0% to 11.3%. Resistance to tetracycline decreased from 89% to 79% but with MIC_{50} and MIC_{90} values outside the susceptible range. While resistance to ciprofloxacin and ofloxacin remained nil with MIC_{50} and MIC_{90} values of 0.008 and 0.0016 µg/mL respectively. The results of this study revealed the endemicity of shigellosis with *S. flexneri* as the predominant serogroup in Lagos. Children and young adults were at a higher risk of severe shigellosis. The results also suggest that ampicillin, tetracycline, co-trimoxazole, and streptomycin should not be used as the first-line drugs in the treatment of shigellosis. Nalidixic acid should still be selectively used for treatment, while ciprofloxacin and ofloxacin can be ideal alternatives.

Key words: Dysentery, Bacillary; *Shigella*; *Escherichia coli*; Drug resistance, Microbial; Antibiotic resistance; Nigeria

INTRODUCTION

Shigellosis still remains a public-health problem in most developing countries where communities are ravaged by poverty, war, poor sanitation, personal hygiene, and water supplies (1). Epidemiologic reports show that about 140 million people suffer from shigellosis with estimated 600,000 deaths per year worldwide (2,3).

In Nigeria, shigellosis is still the major cause of dysentery in children aged 0-9 year(s), and many older children are hospitalized almost immediately after the onset of the disease (4). Although antibiotic therapy is important in the treatment of shigellosis in most endemic

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countries, antibiotics, including ampicillin, trimethoprim-sulphamethoxazole, and nalidixic acid, have been banned in the treatment of shigellosis in countries of Asia and sub-Saharan Africa (5,6). Furthermore, drugs, such as fluoroquinolones, azithromycin, and pivamdinocillin, have been found to be efficacious both in vitro and in vivo in the treatment of shigellosis in children and adults (7,8). These drugs have helped control and manage shigellosis in many countries (9).

In Nigeria, an active surveillance study on shigellosis was carried out over a decade ago (10). Since then, no studies were conducted to examine the status of drug resistance in shigellosis. This situation might have been responsible for the severity, prolonged illness, and high hospitalization rate of patients with shigellosis in Lagos recently (4). If this trend continues too long, the mortality rate due to shigellosis may rise in Lagos. Furthermore, there is a paucity of information on co-infection of shigellae with other pathogenic *Enterobacteriaceae*.

This study was, therefore, carried out to determine the antibiotic susceptibility profile of *Shigella* spp. and to determine the incidence of co-infection of shigellae with enteropathogenic *Escherichia coli* (EPEC).

MATERIALS AND METHODS

Patients and sample collection

During March 1999-February 2000, 1,020 stool samples were collected from diarrhoeal patients who sought treatments at the Massey Children's Hospital, Mainland Infectious Diseases Hospital, Central Medical Health Laboratory Services, Yaba, Lagos, and Randle Medical Health Centre, Lagos. These hospitals/clinics serve as referral centres for most communities and towns in Lagos metropolis. Patients included children, adults, and elderly people.

Bacteriology

Fresh faeces of patients were collected into Cary-Blair tubes (10 mL per tube) and transported to Microbiology Laboratory of Nigerian Institute of Medical Research, Lagos, for immediate culture. The specimens were inoculated on MacConkey and Salmonella-Shigella agar. Colonies suspected to be shigellae were further subcultured on Simmon-citrate, motility-indole-urea and Kligler-iron agar. The *Shigella* isolates were speciated biochemically as outlined by Cowan (11). Colonies on MacConkey plates suspected to be *E. coli* were further tested for enteropathogenicity by slide and tube agglutination tests using *E. coli* polyvalent antisera A, B, and C (Biotec Laboratories, UK).

Antimicrobial susceptibility testing

Resistance patterns of the isolated *Shigella* strains to 12 antibiotics were determined by the agar-diffusion technique (12). Every inoculum was prepared by inoculating 5 mL of Mueller-Hinton broth with five colonies of 18-hour old pure *Shigella* culture followed by incubation in ambient air and at 37 ^{0}C for 16 hours. The resulting turbid culture was standardized to the turbidity of 0.5 McFarland (A_{625nm}=0.09) using 0.85% NaCl as the diluent. A sterile cotton swab was dipped into the standardized suspension, drained, and used for inoculating 25 mL of Mueller-Hinton agar in a 100-mm plate (Sterilin, UK). The inoculated plates were air-dried, and antibiotic disks from Oxoid (UK) and Mast Laboratories (Merseyside, UK) were mounted on them. The 12 antibiotics that were tested for susceptibility included: ampicillin, tetracycline, colistin sulphate, co-trimoxazole, cefotaxime, nitrofurantoin, nalidixic acid, streptomycin, ofloxacin, ciprofloxacin, chloramphenicol, and gentamicin. The plates were inverted and incubated in ambient air at 37 $^\circ$C for 18 hours. Zones of inhibition were recorded in millimetres and were compared with those of *E. coli* ATCC25922, which served as the control.

Determination of minimum inhibitory concentration

Minimum concentration of each antibiotic inhibitory to the growth of 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates was determined on isosensitivity agar (Oxoid, UK). The agar contained concentration ranges of the antibiotics prepared by two-fold serial dilution according to the National Committee for Clinical Laboratory Standards (13). A multipoint inoculator was used for dispensing 20 mL of adjusted inoculum (10^7 cfu/mL) of each isolate onto the surface of the antibiotic plate to obtain a final inoculum size of 10^4–10^5 cfu/spot. Antibiotic-free plates were inoculated last and were used as negative controls. The positive controls were the plates (one plate per antibiotic tested) inoculated with the reference strain *E. coli* ATCC25922. MIC_{50} and MIC_{90} of each antimicrobial agent against the *Shigella* serogroups were evaluated after incubating the plates, containing completely absorbed inocula, in ambient air at 37 ^{0}C for 18 hours (14).

RESULTS

Of the 1,020 diarrhoeal stool samples screened, 397 *E. coli* strains and 62 shigellae strains were identified. While shigellae were isolated from patients ranging in age from 0 to >40 year(s), 35.5% of the patients were aged 10–19 years. The males outnumbered the females in nearly all age groups (Table 1).

(17.7%) cases of shigellosis each (Table 2). Eight cases of shigellosis were caused by *S. sonnei*.

Of the 11 EPEC strains isolated, 7 were isolated with shigellae from diarrhoeal stools of patients aged 0–9 years and 10–19 year(s) (Table 3). The two age groups were responsible for 71.4% (5 strains) and 28.6% (2 strains) co-infections.

Table 1. Age and sex distribution of shigellae and *E. coli* isolated from diarrhoeal patients

	E. coli (n=397)				*Shigella spp.* (n=62)			
Age group (in years)	Male	%	Female	%	Male	%	Female	%
0-4	33	8.3	21	5.3	1	1.6	1	1.6
5-9	46	11.6	28	7.1	5	8.0	4	6.5
10-19	41	10.3	37	9.3	15	24.2	7	11.3
20-29	37	9.3	55	13.9	6	9.7	8	12.9
30-39	27	6.8	38	9.6	6	9.7	2	3.2
≥40	15	3.8	19	4.8	3	4.8	4	6.5

Shigellae were isolated in each month from March 1999 to February 2000 (Fig.). The highest number of isolates was obtained in March, while the lowest numbers were obtained in January and July. *Shigella flexneri* strains were identified in 32 (51.6%) shigellae-positive cultures, while *S. dysenteria*e and *S. boydii* accounted for 11

Resistance to seven antibiotics, such as ampicillin, tetracycline, colistin sulphate, co-trimoxazole, chloramphenicol, streptomycin, and cefotaxime, ranged from 43.5% to 90.3% (Table 4).

Resistance to nalidixic acid was 11.3% and to nitrofurantoin and gentamicin 3.2% each. No isolates

Fig. Monthly distribution of *Shigella* isolates obtained from stools of diarrhoeal patients, March 1999-February 2000

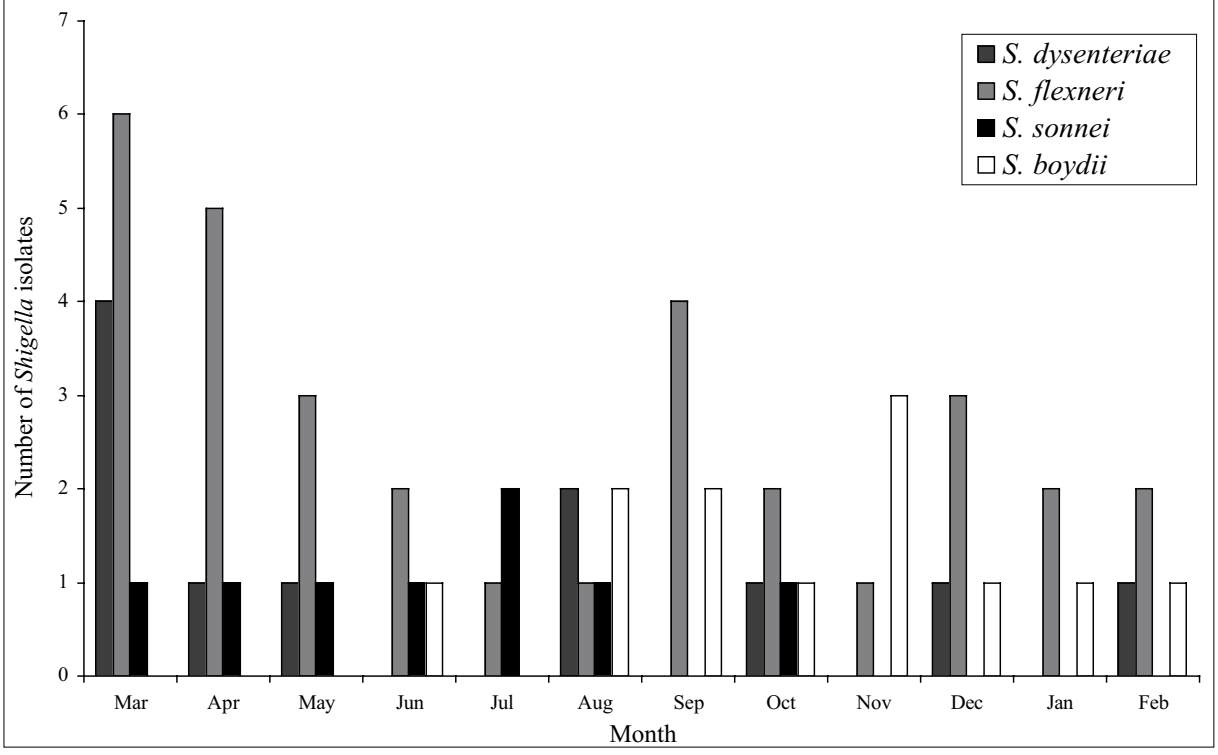

Table 2. Age and sex distribution of patients with *Shigella* serogroups isolated

Age group (in years)	Isolates		Sex				Serogroups							
	No.	%	No. of males	%	No. of females	%	*S. dysenteriae*		*S. flexneri*		*S. sonnei*		*S. boydii*	
							No.	%	No.	%	No.	%	No.	%
0-4	2	3.2	1	1.6	1	1.6	1	1.6	1	1.6	0	0.0	0	0.0
5-9	9	14.5	5	8	4	6.5	3	4.8	5	8.1	1	1.6	0	0.0
10-19	22	35.5	15	24.2	7	11.3	3	4.8	14	22.6	2	3.2	3	4.8
20-29	14	22.6	6	9.7	8	12.9	2	3.2	7	11.3	1	1.6	4	6.5
30-39	8	12.9	6	9.7	2	3.2	1	1.6	3	4.8	2	3.2	2	3.2
≥40	7	11.3	3	4.8	4	6.5	1	1.6	2	3.2	2	3.2	2	3.2
Total	62	100.0	36	58.0	26	42.0	11	17.7	32	51.6	8	13.0	11	17.7

Table 3. Relative risk of co-infection of shigellae with enteropathogenic *E. coli* in different age groups

Age group (in years)	Patients with EPEC		Patients with *Shigellae*	
	With *Shigella* isolates	Without *Shigella* isolates	With EPEC isolates	Without EPEC isolates
0-9	5	1	5	6
10-19	2	1	2	20
≥20	0	2	0	29

Table 4. Comparison and incidence of resistance to 12 antibiotics among 62 *Shigella* isolates and serogroups from Lagos, Nigeria

Antibiotic	% of resistant *Shigella* isolates in 1990 (10)	Resistant isolates		Serogroups							
				S. dysenteriae		*S. flexneri*		*S. sonnei*		*S. boydii*	
		No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	70.0	56	90.3	9	81.8	30	93.7	7	87.5	10	90.9
Tetracycline	89.0	49	79.0	9	81.8	25	78.1	6	75.0	9	81.8
Colistin sulphate	91.0	51	82.3	10	90.9	27	84.4	6	75.0	8	72.7
Co-trimoxazole	74.0	53	85.5	9	81.8	27	84.4	7	87.5	10	90.9
Chloramphenicol	71.0	48	77.4	10	90.9	26	81.3	4	50.0	8	72.7
Streptomycin	71.0	49	79.0	10	90.9	26	81.3	5	62.5	8	72.7
Cefotaxime	-	27	43.5	5	45.5	15	46.9	3	37.5	4	36.3
Nalidixic acid	0.0	7	11.3	1	9.1	5	15.6	1	12.5	1	9.1
Nitrofurantoin	0.0	2	3.2	0	0.0	2	6.3	0	0.0	0	0.0
Gentamicin	10.0	2	3.2	1	9.1	0	0.0	0	0.0	0	0.0
Ofloxacin	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Ciprofloxacin	-	9	0.0	0	0.0	0	0.0	0	0.0	0	0.0

showed any resistance to ofloxacin and ciprofloxacin. In all, 21 patterns of antibiotic resistance were found among the *Shigella* serogroups, and further analysis revealed that over 70% of the isolates were resistant to two or more drugs, including ampicillin and tetracycline (Table 5).

Minimum inhibitory concentrations of each of the 12 antibiotics that made 50% and 90% of shigellae susceptible in vitro are shown in Table 6. At 0.008 µg/mL and 0.016 µg/mL, ofloxacin and ciprofloxacin inhibited the growth of 50% and 90% of the isolates.

Generally, there was a wide variation in MIC_{50} and MIC_{90} values of the antimicrobial agents, and those of streptomycin, colistin sulphate, tetracycline, chloramphenicol, co-trimoxazole, and ampicillin were very high.

DISCUSSION

In this study, we isolated 62 *Shigella* strains from diarrhoeal stools of 1,020 patients, and speciation showed that *S. flexneri* caused 32 (51.6%) of the total cases of shigellosis. *S. dysenteriae* and *S. boydii* were identified in 11 (17.7%) cases each, while eight (13%) cases were caused by *S. sonnei*. This pattern of shigellosis indicates that *S. flexneri* is the predominant and most active serogroup in Lagos. Our results agree with the pattern reported by Olukoya and Oni (10). Thus, the predominance of *S. flexneri* in Lagos has not changed

Table 5. Antibiotic resistance patterns of *Shigella* isolates

Antibiotic resistance pattern	No. of resistant strains			
	S. dysenteriae (n=11)	*S. flexneri* (n=32)	*S. sonnei* (n=8)	*S. boydii* (n=11)
Amp Tet	0	0	1	0
Amp Tet Cot	0	1	1	1
Amp Tet Str Chl	1	2	0	1
Amp Cot Str Chl Tet	0	1	0	1
Amp Chl Cot Col Tet	0	1	0	1
Amp Cot Col Tet Cef	0	1	1	1
Amp Cot Col Tet Chl	1	2	0	0
Amp Cot Col Str Chl	0	2	1	1
Amp Tet Str Chl Col	1	2	0	0
Str Cot Cef Chl Nal	0	1	0	0
Tet Str Cot Col Chl	1	1	0	1
Amp Cot Col Cef Str Chl	1	2	1	1
Amp Tet Str Cot Col Cef	1	2	1	1
Amp Str Col Cot Cef Chl	1	2	0	0
Amp Cot Col Str Tet Chl	1	5	1	1
Amp Tet Cef Str Nal Nit Col	0	1	0	0
Amp Tet Chl Str Col Cot Nal Cef	1	2	0	1
Amp Tet Col Cot Cef Nal	0	1	0	0
Tet Str Col Cot Chl Gen	1	0	1	0
Amp Tet Col Cot Chl Cef Str	1	2	0	0
Amp Tet Str Col Cot Cef Chl Nit	0	1	0	0

Amp=Ampicillin; Cef=Cefotaxime; Chl=Chloramphenicol; Col=Colistin sulphate; Cot=Co-trimoxazole; Gen=Gentamicin; Nal=Nalidixic acid; Nit=Nitrofurantoin; Str=Streptomycin; Tet=Tetracycline

since 1990. This is unlike the situation in the islands of Bengal (15) where *S. flexneri* and *S. dysenteriae* alternate as most active agents of shigellosis or in endemic communities of Israel and Pakistan where *S. sonnei* persists as the predominant aetiologic agent (16,17).

Table 6. Minimum inhibitory concentrations of each of 12 antimicrobial agents for *Shigella* isolates (n=62)

Antimicrobial agent	Range µg/mL	MIC_{50} µg/mL	MIC_{90} µg/mL
Tetracycline	1-128	64.000	128.000
Gentamicin	0.25-32	1.000	6.000
Cefotaxime	0.004-32	8.000	18.000
Co-trimoxazole	0.02-64	10.000	32.000
Ampicillin	2-128	64.000	128.000
Nalidixic acid	1-64	4.000	8.000
Nitrofurantoin	1-32	2.000	4.000
Colistin sulphate	1-128	64.000	128.000
Ofloxacin	0.003-1	0.008	0.016
Ciprofloxacin	0.003-1	0.008	0.016
Streptomycin	1-128	60.000	120.000
Chloramphenicol	0.25-64	64.000	64.000

Unlike Olukoya and Oni (10), we stratified 62 cases of shigellosis by age and sex, and the pattern obtained shows that both children and young adults were at a higher risk of contacting the disease. This is similar to the distribution pattern found by Khan *et al.* in Bangladesh (18). The high incidence of shigellosis in young adults might be a reflection of secondary infection caused by contacts with the lower age groups, i.e. 0–4 year(s), 5–9 years, and within 10 to 19-year and 20 to 29-year age groups. Khan *et al.* reported secondary infection rates of 30.6% and 28.3% in the age groups of 0–4 year(s) and 5–9 years, and secondary infection was observed more in males than females (18). Similarly, the total number of males with shigellosis was more than that of females in the first three groups in our study. Poor personal hygiene and food intake have been documented in these age groups. In a recent study conducted in Lagos, Akinyemi *et al.* found children (with males dominating) as potential reservoirs of pathogenic *Enterobacteriaceae* (19).

We also examined the incidence of co-infection with EPEC. We found 7 EPEC and *Shigella* co-infections in

the age groups of 0–9-year(s) (71.4%) and 10–19 years (28.6%). EPEC has been reported as the major cause of acute diarrhoea in children aged ≥2 years in Latin America (20). Gomez *et al.* further revealed the significance of EPEC and *Shigella* co-infections among children who died due to diarrhoea (21). In Nigeria, infant mortality due to diarrhoea was attributed mostly to EPEC in the late 1980s (22). The present trend in Lagos calls for urgent measures to reduce deaths of children due to shigellosis. More so is that shigellosis is endemic in Lagos (10), which is further supported by our results; most of our study children with shigellosis were hospitalized (4). Therefore, public-health strategy should ensure clean water supply, good sewage management, and a clean environment.

Furthermore, the results of the antimicrobial susceptibility testing make the management of shigellosis in the population studied complicated. Our antibiograms are at variance with those of Olukoya and Oni (10). When compared with the latter, we found increased resistance rates for ampicillin (70–90.5%), streptomycin (71–79%), trimethoprim-sulphamethoxazole (i.e. co-trimoxazole; 77.4–85.5%), and nalidixic acid (0–11.3%). The decreased resistance rate for tetracycline (89–79%) was not significant for its use, because its MIC_{50} and MIC_{90} values of 64 µg/mL and 128 µg/mL were outside the susceptibility range (13). These antibiotics have been grossly abused in Nigeria (23,24). Since there are no antibiotics with poor in-vitro efficacy against shigellae that have good clinical cure rate, our results suggest that these drugs, except nalidixic acid, should not be used in the treatment of shigellosis in any age groups in Lagos. Nalidixic acid was exempted, because it demonstrated over 78% bacteriologic cure (13) with MIC_{50} and MIC_{90} values within the susceptibility range (Table 6). However, the upsurge of resistance to nalidixic acid by shigellae, as observed in our study, mimics trends in communities where nalidixic acid was introduced to cure shigellosis caused by ampicillin and trimethoprim-sulphamethaxazole-resistant strains (7). Henceforth, to prevent a situation where the non-use of nalidixic acid in the treatment of shigellosis will be considered, it is imperative that the drug should be used in patients, especially in children, whose aetiologic agents are susceptible in vitro. Nalidixic acid remains the drug of choice for shigellosis in Pakistan (17). The drug maintains appreciable intracellular concentrations that are ideal for efficacy against inflammatory diarrhoeal illnesses, such as shigellosis (25).

Our results further confirm the in-vitro bacteriologic efficacy of gentamicin and nitrofurantoin as reported previously (10). Since these drugs possess poor intracellular concentrations, these are ineffective for a clinical cure (26).

Unlike nalidixic acid, ciprofloxacin and ofloxacin retain their 100% efficacies that they possessed 10 years ago against bacteriologic shigellae. They are, thus, excellent drugs either for first-line treatment or for 'back-up' treatment of shigellosis in Lagos. However, the drugs are restricted for use in children due to their reported toxicity (27) and are relatively expensive when used in adults. In Lagos, the cost of ciprofloxacin or ofloxacin ranges from $5.00 to $7.00 for a five-day therapy in adults. Since nalidixic acid is apparently the only available effective drug in use in the treatment of shigellosis in Lagos, there is a need to employ other antibiotics with proven bacteriologic and clinical efficacy against shigellosis in other endemic communities. Such drugs include macrolides, such as azithromycin (7), pivmecillinam (8), and third-generation cephalosporins, such as cefixime and ceftazidime (9). Our result was inconclusive to suggest the non-use of cefotaxime as the first-line drug, although the resistance rate of 43.5% was obtained. More than 62% of the *S. sonnei* and *S. boydii* strains were still susceptible to cefotaxime, suggesting that this drug could still be clinically relevant when used selectively and as required in the treatment of shigellosis in Nigeria.

We conclude that government and non-government organizations have a significant role to play in ensuring the procurement and distribution of new shigellocidal drugs at affordable prices, providing funds and machinery for continuous surveillance, and making long-lasting policies that will solve the problems of illegal drug trade, inappropriate drug prescription, and self-medication in Nigeria. On a global scale, the current tempo toward the production of live oral and parenteral subunit vaccines against shigellosis should be raised. Vaccines, such as SC602 (*Shigella flexneri* 2a), which have been subjected to phase-1 clinical trials and found safe, immunogenic, and protective should be considered for further trials, and their usage be legalized in most endemic communities (28). It is only then that the public-health burden of shigellosis can be curtailed drastically.

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