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High level resistance to trimethoprim, cotrimoxazole and other antimicrobial agents among clinical isolates of *Shigella* species in Ontario, Canada – an update

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SUMMARY

A total of 598 isolates of *Shigella* species (24 *S. dysenteriae*, 254 *S. flexneri*, 30 *S. boydii*, 290 *S. sonnei*) submitted to the Ontario Public Health Laboratories in 1990 were tested for their susceptibility to 14 antimicrobial agents by the agar dilution method. Overall 79·6% of isolates were resistant to one or more antimicrobial agents and 52·0% were resistant to four or more. Trimethoprim resistance ranged from 26·7% among isolates of *S. boydii* to 39·4% among *S. flexneri* strains. The majority of the 224 TMP resistant isolates (88·8%) demonstrated high level resistance (MIC > 1000 mg/l) to trimethoprim. Resistance to cotrimoxazole increased from 3% in 1978 to between 26·7 and 37·6% in 1990. MICs for 90% of isolates (MIC_{90}s) for ampicillin, ticarcillin and piperacillin were 128 to > 256 mg/l, > 256 for tetracycline and chloramphenicol, and > 2·0/38·0 for cotrimoxazole. These results from the Canadian Province of Ontario emphasize the need for prudent use of antimicrobial agents in the treatment of shigellosis.

INTRODUCTION

Shigellosis is a disease of the gastrointestinal tract which is recognized as an important problem with high morbidity, particularly in developing countries [1–3]. *Shigella* organisms resistant to trimethoprim and other antimicrobial agents have been found in many parts of the world resulting in complications in the treatment of the disease [4–6]. R-factor mediated antimicrobial resistance, well known for the potential to spread from strain to strain, plays a major role in the development of resistance in *Shigella* species [7, 8].

The first trimethoprim and cotrimoxazole resistant isolates in Ontario was reported by Bannatyne and co-workers [9] in 1980. In a previous communication [10] we described the incidence of trimethoprim resistance among shigella isolates submitted to our Diagnostic Bacteriology Laboratory. The present report describes the antimicrobial susceptibility patterns of the total number of isolates submitted to both the Diagnostic and Reference Bacteriology Laboratories for the period January to December 1990. This investigation is part of an epidemiologic surveillance of shigella isolates in Ontario and other provinces in Canada, to determine the incidence of antimicrobial resistance among these organisms and to

investigate particularly the genetic and molecular traits of trimethoprim-resistant clones.

MATERIALS AND METHODS

Bacterial strains

A total of 598 isolates of *Shigella* spp. received in both the Diagnostic and Reference Bacteriology Laboratories of the Central Public Health Laboratory during January to December 1990 were used in this study. Of the 598 isolates, 24 were *S. dysenteriae*, 254 were *S. flexneri*, 30 *S. boydii* and 290 *S. sonnei*. Isolation identification and serotyping were performed using standard procedures [11, 12].

Antimicrobial agents

The antimicrobial agents used in this investigation were obtained from their respective distributors as follows: amikacin (Bristol Labs of Canada, Belleville, Ontario, Canada), ampicillin, chloramphenicol, sulfamethoxazole and tetracycline (Sigma Chemical Co., St Louis, Mo.), ciprofloxacin (Miles Inc., West Haven, Conn.) gentamicin (Schering Canada Inc., Pointe Claire, Quebec, Canada), nalidixic acid (Sterling Drug, Aurora, Ontario, Canada), norfloxacin (Merck Frost Canada Inc., Kirkland, Quebec, Canada), piperacillin (Lederle Cyanamid Canada Inc., Montreal, Quebec, Canada), ticarcillin (SmithKline Beecham Pharma Inc., Oakville, Ontario, Canada), tobramycin (Eli Lilly Canada Inc., Toronto, Ontario, Canada), trimethoprim (Burroughs Wellcome Inc., Kirkland, Quebec, Canada).

Antimicrobial susceptibility testing

The susceptibilities of all strains were determined by an agar dilution technique with Mueller–Hinton (M–H) agar (BBL, Becton Dickinson, Canada). Inocula were prepared by culture in brain heart infusion (BHI) broth (Difco Labs, Detroit, Michigan, USA) at 35 °C in air for 2 h. The cultures were further diluted in BHI broth to match the turbidity of a 1:10 dilution of a 0·5 McFarland standard [13] and inoculated with a Steers replicator [14], delivering 2 μl, to obtain a final inoculum of 10^4–10^5 c.f.u. The standard inoculum was diluted an additional 1:200 and M–H agar supplemented with lysed blood (5% v/v), for susceptibility testing of trimethoprim, sulfamethoxazole and cotrimoxazole. Control drug-free plates were similarly inoculated and the plates were read after incubation at 35 °C in air for 24 h. Susceptibility breakpoints were as outlined by the National Committee of Clinical Laboratory Standards [13] as follows: ampicillin, chloramphenicol, tetracycline, and trimethoprim, 8 mg/l; gentamicin, norfloxacin and tobramycin, 4 mg/l; ciprofloxacin, 1 mg/l; amikacin, 16 mg/l; piperacillin and ticarcillin, 16 and 64 mg/l; nalidixic acid 6 mg/l; sulfamethoxazole, 256 mg/l; cotrimoxazole (trimethoprim-sulfamethoxazole) 0·5/9·5 mg/l.

Measurement of MICs

The MICs of ampicillin, chloramphenicol, cotrimoxazole, piperacillin, sulfamethoxazole, tetracycline, ticarcillin and trimethoprim were determined by the agar dilution method. Basically, serial twofold dilutions of antibiotic powders were incorporated into duplicate M–H agar plates. MICs of trimethoprim, sulfamethoxazole and cotrimoxazole were determined on M–H agar supplemented with lysed horse blood (5%, v/v). Fresh plates were seeded with inocula as

described above and MICs were determined after incubation of 24 h at 35 °C. The MIC was defined as the lowest concentration of an antibiotic that completely inhibited the visible growth of the test organism after incubation.

RESULTS

Antimicrobial activity

Differences in antimicrobial resistance patterns among the *Shigella*, spp. are shown in Table 1. Isolates resistant to trimethoprim were also resistant to cotrimoxazole in all species except for *S. flexneri* where the percentage of isolates resistant to trimethoprim (39·4%) was higher than those resistant to cotrimoxazole (30·3%). The percentage of sulfamethoxazole resistance was highest among *S. boydii* (76·7%) and lowest among isolates of *S. flexneri* (44·5%). Resistance to ampicillin, one of the first-line therapeutic agents, and to tetracycline, was highest among *S. boydii* and *S. flexneri*; 56·7 and 73·3% for *S. boydii* versus 66·5 and 80·3% for *S. flexneri*.

An interesting finding with the β-lactam antimicrobial agents is illustrated in Table 1 and Fig. 1. All strains resistant to ampicillin were also resistant to ticarcillin but only a few isolates of *S. dysenteriae*, *S. flexneri* and *S. boydii* were resistant to piperacillin; on the other hand, a large number of *S. sonnei* strains were resistant to piperacillin. Chloramphenicol resistance was less frequent in *S. sonnei* (5·2%) than in the other three species. Overall 79·6% of isolates were resistant to one or more antimicrobial agents (*S. dysenteriae*, 75%; *S. flexneri*, 89%; *S. boydii*, 90%; *S. sonnei* 71%), and 52% were resistant to four or more.

The frequency of antimicrobial resistance among different serotypes of *S. flexneri* are shown in Table 2. The majority of isolates belonged to serotype 2A, this group had the highest percentage of TMP resistance (63·2%) and the second highest resistance to cotrimoxazole (36·8%) and sulfamethoxazole (46·1%). A relatively large number of isolates of serotype 6 (90·3%) showed resistance to sulfamethoxazole and slightly less than half the number (41·9%) to trimethoprim and cotrimoxazole. Strains from all serotypes showed a high percentage of resistance to ampicillin and tetracycline, two of the first-line treatment drugs, the highest percentage occurring in serogroup 1b, 92·5 and 94·3% respectively. Isolates of serogroup 1b also showed the highest percentage of resistance to chloramphenicol, 90·6%. The results of resistance to piperacillin as compared to ampicillin and piperacillin among the different serotypes of *S. flexneri* are shown quite dramatically in Table 2, fewer isolates were resistant to piperacillin than to ampicillin and ticarcillin.

The differences in the pattern of resistances associated with TMP-susceptible and TMP-resistant isolates of each of the *Shigella* spp. are shown in Table 3. A large proportion of TMP-susceptible *S. dysenteriae* was resistant to tetracycline and sulfamethoxazole (63·6 and 54·5%), a smaller percentage to ampicillin and chloramphenicol (36·4 and 27·3%), and none to piperacillin. On the other hand, all TMP-resistant *S. dysenteriae* were resistant to sulfamethoxazole, cotrimoxazole and ampicillin, and a high percentage to tetracycline and chloramphenicol (85·7%).

TMP-susceptible *S. flexneri* were mostly resistant to tetracycline (84·9%)

Table 1. *Antimicrobial resistance among isolates of* Shigella *species submitted to our laboratory in 1990*

Antimicrobial agent	Percentage of resistant isolates			
	S. boydii (n = 30)	*S. dysenteriae* (n = 24)	*S. flexneri* (n = 254)	*S. sonnei* (n = 290)
Trimethoprim	26·7	29·2	39·4	37·6
Sulfamethoxazole	76·7	54·2	44·5	55·9
Cotrimoxazole	26·7	29·2	30·3	37·6
Ampicillin	56·7	45·8	66·5	39·3
Ticarcillin	56·7	45·8	66·5	39·3
Piperacillin	16·7	8·3	14·2	32·4
Tetracycline	73·3	54·2	80·3	30·7
Chloramphenicol	56·7	37·5	65·0	5·2

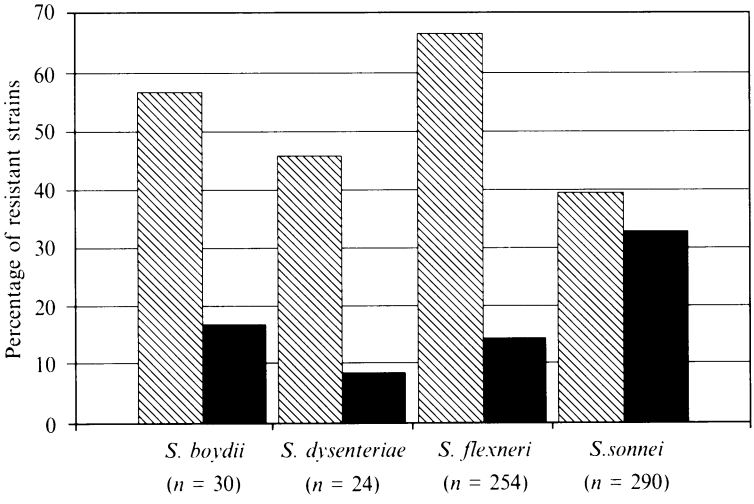

Fig. 1. Comparison of the activity of ampicillin and ticarcillin (▨) versus piperacillin (■) against isolates of *Shigella* species.

Table 2. *Numbers and percentages of antimicrobial-resistant* Shigella flexneri *1b, 2A, 3A, 4A, 6 and other serotypes in 1990, a comparison*

Antimicrobials	Serotypes					
	1b (n = 53)	2A (n = 76)	3A (n = 43)	4A (n = 28)	6 (n = 31)	Others* (n = 23)
TMP	26·4	63·2	25·6	32·1	41·9	21·7
SMX	32·1	46·1	37·2	25·0	90·3	43·5
TMP/SMX	26·4	36·8	25·6	21·4	41·9	21·7
Ap	92·5	77·6	51·2	64·3	32·3	47·8
Tic	92·5	77·6	51·2	64·3	32·3	47·8
Pip	20·8	18·4	7·0	7·1	6·5	17·4
Te	94·3	78·9	76·7	82·1	61·3	82·6
Cm	90·6	72·4	44·2	67·9	35·5	56·5

* 2B, (n = 4); 1a (n = 4); X (n = 2); Y (n = 13).

TMP, trimethoprim; SMX, sulfamethoxazole; TMP/SMX, trimethoprim/sulfamethoxazole (cotrimoxazole); Ap, ampicillin; Tic, ticarcillin; Pip, piperacillin; Te, tetracycline; Cm, chloramphenicol.

Table 3. *Antimicrobial resistances associated with trimethoprim-resistant and trimethoprim-sensitive* Shigella *species*

Species	No. studied	% of isolates resistant to					
		SMX	TMP/SMX	Ap*	Pip	Tc	Cm
S. boydii							
TMP resistant	8	100·0	100·0	62·5	50·0	87·5	62·5
TMP susceptible	19	78·9	0	63·2	5·3	78·9	63·2
S. dysenteriae							
TMP resistant	7	100·0	100·0	100·0	28·6	85·7	85·7
TMP susceptible	11	54·5	0	36·4	0	63·6	27·3
S. flexneri							
TMP resistant	100	76·0	77·0	87·0	21·0	97·0	84·0
TMP susceptible	126	29·4	0	65·1	11·9	84·9	64·3
S. sonnei							
TMP resistant	109	99·1	100·0	60·6	58·7	46·8	5·5
TMP susceptible	96	56·3	0	50·0	31·3	39·6	9·4

* Isolates resistant to ampicillin were also resistant to ticarcillin.

Ap, ampicillin; Cm, chloramphenicol; Pip, piperacillin; SMX, sulfamethoxazole; Tc, tetracycline; TMP/SMX, trimethoprim-sulfamethoxazole (cotrimoxazole).

ampicillin and chloramphenicol (65·1 and 64·3%). Fewer isolates showed resistance to sulfamethoxazole and piperacillin (29·4 and 11·9%). Resistance to sulfamethoxazole and cotrimoxazole however was not always associated with resistance to trimethoprim among isolates of *S. flexneri*. An equal proportion of TMP-susceptible *S. boydii* was resistant to sulfamethoxazole and tetracycline (78·9%); a similar picture was seen for ampicillin and chloramphenicol (63·2%). Fewer isolates were resistant to piperacillin. Among TMP-susceptible *S. sonnei* a larger proportion was resistant to chloramphenicol than was seen with TMP-resistant isolates (9·4 compared to 5·5%) and a higher percentage was resistant to sulfamethoxazole and ampicillin (56·3 and 50·0%) than to tetracycline and piperacillin (39·6 and 31·3%).

Overall TMP-susceptible isolates were mostly resistant to the β-lactams, sulfamethoxazole, tetracycline and chloramphenicol (Table 3). The majority of TMP-susceptible isolates of *S. boydii* and *S. flexneri* were resistant to ampicillin-ticarcillin, tetracycline and chloramphenicol. *S. flexneri* serogroup 6 was somewhat different, with most isolates resistant only to sulfamethoxazole. TMP-susceptible isolates of *S. dysenteriae* were mostly resistant also to sulfamethoxazole while those of *S. sonnei* had the phenotype ampicillin-ticarcillin, piperacillin, followed closely by sulfamethoxazole only.

The patterns of other resistances associated with TMP-resistant *Shigella* spp. and the levels of resistance to TMP are shown in Table 4. Of the 109 *S. sonnei* strains resistant to TMP, 82·6% showed high-level resistance (MIC > 1000 mg/l) while low-level resistance (MIC > 256 < 1000 mg/l) was demonstrated in 17·4%. The most common resistant phenotype associated with TMP-resistance among *S. sonnei* isolates was that of ampicillin/ticarcillin, piperacillin, sulfamethoxazole and cotrimoxazole, 51·4% of isolates had this pattern; this was followed by resistance to sulfamethoxazole, cotrimoxazole and tetracycline which was seen in 37·6% of isolates. There was a wider variety of resistance patterns associated with

Table 4. *Patterns of resistance associated with trimethoprim-resistant* Shigella *species*

Species	Resistance pattern	TMP MIC (mg/l) > 1000	TMP MIC (mg/l) > 256 < 1000
S. sonnei	Ap* Pip Smx Tmp/Smx	45	11
(n = 109)	Ap Pip Smx Tmp/Smx Tc	0	3
	Ap Pip Smx Tmp/Smx Tc Cm	0	5
	Ap Smx Tmp/Smx Tc Cm	1	0
	Ap Smx Tmp/Smx Tc	1	0
	Smx Tmp/Smx Tc	41	0
	Smx Tmp/Smx	1	0
	Tmp/Smx	1	0
S. flexneri	Ap Pip Smx Tmp/Smx	3	2
(n = 100)	Ap Pip Smx Tmp/Smx Tc	2	0
	Ap Pip Smx Tmp/Smx Tc Cm	11	2
	Ap Smx Tmp/Smx Tc Cm	38	2
	Ap Smx Tmp/Smx Tc	1	0
	Smx Tmp/Smx Tc	8	0
	Smx Tmp/Smx Tc Cm	5	0
	None†	1	0
	Ap Tc Cm	22	0
	Ap Tmp/Smx Tc Cm	1	0
	Ap Smx Tmp/Smx	1	0
	Ap Pip Smx Tmp/Smx Cm	1	0
S. boydii	Ap Pip Smx Tmp/Smx Tc Cm	4	0
(n = 8)	Ap Pip Smx Tmp/Smx	1	0
	Smx Tmp/Smx Tc Cm	1	0
	Smx Tmp/Smx Tc	2	0
S. dysenteriae	Ap Pip Smx Tmp/Smx Tc Cm	1	0
(n = 7)	Ap Smx Tmp/Smx Tc Cm	5	0
	Ap Pip Smx Tmp/Smx	1	0

* All isolates resistant to ampicillin were also resistant to ticarcillin.

† Resistant to trimethoprim only.

Ap, ampicillin; Cm, chloramphenicol; Pip, piperacillin; Smx, sulfamethoxazole; Tc, tetracycline; Tmp/Smx, trimethoprim-sulfamethoxazole (cotrimoxazole).

TMP resistance among isolates of *S. flexneri*. Ninety-four percent had high-level resistance and low-level resistance was seen in 6·0% of isolates. The three most common resistant patterns were ampicillin/ ticarcillin, sulfamethoxazole, cotrimoxazole, tetracycline and chloramphenicol (40%), ampicillin/ticarcillin, tetracycline, chloramphenicol (22%) and ampicillin/ticarcillin, piperacillin, sulfamethoxazole, cotrimoxazole, tetracycline and chloramphenicol (11%). One isolate of *S. flexneri* was resistant only to trimethoprim. All TMP-resistant isolates of *S. dysenteriae* and *S. boydii* were at the high level of > 1000 mg/l. The majority of TMP-resistant *S. dysenteriae* strains, 5 of 7 (71·4%), was associated with the resistance phenotype ampicillin/ticarcillin, sulfamethoxazole, cotrimoxazole, tetracycline and chloramphenicol, a pattern most common among *S. flexneri*. Fifty percent of isolates of *S. boydii* demonstrated a similar resistance pattern in addition to resistance to piperacillin.

The MICs for 50 and 90% of isolates and the ranges of resistances of ampicillin, chloramphenicol, piperacillin, tetracycline and ticarcillin for all resistant *Shigella*

Table 5. *MIC range, MIC_{50} and MIC_{90} of ampicillin, chloramphenicol, piperacillin, tetracycline and ticarcillin for resistant isolates of* Shigella *species*

Species	Antibiotic	MIC (mg/l)* Range	50%	90%
S. boydii	Ap	> 256	> 256	> 256
	Cm	> 256	> 256	> 256
	Pip	128–> 256	256	256
	Tc	16–> 256	> 256	> 256
	Tic	256–> 256	> 256	> 256
S. dysenteriae	Ap	32–> 256	256	256
	Cm	128–> 256	> 256	> 256
	Pip	32–> 256	128	128
	Tc	128–> 256	> 256	> 256
	Tic	128–> 256	256	256
S. flexneri	Ap	32–> 256	> 256	> 256
	Cm	32–> 256	> 256	> 256
	Pip	32–> 256	> 256	> 256
	Tc	64–> 256	> 256	> 256
	Tic	128–> 256	256	256
S. sonnei	Ap	16–> 256	> 256	> 256
	Cm	> 256	> 256	> 256
	Pip	64–> 256	> 256	> 256
	Tc	16–> 256	> 256	> 256
	Tic	32–> 256	> 256	> 256

* 50% and 90%, MIC for 50 and 90% of strains, respectively.
Ap, ampicillin; Cm, chloramphenicol; Pip, piperacillin; Tc, tetracycline; Tic, ticarcillin.

species are shown in Table 5. The majority of isolates showed high level resistance to these five antimicrobial agents (MIC_{90} > 256 mg/l).

DISCUSSION

As recently as a decade ago most isolates of *Shigella* species in Ontario were susceptible to trimethoprim (TMP) and cotrimoxazole (TMP/SMX) [9]. Since that time resistance to both these agents have been increasing [10]. The present study describes the results of antibiograms of all isolates received in both the Diagnostic and Reference Bacteriology Divisions of the Ontario Public Health Laboratories during the 12-month period of 1990. This investigation has revealed a significant increase in both TMP and TMP/SMX among isolates of *S. dysenteriae* and *S. boydii* (29·2 and 26·7%) where none existed in the previous study [9]; also evident is the sharp rate of increase among *S. sonnei* strains from 3 to 37·6% (Table 1). Isolates of serotype 2A of *S. flexneri*, the most frequently isolated serotype of *S. flexneri* (Table 2), had developed a 40% higher incidence of resistance to TMP (63·2%) than to the combination TMP/SMX (36·8%); these results are a significant increase over those previously reported [9]. Furthermore, other serotypes of *S. flexneri* previously all susceptible to these drugs [9] are now showing a high proportion of resistance ranging from 41·9% among serotype 6 to 21·4% among serotype 4A. Isolates of serotype 4A, like those of serotype 2A, also had a higher incidence of resistance to TMP (32·1%) than to TMP/SMX (21·4%) (Table 2). It is now evident from this investigation that the rate of resistance to

both TMP and TMP/SMX among shigellae in Ontario is as high as that seen in other parts of the world [4–7].

This report also revealed that multiresistance to various other antimicrobial agents was common among the TMP-resistant isolates investigated. In most instances these resistances included one or more agents commonly used for the treatment of shigellosis (Tables 1–4). The most common antimicrobial susceptibility patterns associated with TMP-resistance were resistance to ampicillin/ticarcillin, piperacillin, sulfamethoxazole, cotrimoxazole, and resistance to sulfamethoxazole, cotrimoxazole and tetracycline. Although overall 28·1% of TMP-resistant isolates had the resistance phenotype of ampicillin/ticarcillin, piperacillin, sulfamethoxazole and cotrimoxazole and 22·8% had the pattern sulfamethoxazole, cotrimoxazole, tetracycline; these patterns were observed mostly among *S. sonnei* isolates; 56 of 109 (51·4%) showed the former pattern, while 41 of 109 (37·6%) showed the latter (Table 4). Multiresistance to antimicrobial agents used for shigellosis has been reported in several countries [5–7, 15]. This report differs mainly in the incidences of resistance to different therapeutic agents and the resistance patterns evident among the majority of isolates.

The resistance pattern ampicillin/ticarcillin, sulfamethoxazole, cotrimoxazole, tetracycline, chloramphenicol was present among 20·5% of all four species, however, this phenotype occurred mainly among isolates of *S. flexneri*, 40 of 100 (40%), and *S. dysenteriae*, 5 of 7 (71·4%) (Table 4). The similarity in resistance phenotype among these two species of *Shigella* is interesting and is under investigation to determine whether there is a spread of the same clone between these two species. The frequency of resistance to chloramphenicol was higher in *S. flexneri* than in isolates of *S. sonnei*, the higher incidence of chloramphenicol resistance among *S. flexneri* compared with *S. sonnei* isolates has been reported by other investigators [5, 15]. Only one trimethoprim-resistant *S. sonnei* strain was susceptible to sulfamethoxazole, on the other hand 24·0% of trimethoprim-resistant *S. flexneri* were susceptible to sulfamethoxazole and 23·0% to cotrimoxazole, one isolate being resistant only to trimethoprim (Table 3). Resistances to sulfamethoxazole and cotrimoxazole were also found together with TMP in the same isolates of *S. dysenteriae* and *S. boydii*.

An examination of the resistance patterns associated with TMP-susceptible isolates of all four *Shigella* species revealed a number of differences when compared to those resistances associated with the TMP-resistant isolates. The exception to this rule was among isolates of *S. flexneri* where the most common phenotype among TMP-susceptible strains (ampicillin, ticarcillin, tetracycline, chloramphenicol) was the second most common pattern among TMP-resistant strains. These findings differ from those of Heikkila and colleagues [5] who found TMP-resistance most commonly linked to the most common resistance pattern observed in the TMP-susceptible population.

High level resistance to TMP among *Shigella* species has increased considerably in various parts of the world [4–7, 16]. In the present study all isolates of *S. dysenteriae* and *S. boydii* and the majority (94·0%) of *S. flexneri* demonstrated a high level of resistance to trimethoprim (MIC $>$ 1000 mg/l). MIC for trimethoprim among *S. sonnei* was $>$ 1000 mg/l for 82·6% of isolates and $>$ 256 $<$ 1000 mg/l

for 17·4% of strains. The majority of isolates resistant to the lower level of trimethoprim had the most common resistance pattern (Table 4). Resistance to cotrimoxazole and sulfamethoxazole were also at high levels, MIC > 2·0/38·0 mg/l and > 1024 mg/l respectively. High level resistance to the β-lactams, tetracycline and chloramphenicol was also common among the shigella isolates (Table 5). The MICs for 90% of isolates (MIC_{90}s) were > 256 mg/l. The differences in activity of piperacillin against the isolates is interesting (Table 1, Fig. 1); the level of activity against *S. sonnei* was similar to that recently observed among *Escherichia coli* from urinary tract infections [17]. These findings are currently under investigation.

All the isolates remained susceptible to nalidixic acid and the 4-fluoroquinolones, ciprofloxacin and norfloxacin. These results are consistent with the majority of antimicrobial susceptibility studies of *Shigella* species [2, 5, 6]. Nalidixic acid has been used quite successfully for the treatment of enteric infections with organisms resistant to other antimicrobials [18, 19]. Recently however, resistance to nalidixic acid has appeared in some areas [20, 21] hence close monitoring of Ontario strains is necessary to detect resistant isolates when they arise. The newer quinolones, ciprofloxacin and norfloxacin, have enhanced *in vitro* activity against *Shigella* species [18, 22, 23] and have been suggested as an alternative therapeutic option, but these drugs are costly and have not been approved for use in children due to their potential toxicity for developing joints [15]. The aminoglycosides, gentamicin, tobramycin and amikacin, were also effective against the strains in this study, resistance of shigellae to some aminoglycosides have been reported [2, 6] but a number of in vitro studies have shown shigella to be susceptible to these agents [9, 15, 20].

Antimicrobial resistance among *Shigella* species is a growing concern both for public health authorities and for physicians. This study has shown that multiresistant strains of shigellae are increasing steadily in Ontario, that high-level resistance is common among these strains and that there is a need to maintain surveillance in order to assess local susceptibility patterns. The results presented here also emphasize the need for prudent use of antimicrobial agents in the treatment of shigellosis. Preliminary studies have shown that a fair number of these TMP-resistant strains can transfer their resistances conjugally [10]. Further studies are being conducted on the molecular aspects of TMP resistance in Ontario.

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