ORIGINAL ARTICLE

Species Distribution and Antibiotic Resistance of *Shigella* Isolates in an Urban Community in Malaysia

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Summary

There is an increasing trend for *Shigella* isolates worldwide to be resistant to commonly prescribed antibiotics. The species distribution and antibiotic resistance of *Shigella* species isolated from children in Kuala Lumpur, Malaysia from 1978 to 1997 was reviewed. Three hundred and eighty six isolates were positive for *Shigella* species, representing 1.4% (95% CI: 1.3% - 1.6%) of the 26320 total stool specimens and 13% (95% CI: 11.8% - 14.2%) of 2986 isolates positive for bacterial pathogens. *Shigella flexneri*, constituting 74% of all isolates in the first five years of the study, decreased by 40% during the last five years (95% CI of decrease: 22.1% - 57.9%, p-value < 0.001) to 34%. There was a significant reduction (chi^2 for linear trend = 77.6, p-value < 0.001) in the number of *Shigella* isolates as a percentage of total stool isolates obtained. 58% of the 241 isolates tested for antibiotic sensitivity were resistant to at least one antibiotic, and 42% were multi-resistant to three or more antibiotics. *Shigella* species was not a common pathogen among children admitted with diarrhoea in Kuala Lumpur, and was more likely to be resistant to commonly prescribed antibiotics.

Key Words: Shigellosis, Children, Antibiotic resistance

Introduction

Shigellosis is a major problem in developing countries with high morbidity and mortality,[1] and presents a pressing challenge for providing effective antibiotic therapy because of its invasive nature. But *Shigella* species are also more prone to acquire antibiotics resistance[2]. Up to 51% of *Shigella* isolates obtained from children with acute gastroenteritis in Kenya were resistant to at least one antibiotic[3]. Common antibiotics used to treat bacterial enteric infection, such as trimethoprim-sulfamethoxazole (TMP-SMX), ampicillin, tetracycline, and nalidixic acid are no longer effective in shigellosis[4]. Many countries in the Southeast Asian region have reported the emergence of multi-resistant strains of *Shigella*[4-6].

Shigella flexneri is the commonest species in developing countries while *S. sonnei* is the commonest in developed countries[1]. In Malaysia, *S. flexneri* was the most predominant *Shigella* species isolated in the 1970's and 1980's[7, 8]. The

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reported resistance rate to antibiotics was high[8]. However, no recent data is available to ascertain the predominant *Shigella* strain and the antibiotic sensitivity pattern. The aim of the present study was to report the species distribution and pattern of antibiotic resistance of *Shigella* strains isolated in children less than 16 years old admitted with acute diarrhoea in an urban hospital in Kuala Lumpur, Malaysia, from 1978 to 1997.

Materials and Methods

The results of all stool cultures processed at the Department of Medical Microbiology, University of Malaya Medical Centre (UMMC), Kuala Lumpur from 1978 to 1997 were retrieved and reviewed. All results positive for bacterial pathogens obtained from children younger than 16 years of age, admitted for acute gastroenteritis during the study period were included. The pattern of antibiotics sensitivity of these pathogens was analysed.

During the study period, stool cultures were processed for bacterial pathogens according to routine microbiological laboratory procedures. *Shigella* species were identified by standard bacteriological techniques. Serotyping was performed by slide agglutination using commercially prepared antisera.

Susceptibility to antibiotics was tested using the standard disc diffusion method according to NCCLS guidelines. Commercially available discs were used and zone diameters were read as sensitive or resistant according to the manufacturer's recommendation. Ampicillin, chloramphenicol, trimethoprim-sulphamethoxazole (TMP-SMX), tetracycline, netilmicin and gentamicin were routinely tested from 1979, cefriaxone from 1989, and ciprofloxacin from 1992. Multi-resistance was defined as resistance to more than one antibiotic tested.

Student t-test was used for statistical analysis and was expressed in proportions and 95% confidence intervals where appropriate. p-value of < 0.05 was considered to be significant.

Results

During the study period, a total of 26320 stool specimens were obtained from children less than 16 years of age admitted to UMMC for acute gastroenteritis (Table I). Of these, 2986 (11%, 95% CI: 10.6% - 11.4%) were positive for common bacterial pathogens. The four most common bacterial pathogens isolated were non-typhoidal *Salmonellae* (57%), enteropathogenic *E. coli* (14%), *Shigella* species (13%), and *Campylobacter* species (5%).

Pattern of isolation of Shigella species (Tables I & II): Three hundred and eighty six isolates were positive for *Shigella* species, representing 1.4% (95% CI: 1.3% - 1.5%) of all stool specimens and 13% (95% CI: 11.8% - 14.2%) of isolates positive for bacterial pathogens (Table I & II). The number of stool cultures obtained from children < 16 years of age was fairly constant throughout the study period. There was, however, a significant reduction in the isolation rate of *Shigella* species (Table I, chi^2 for linear trend = 77.585, p-value 0.001).

Shigella flexneri (63%) was the commonest species isolated during the study period, followed by *S. sonnei* (34%) while *S. dysenteriae* (2%) and *S. boydii* (0.5%) were uncommon (Table II). There was a change in the pattern of isolation of various *Shigella* species throughout the study period (Table II). *Shigella flexneri* was the commonest species isolated in the first five years of the study (1978 – 1982), constituting 74% of all *Shigella* isolates during this period. This pattern then declined to 34% during the last 5 years (1993 – 1997) of the study period (reduction of 40%, 95% CI 22.1% - 57.9%; $p < 0.001$). Instead, *S. sonnei* emerged as the commonest species isolated during the last 5 years (59%). There was a trend for *S. flexneri* to be less common as compared to the total *Shigella* isolates (chi^2 for linear trend = 5.535, p-value 0.018).

Serotyping of *S. flexneri* was performed on 55 isolates. The commonest serotype isolated was 2a (n=30, 55%), followed by 1b (n=11, 20%) and 3a (n=11, 20%). There was one isolate each for serotypes 1a, 5b and 6.

Antibiotic sensitivity (Table III): Antibiotic sensitivity pattern of 241 of the 346 isolates (70%) from 1979 onwards was available for review. Resistance rates (RR) to ampicillin and tetracycline exceeded 50% while that of chloramphenicol was 47%. There was no significance difference in the RR for ampicillin, tetracycline and chloramphenicol between the first and second ten years of the study. The overall RR for TMP-SMX was 31%, but the RR increased from 10% before 1990 to 58% after 1990 ($p< 0.001$). The RR to gentamicin and ceftriaxone was low, less than 5%. No resistance to ciprofloxacin was noted.

Only 102 isolates (42%) were sensitive to all the antibiotics tested. The remaining 139 (58%) were resistant to at least one antibiotic; 39 isolates (16%) were resistant to two antibiotics while 100 isolates (41%) were resistant to three or more antibiotics. The percentage of multi-resistance was 50% in the first ten years, and 48% in the second ten years of the study.

Table I: Shigella-positive isolates from children < 16 years of age with acute diarrhoea: University of Malaya Medical Centre, Kuala Lumpur, 1978 - 1997

Year	No. of stool samples processed (a)	No. of isolates +ve for bacterial pathogens* (b)	No. of isolates +ve for Shigella spp.	Shigella-positive isolates as a % of (a)	Shigella-positive isolates as a % of (b)
1978	1033	167	40	3.9	24.0
1979	1002	145	22	2.2	15.2
1980	1203	214	51	4.2	23.8
1981	1328	196	43	3.6	21.9
1982	1401	183	43	3.2	23.4
1983	1728	209	32	1.8	15.3
1984	1349	254	26	1.9	10.2
1985	1474	217	17	1.1	7.8
1986	1661	221	27	1.6	12.2
1987	1440	146	19	1.3	8.6
1988	1415	154	6	0.4	3.8
1989	1325	131	11	0.8	8.4
1990	1377	135	5	0.3	3.7
1991	1053	82	8	0.7	9.7
1992	1513	96	4	0.3	4.2
1993	1378	108	9	0.7	8.3
1994	1226	100	10	0.8	10.0
1995	1327	108	8	0.6	7.4
1996	1058	74	2	0.2	2.7
1997	1029	46	3	0.3	6.5
Total	**26320**	**2986**	**386**	**1.4**	**12.9**

** including non-typhoidal Salmonella; enteropathogenic E. coli; Shigella species, Aeromonas species, Campylobacter spp. and Vibrio spp.,*

Table II: Distribution of various Shigella serogroups in Malaysia

Study (reference)	Year	Total number of isolates	S. flexneri (%)	S. sonnei (%)	S. dysenteriae (%)	S. boydii (%)
Kan SP *et al*(7)	1974-1978	241	168 (70)	71 (29)	0 (0)	2 (1)
Jegathesan (8)	1980-1981	406	351 (86)	54 (13)	1 (0.2)	0 (0)
Present study*	1978-1997	386	244 (63)	132 (34)	8 (2)	2 (0.5)
	1978-1982	199	147 (74)	46 (23)	6 (3)	0 (0)
	1983-1987	121	67 (55)	53 (44)	1 (1)	0 (0)
	1988-1992	34	19 (56)	14 (41)	0 (0)	1 (3)
	1993-1997	32	11 (34)	19 (59)	1 (3)	1 (3)

** There was a significant reduction in the proportion Shigella flexneri isolates as compared to total Shigella isolates (chi2 for linear trend = 5.535, p-value 0.018)*

Table III: Resistance rates of Shigella isolates to antibiotics, Kuala Lumpur, 1978 - 1997

Antibiotics	Overall (n=241)			1978 - 1987 (n=174)			1988 - 1997 (n=67)			
	Tested	Resistant	RR (%)[a]	Tested	Resistant	RR (%)	Tested	Resistant	RR (%)	p-value[b]
Ampicillin	231	124	54	165	89	54	66	33	50	0.69
Tetracycline	209	117	56	162	91	56	47	26	55	0.91
Chloramphenicol	215	102	47	157	80	51	58	22	38	0.09
TMP-SMXc	189	31	16	153	14	9	36	17	47	< 0.001
Netilmicin	200	20	10	164	8	5	36	2	6	0.89
Gentamicin	49	1	2	29	1	3	20	0	0	0.40
Cefriaxone	31	1	3	31	1	3				
Cirpofloxacin	28	0	0	28	0	0				

a: RR: resistance rate
b: comparison between the first and second decades
c: TMP-SMX: trimethoprim-sulfamethoxazole

Discussion

Shigella species was not a common bacterial pathogen causing acute diarrhoea among children in Kuala Lumpur. There was a significant trend for *Shigella* species to become less common in children from Kuala Lumpur during the two decades of study period. Overall, it constituted 13% of all stool specimens positive for any bacterial pathogen and 1.4% of all stool specimens obtained from children with acute diarrhoea. Similar patterns of bacterial gastroenteritis in children where *Shigella* species is uncommon were reported in many developed countries such as Hong Kong,[9] Italy,[10] and Australia[11].

This study has shown that shigellosis has become an uncommon childhood gastrointestinal infection in an urban area in Malaysia as the standard of living of a community has improved. The per capita gross national product in Malaysia (at market prices in Malaysian currency, Ringgit (RM); one US Dollar equalled to RM 2.40 on average during the study period) increased from RM 2689

in 1978 to RM 12102 at 1997[12, 13]. Death attributed to acute gastroenteritis among children was low, the case mortality rate was 2.1/1000 admissions[14]. Hospital admission of childhood diarrhoea usually has a low morbidity[15]. There was a strong health care infrastructure and little malnutrition.

Shigella flexneri is more common in developing countries while *S. sonnei* is more common in developed communities[1]. In Malaysia, *S. flexneri* was the commonest species isolated from two studies conducted in 1970's and 80's[7, 8]. In this study, *S. flexneri*, the commonest species isolated became relatively uncommon during the later part of the study period, and was replaced by *S. sonnei*. This pattern is seen in other more developed communities. Shigella dysenteriae, the most virulent of all Shigella species, was the least common. It constituted only 2% of all *Shigella* species isolated. Only one isolate was seen in the last eleven years of the study period.

There is a trend for *Shigellae* isolates to be multi-resistant in many parts of Asia[16-19]. Antibiotics that are cheap but previously effective in shigellosis may no longer be useful, and have to be replaced by more expensive ones such as the third generation cephalosporins, the quinolones, or the potentially more toxic aminoglycoside[19]. In this study, there was a high RR for *Shigella* isolates to commonly prescribed antibiotics. Only 42% of the isolates were sensitive to all the antibiotics tested. RR to ampicillin, chloramphenicol and tetracycline exceeded 40%, while the RR to TMP-SMX rose significantly. Multi-resistance was seen in 41% of the isolates throughout the study period.

However, there was no significant difference in the RR to ampicillin, chloramphenicol or multi-resistance rate during the first ten years when compared with that of the second ten years. Two reasons can be offered for this observation. Firstly there was already a high RR to ampicillin and chloramphenicol during the first decade of the study. Therefore it was unlikely that RR would even be higher during the subsequent years of the study. Secondly, as shown in Table III, there was a shift in the antibiotics chosen to be tested for sensitivity in the later years of the study, reflecting the prevalent choice of antibiotics used in the latter years of the study. Thus ampicillin and chloramphenicol were no longer routinely tested for their sensitivity as it was expected that the *Shigella* isolates would be resistant to them. Other antimicrobials such as third generation cephalosporins and the quinolones were tested instead.

In conclusion, *Shigella* has become an uncommon bacterial pathogen causing diarrhoea in children in and around Kuala Lumpur, Malaysia. There is a high RR to commonly prescribed antibiotics, as similarly observed in many parts of the Asia. Third generation cephalosprins and the quinolones may be alternative antibiotics for the treatment of shigellosis in Malaysia.

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References

1. Kotloff KL, Winickoff JP, Ivanoff B, et al. Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. Bull World Health Org 1999; 77: 651-66.
2. Tauxe RV, Puhr ND, Wells JG, Hargett-Bean N, Blake PA. Antimicrobial resistance of Shigella isolates in the USA: the importance of international travellers. J Infect Dis 1990; 162: 1107-11.
3. Shapiro RL, Kumar L, Philips-Howard P, et al. Antimicrobial-resistant bacterial diarrhea in rural western Kenya. J Infect Dis 2001; 183: 1701-4.
4. Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Clin Infect Dis 1998; 26: 341-5.
5. Lim YS, Tay L. Serotype distribution and antimicrobial resistance of Shigella isolates in Singapore. J Diarrhoeal Dis Res 1991; 9: 328-31.
6. Chu YW, Houang ETS, Lyon DJ, Ling JM, Ng TK, Cheng AFB. Antimicrobial resistance in Shigella flexneri and Shigella sonnei in Hong Kong, 1986 to 1996. Antimicrob Agents Chemother 1998; 42: 440-3.
7. Kan SKP, Chan MKC. Annual and geographical distribution of Shigella serotypes in Sabah, Malaysia from 1974 to 1978. Med J Malaysia 1980; 35: 9-13.
8. Jegatheson M. Serotype prevalence and antibiotic susceptibility of Shigella strains isolated in Malaysia during 1980 and 1981. J Diarrhoeal Dis Res 1984; 2: 102-4.
9. Biswas R, Lyon DJ, Nelson EAS, Lau D, Lewindon PJ. Aetiology of acute diarrhoea in hospitalised children in Hong Kong. Trop Med Int Health 1996; 1: 679-83.
10. Caprioli A, Pezzella C, Morelli R, et al. Enteropathogens associated with childhood diarrhoea in Italy. Pediatr Infect Dis J 1996; 15: 876-83.
11. Barnes GL, Uren E, Stevens KB, Bishop RF. Etiology of acute gastroenteritis in hospitalized children in Melbourne, Australia, from April 1980 to March 1993. J Clin Microbiol 1998; 36: 133-8.
12. Vital statistics of Malaysia. In: Social Statistics Bulletin of Malaysia, 1978; Department of Statistics, Malaysia.
13. Vital statistics of Malaysia. In: Social Statistics Bulletin of Malaysia, 1997; Department of Statistics, Malaysia.
14. Lee WS, Ooi TN. Deaths following acute diarrhoeal illness among hospitalised infants in Kuala Lumpur. Med J Malaysia 1999; 54: 303-9.
15. Lee WS, Lee SP, Boey CCM. Admission to hospital with acute gastroenteritis in Malaysia. Singapore Paediatr J 1997; 39: 185-90.
16. Sohail M, Sultana K. Antibiotic susceptibilities and plasmid profiles of Shigella flexneri isolates from children with diarrhoea in Islamabad, Pakistan. J Antimicrob Chemother 1998; 42: 838-9.
17. Ghosh AR, Sehgal SC. Shigella infections among children in Andaman: an archipelago of tropical islands in Bay of Bengal. Epidemiol Infect 1998; 121: 43-8.
18. Jahan Y, Hossain A. Multiple drug-resistant Shigella dysenteriae type 1 in Rajbari district, Bangladesh. J Diarrhoeal Dis Res 1997; 15: 17-20.
19. Mamun KZ, Tabassum S, Hussain MA, Shears P. Antimicrobial susceptibility of Shigella from a rural community in Bangladesh. Ann Trop Med Parasitol 1997; 91: 59-61.