PILOT STUDY OF THE ANTIMICROBIAL SUSCEPTIBILITY OF *SHIGELLA* IN NEW ZEALAND IN 1996

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by

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SUMMARY

Shigella species are an important cause of gastrointestinal disease manifested by watery diarrhoea that may progress to mucoid bloody diarrhoea or dysentery. The emergence of antibiotic resistance in *Shigella*, particularly multiresistance to ampicillin and trimethoprim-sulphamethoxazole (TMP-SMZ), the drugs of choice, has complicated antibiotic treatment of shigellosis. Studies in several countries have shown an increase in the prevalence of resistance among *Shigella* spp.

As there is little current information on the prevalence of resistance among *Shigella* isolated in New Zealand, this pilot project aimed to examine the antimicrobial susceptibilities of recent isolates of *Shigella*. A total of 107 isolates were referred to ESR Communicable Disease Centre in January to June 1996. All isolates were serotyped, biotyped and tested for susceptibility to ampicillin, ciprofloxacin, amoxicillin/clavulanic acid, tetracycline and TMP-SMZ by an agar dilution method.

The predominant species was *Shigella sonnei* which accounted for nearly 70% of the isolates. *Shigella flexneri* accounted for 23% and *Shigella boydii* and *Shigella dysenteriae* were relatively rare. Resistance to ampicillin and TMP-SMZ was detected in 42.1% and 57.0% respectively. Amoxicillin/clavulanic acid resistance occurred in 15.9% and cephalothin resistance in 5.6% of the isolates. Combined resistance to ampicillin and TMP-SMZ was detected in 30.8% of the isolates. Resistance to cefotaxime, ciprofloxacin, gentamicin and imipenem was not detected and 31.8% of the isolates were sensitive to all agents tested. Ampicillin resistance was significantly more prevalent in *S. flexneri* than *S. sonnei* and in isolates of known overseas origins.

This pilot study demonstrated that a good proportion of *Shigella* isolated in New Zealand are resistant to the drugs of choice, ampicillin and TMP-SMZ, and nearly a third of *Shigella* exhibited combined resistance to ampicillin and TMP-SMZ. This combined resistance complicates empirical treatment of shigellosis as alternative antimicrobial agents are more costly and some agents like quinolones are not yet approved for use in children.

The findings indicate that there is a need to monitor the prevalence of antimicrobial resistance in *Shigella* and suggest that susceptibility testing of *Shigella* might be needed to aid empirical therapy. It was reassuring that resistance to the other alternative antimicrobial agents like quinolones and third generation cephalosporins was not detected.

INTRODUCTION

Shigella species are an important cause of gastrointestinal disease manifested by watery diarrhoea that may progress to mucoid bloody diarrhoea or dysentery.[1] The severity of the disease is related in part to the infecting species; *Shigella dysenteriae* and *Shigella flexneri* infections can progress to dysentery while *Shigella boydii* and *Shigella sonnei* generally cause a self-limited, watery diarrhoea. Effective antimicrobial therapy has been shown to reduce the duration and severity of the disease as well as shortening the period in which organisms are shed.[2]

Shigella isolates resistant to a variety of antimicrobial agents have been reported in Asia,[3] Europe,[4] and the Americas.[5,6] Past experience has shown that *Shigella* spp. have a propensity to develop resistances to antimicrobial agents. In response to increases in ampicillin and trimethoprim-sulphamethoxazole (TMP-SMZ) resistances among *Shigella* isolates in Bangladesh, nalidixic acid was introduced for the treatment of shigellosis. Within four years, there was a high rate of nalidixic acid resistance among *S. dysenteriae* type 1.[3] In developed countries like the United States and Finland, multiply resistant strains have been largely linked with foreign travel and day care centres.[4,7]

The emergence of antibiotic resistance in *Shigella*, particularly to the drugs of choice ampicillin and TMP-SMZ, has complicated antibiotic treatment of shigellosis. Studies in several countries have shown an increase in the prevalence of resistance among *Shigella* spp.[3,4,5] There is little current information on the antimicrobial susceptibilities of *Shigella* isolated in New Zealand. This pilot project aimed to examine the antimicrobial susceptibility of *Shigella* referred to ESR in January to June 1996.

METHODOLOGY

Bacterial strains

A total of 107 clinical isolates of *Shigella* spp. referred to ESR Communicable Disease Centre during January to June 1996 were tested. The isolates were referred by 20 hospital and community laboratories throughout New Zealand and were identified and serotyped by standard procedures.

Antimicrobial susceptibility tests

The susceptibility of the isolates was tested by an agar dilution method following NCCLS guidelines[8] to the following antimicrobial agents: ampicillin, amoxicillin/clavulanic acid, cephalothin, cefotaxime, ciprofloxacin, TMP-SMZ, gentamicin and imipenem. Mueller-Hinton agar was used to test for all the antimicrobial agents except for TMP-SMZ where Mueller-Hinton agar supplemented with 5% lysed horse blood was used. An inoculum of 10^4 cfu/spot was applied to plates using a multipoint inoculator. The plates were incubated at 35°C for 18 hours. MIC endpoints were read as recommended by NCCLS and the susceptibility results were interpreted according to NCCLS recommendations.[9] The following controls were included with every batch of tests:

- *Escherichia coli* NZRM Acc 916 (ATCC 25922), sensitive control
- *E. coli* NZRM Acc 2749 (NCTC 11560), β-lactamase positive control

RESULTS

Distribution of *Shigella* species

Of 107 *Shigella* isolates referred in January to June 1996, *S. sonnei* accounted for 69.2% (74) and *S. flexneri* for 23.3% (25). *S. boydii* and *S. dysenteriae* were relatively rare, accounting for 4.7% (5) and 2.8% (3) respectively. The distribution of serotypes and biotypes among the 107 isolates is shown in Table 1. Among these isolates was a cluster of eight isolates of *S. sonnei* biotype g from an outbreak.

Table 1. Distribution of biotypes and serotypes of Shigella isolates

Species	**Number**	**%**
Shigella sonnei		
biotype a	50	46.7
biotype e	3	2.8
biotype g	21	19.6
Shigella flexneri		
type 1	1	0.9
type 1a	1	0.9
type 2	3	2.8
type 2a	14	13.1
type 3	1	0.9
type 3a	2	1.9
type 4b	1	0.9
type 6	2	1.9
Shigella boydii		
type 2	2	1.9
type 13	2	1.9
type 18	1	0.9
Shigella dysenteriae		
type 1	1	0.9
type 2	2	1.9

Antimicrobial susceptibilities of *Shigella*

The ranges, MIC_{50}, MIC_{90} and percent resistance of each antibiotic tested are shown in Table 2. Resistance to ampicillin and TMP-SMZ was detected in 42.1% and 57.0% respectively. The prevalence of resistance to amoxicillin/clavulanic acid and cephalothin was 15.9% and 5.6%. One isolate was intermediate resistant to cefotaxime and another isolate was intermediate resistant to imipenem. No resistance to cefotaxime, ciprofloxacin, gentamicin or imipenem was detected.

Table 2. Ranges, MIC_{50}, MIC_{90} of 107 Shigella isolates

Antimicrobial agent	MIC (mg/L)			% resistant
	range	MIC_{50}	MIC_{90}	
Ampicillin	1-256	4	256	42.1
Amoxicillin/clavulanic acid	1-256	4	16	15.9
Cefotaxime	0.03-16	0.03	0.06	0
Cephalothin	1-128	4	8	5.6
Ciprofloxacin	0.008-0.12	0.016	0.016	0
Gentamicin	0.25-2	2	2	0
Imipenem	0.12-8	0.5	0.5	0
Trimethoprim-sulphamethoxazole	0.06/1.2-16/304	16/304	16/304	57.0

Resistance trends among *Shigella* species

The resistance trends among *Shigella* species were analysed for *S. sonnei* and *S. flexneri* only as there were few isolates of *S. boydii* and *S. dysenteriae.* Table 3 depicts the distribution of the antimicrobial resistances among isolates of *S. sonnei* and *S. flexneri*. There were no significant differences between these two species except for ampicillin resistance. *S. flexneri* was significantly more resistant to ampicillin than *S. sonnei* (χ^2 test, $p<0.001$).

Table 3. Antimicrobial resistances (%), by species

Antimicrobial agent	*Shigella sonnei* n=74	*Shigella flexneri* n=25
Ampicillin	32.4	76.0
Amoxycillin/clavulanic acid	4.1	4.0
Cephalothin	2.7	4.0
Trimethoprim-sulphamethoxazole	56.8	64.0

Antibiograms and multiresistance

The prevalence of multiple resistance and distribution of resistance patterns is shown in Table 4. A total of 31.8% of the isolates were sensitive to all the antimicrobial agents tested, 35.5% were resistant to one agent, 29% were resistant to two agents, 3.7% were resistant to at least three agents. Resistance to both ampicillin and TMP-SMZ occurred in 30.8% of the isolates. Combined ampicillin and TMP-SMZ resistance was detected in *S. sonnei* (17), *S. flexneri* (14), *S. boydii* (1) and *S. dysenteriae* (1). Fifty six % of *S. flexneri* isolates were resistant to ampicillin and TMP-SMZ.

Table 4. Prevalence of multiple resistance and resistance patterns

	%	(Number)	Patterns	% with each pattern
Fully sensitive	31.8	(34)		
Resistant to 1 agent	35.5	(38)	Amp	9.3
			TMP-SMZ	26.2
Resistant to 2 agents	29.0	(31)	Amp TMP-SMZ	29.0
Resistant to 3 agents	2.8	(3)	Amp Amc TMP-SMZ	0.9
			Amp Amc Cep	1.8
Resistant to 4 agents	0.9	(1)	Amp Amc Cep TMP-SMZ	0.9

Amp = ampicillin; Amc = amoxicillin/clavulanic acid;
Cep = cephalothin; TMP-SMZ = trimethoprim-sulphamethoxazole

***Shigella* isolates of known overseas origin**

Among the 107 *Shigella* isolates, 19 were from patients who had either been overseas recently, or were overseas visitors, refugees or immigrants. Thirteen were *S. sonnei* (nine biotype a, four biotype g), three were *S. flexneri*, two were *S. boydii* and one was *S. dysenteriae*. The prevalence of resistance is shown in Table 5. Except for ampicillin, there were no significant differences in the prevalence of resistance between isolates of known overseas origin and isolates that were not known to have overseas origins. Isolates of known overseas origins were significantly more resistant to ampicillin (χ^2 test, $p < 0.04$).

Table 5. Association between overseas origin and % resistance

Antimicrobial agent	***Shigella* isolates of known overseas origins n=19**	***Shigella* isolates without known overseas origins n=88**
Ampicillin	63.2	37.5
Amoxicillin/clavulanic acid	5.3	3.4
Cephalothin	5.3	2.3
TMP-SMZ	57.9	56.8
Ampicillin + TMP-SMZ	42.1	28.4

TMP-SMZ = trimethoprim-sulphamethoxazole

DISCUSSION

Shigella sonnei and *S. flexneri* have been the main *Shigella* species isolated in New Zealand. In recent years, *S. sonnei* has been the predominant species. In this study, nearly 70% of the *Shigella* isolates were *S. sonnei* and 23% were *S. flexneri*. Almost 47% of the isolates were *S. sonnei* biotype a, which is considered to be endemic in New Zealand. In developed countries in recent years, *S. sonnei* has been reported to be the predominant species.[10,11]

The sensitivities of *Shigella* isolates in New Zealand were routinely determined in the 1970s and early 1980s by the Antibiotic Reference Laboratory, ESR.[12] Between 1969 and 1975 the prevalence of ampicillin resistance varied from 20% to 78%.[12,13] However, from 1976 to 1982, ampicillin resistance decreased to under 10% among the *Shigella* isolates.[12]

In this study, 42.1% of recent *Shigella* isolates were ampicillin-resistant and 57% were TMP-SMZ-resistant. Almost 31% were resistant to both ampicillin and TMP-SMZ, the drugs of choice for shigellosis. No resistance to quinolones was detected. Quinolones have become first line agents for shigellosis in countries with high prevalences of resistances to ampicillin and TMP-SMZ. However, quinolones are not yet approved for use in children.

In Bangladesh and Africa, *S. flexneri* and *S. dysenteriae* are the predominant species isolated and *S. dysenteriae* type 1 in particular has been associated with multiple antibiotic resistance.[3,14,15] In Bangladesh, *S. flexneri* was often more resistant to antibiotics than the other non-*S. dysenteriae* type 1 isolates.[3] Our study showed that ampicillin resistance, but not other antibiotic resistance, was significantly higher in *S. flexneri* than in *S. sonnei*. In addition, 56% of the *S. flexneri* isolates manifested resistance to both ampicillin and TMP-SMZ. This finding is in contrast to the study carried out in Israel by Ashkenazy *et al.*[10] who showed that *S. sonnei* was significantly more resistant to ampicillin and TMP-SMZ than *S. flexneri*. Ashkenazy *et al.* reported increases in resistance rates to ampicillin and TMP-SMZ to nearly 90% among *S. sonnei* between 1984 and 1992.

In developed countries, infection with multiply resistant *Shigella* has been associated with travel overseas.[7,16,17] Results from this study showed that ampicillin resistance was significantly higher in isolates with known overseas origin. Also, 42.4% of the isolates of known overseas origin had combined resistance to ampicillin and TMP-SMZ. However, resistance to TMP-SMZ and ampicillin was detected in almost 57% and 38% of *Shigella* isolated from patients with no known history of travel overseas.

This pilot study demonstrated that a good proportion of *Shigella* isolated in New Zealand are resistant to the drugs of choice, ampicillin and TMP-SMZ and nearly a third of *Shigella* exhibited combined resistance to ampicillin and TMP-SMZ. The prevalence of this combined resistance is of concern as it complicates the empirical treatment of shigellosis. The alternative antimicrobial agents are more costly and some agents like quinolones are not yet approved for use in children.

The findings indicate that there is a need to monitor the prevalence of antimicrobial resistance in *Shigella* and suggest that susceptibility testing of *Shigella* might be needed to aid empirical therapy. It was reassuring that resistance to the other alternative antimicrobial agents like quinolones and third generation cephalosporins was not detected.

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