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Transferable high-level trimethoprim resistance among isolates of *Escherichia coli* from urinary tract infections in Ontario, Canada

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SUMMARY

Of 1171 isolates of *Escherichia coli* isolated from urine samples at the Public Health Laboratory, Toronto, Ontario, Canada, between May 1990 and December 1991, 120 (10·3%) were resistant to trimethoprim (TMP), cotrimoxazole (TMP/SMX), sulfamethoxazole (SMX) and other antimicrobial agents; 110 of the 120 isolates (91·7%) were resistant to four or more agents. The majority of resistant isolates (91·7%) exhibited high-level resistance (MIC > 1000 mg/L) to TMP. The MIC of TMP/SMX for all 120 isolates was > 2·0/38·0 mg/L and for SMX > 1024 mg/L. High-level resistances were also present among the β-lactam antimicrobials with MICs ranging from 16- > 256 mg/L. Forty-three of 120 TMP-resistant (35·8%) isolates conjugally transferred TMP-resistance to *E. coli* K-12. Co-transfer of several other resistances was observed. SMX cotransferred from 86% of the 43 donors and β-lactams together with SMX cotransferred from 70%. Nalidixic acid resistance was present among 22 (18·3%) of the 120 resistant isolates, however, nalidixic acid resistance was not transferred to *E. coli* K-12.

INTRODUCTION

Trimethoprim (TMP) either alone or in combination with sulfamethoxazole (cotrimoxazole) is commonly used as standard therapy for urinary tract infections, and increased resistance to both agents have been reported to occur in isolates of *Escherichia coli* in different parts of the world [1–4]. The introduction of newer alternative antimicrobial agents have also resulted in rapidly developing resistance which threatens the usefulness of these agents [5].

E. coli is the most frequent pathogen isolated from cases of community acquired urinary tract infections received in the Diagnostic Bacteriology Laboratory of the Central Public Health Laboratory in Toronto, Ontario. However, surveillance studies to determine the changes occurring in the patterns of antimicrobial susceptibility have not been conducted. There have also been reports in the literature that the high prevalence of resistance to TMP in *Shigella* species is paralleled by a similar increase among isolates of *E. coli* [6, 7]. Because of our recent observations of the high percentage of antimicrobial resistance among *Shigella* spp. in Ontario [8, 9], the present study was undertaken to determine the incidence of resistance to TMP, cotrimoxazole and other antimicrobial agents among *E. coli* from urinary tract infections. This study is part of an epidemiologic

longitudinal surveillance of *E. coli* isolates in Ontario to determine the incidence of antimicrobial resistance and to assess how this compares with that of *Shigella* spp. and with the incidence of resistant *E. coli* in other parts of the world.

MATERIALS AND METHODS

Bacterial strains

A total of 1171 cultures of *Escherichia coli* isolated from urine samples submitted to the Diagnostic Bacteriology Laboratory of the Central Public Health Laboratory in Toronto, Ontario between May 1990 and December 1991 were investigated. All cultures were identified using standard laboratory procedures [10] and examined for antimicrobial susceptibility patterns. One hundred and twenty of these isolates resistant to trimethoprim were selected for further investigation. Duplicate isolates were not included in this study.

Antimicrobial agents

The following antimicrobial agents were supplied by their distributors as follows: amikacin (Bristol Labs of Canada, Belleville, Ontario, Canada), ampicillin, chloramphenicol, nitrofurantoin, rifampin, 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 (Smith Kline Beecham Pharm. Inc., Ontario, Canada), cefamandole, tobramycin (Eli Lilly Canada Inc., Toronto, Ontario, Canada), trimethoprim (Burroughs Wellcome Inc., Kirkland, Quebec, Canada).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the agar dilution, break point technique as outlined by the National Committee of Clinical Laboratory Standards [11]. Mueller-Hinton (M-H) agar (BBL, Becton Dickinson, Canada) was used for testing all antimicrobials and 5% (v/v) lysed horse blood was added to the M-H agar base for testing trimethoprim, sulfamethoxazole and cotrimoxazole. The following antimicrobials were tested at the concentrations indicated: ampicillin, chloramphenicol, cefamandole, 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; nitrofurantoin, 64 mg/L.

Determination of MIC

The MICs were determined by the agar dilution method. Basically, serial twofold dilutions of antimicrobial powders were incorporated into duplicate M-H agar plates. Fresh plates were seeded with inocula, 10^4 CFU, by use of a Steers replicator [12]. MICs were determined after incubation for 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.

Table 1. *The incidence of resistance to 15 antimicrobial agents among 120 trimethoprim-resistance* Escherichia coli *from urinary tract infections*

Antimicrobial agents	Number (percentage) of resistant isolates
Sulfamethoxazole	120 (100)
Co-trimoxazole	120 (100)
Ampicillin	87 (72·5)
Ticarcillin	87 (72·5)
Piperacillin	85 (70·8)
Tetracycline	83 (69·2)
Chloramphenicol	43 (35·8)
Gentamicin	6 (5·0)
Tobramycin	4 (3·3)
Amikacin	0 (0)
Ciprofloxacin	3 (2·5)
Norfloxacin	3 (2·5)
Nalidixic acid	22 (18·3)
Nitrofurantoin	3 (2·5)
Cefamandole	13 (10·8)

Haemolysin assay

The production of haemolysin was tested on 5% defribinated sheep blood agar plates. Haemolytic strains were identified after overnight incubation by the presence of a clear halo around the colonies.

Genetic transfer

Conjugation was performed using *E. coli* strains 711 (F-*lac his trp proC phe*) Nal^R and RG192 Rif^R, both K-12 derivatives, as recipients. Donor and recipient cultures were grown separately in Brain Heart Infusion broth (Difco Labs, Detroit, Michigan, USA) for 4 h at 37 °C. Matings in liquid medium were carried out by adding 0·1 ml of donor and 0·5 ml of recipient bacteria to 4·5 ml BHIB and incubating the mixtures overnight, without aeration, at 37 °C. Transconjugants were selected by plating 100 μl of the overnight cultures on selective M-H agar plates containing 5% lysed blood and rifampin 100 mg/L plus trimethoprim 16 mg/L or nalidixic acid 50 mg/L plus trimethoprim 16 mg/L. Replica plating of transconjugants was done on M-H agar containing appropriate antimicrobials and on 5% defribinated sheep blood agar plates for analysis of transfer of haemolysin.

RESULTS

Antimicrobial resistance

The activity of the 15 other antimicrobial agents against the trimethoprim-resistant *E. coli* isolates from urinary tract infections are shown in Table 1. Of the 120 isolates 87 (72·5%) were resistant to both ampicillin and ticarcillin while 85 (70·8%) were resistant to piperacillin. All isolates showed resistance to sulfamethoxazole and cotrimoxazole, 83 (69·2%) to tetracycline, and 43 (35·8%) to chloramphenicol. All isolates were susceptible to amikacin and only a small percentage showed resistance to gentamicin (5%), tobramycin (3·3%), cipro-

Table 2. *Antimicrobial resistance patterns associated with trimethoprim-resistant* Escherichia coli

Resistance patterns*	Number (percentage) exhibiting patterns
SMX TMP/SMX Ap† Pip Tc	19 (15·8)
SMX TMP/SMX Ap Pip Tc Cm	16 (13·3)
SMX TMP/SMX Ap Pip	14 (11·7)
SMX TMP/SMX Tc	11 (9·2)
SMX TMP/SMX	10 (8·3)
SMX TMP/SMX Ap Pip Tc Nal	7 (5·8)
SMX TMP/SMX Ap Pip Tc Cm Nal	5 (4·2)
SMX TMP/SMX Tc Cm	4 (3·3)
SMX TMP/SMX Ap Pip Cm	3 (2·5)
SMX TMP/SMX Ap Pip Tc Cm Cip Nor Nal	3 (2·5)
SMX TMP/SMX Ap Pip Tc Cm Ma	3 (2·5)
SMX TMP/SMX Ap Pip Cm Ma	3 (2·5)
SMX TMP/SMX Ap Pip Tc Gm Tb	2 (1·7)
SMX TMP/SMX Ap Pip Tc Ma	2 (1·7)
SMX TMP/SMX Tc Fur	2 (1·7)
SMX TMP/SMX Cm	2 (1·7)
SMX TMP/SMX Tc Gm	2 (1·7)
SMX TMP/SMX Ap Tc Ma	1 (0·8)
SMX TMP/SMX Ap Pip Ma	1 (0·8)
SMX TMP/SMX Ap Pip Cm Fur Nal	1 (0·8)
SMX TMP/SMX Ap Pip Tc Ma Nal	1 (0·8)
SMX TMP/SMX Ap Pip Tc Cm Ma Nal	1 (0·8)
SMX TMP/SMX Ap Pip Cm Ma Nal	1 (0·8)
SMX TMP/SMX Ap Cm	1 (0·8)
SMX TMP/SMX Ap Pip Tc Gm Tb Nal	1 (0·8)
SMX TMP/SMX Tc Gm Tb	1 (0·8)
SMX TMP/SMX Ap Tc	1 (0·8)
SMX TMP/SMX Tc Cm Nal	1 (0·8)
SMX TMP/SMX Ap Pip Nal	1 (0·8)

* Ap, ampicillin; Cip, ciprofloxacin; Cm, chloramphenicol; Fur, nitrofurantoin; Gm, gentamicin; Ma, cefamandole; Nal, nalidixic acid; Nor, norfloxacin; Pip, piperacillin; Smx, sulfamethoxazole; Tc, tetracycline; Tb, tobramycin; Tic, ticarcillin; TMP/SMX, (trimethoprim/sulfamethoxazole) cotrimoxazole.

† Strains resistant to ampicillin are also resistant to ticarcillin.

floxacin (2·5%), nitrofurantoin (2·5%), norfloxacin (2·5%), and cefamandole (10·8%). Nalidixic acid resistance was present in 18·3% of strains, and 3 of these strains were also resistant to ciprofloxacin and norfloxacin.

The most common antimicrobial susceptibility patterns associated with TMP resistance (Table 2) were resistance to sulfamethoxazole, cotrimoxazole, ampicillin, ticarcillin, piperacillin and tetracycline (15·8%) and those resistances in addition to chloramphenicol (13·3%); 91·7% of isolates were resistant to four or more antimicrobial agents.

MICs

The majority of isolates (91·7%) exhibited high level resistance (MIC $>$ 1000 mg/L) to trimethoprim, 7 of 120 isolates (5·8%) were resistant at a level $>$ 256 mg/L but susceptible at 1000 mg/L. One isolate had an MIC of 128 mg/L,

Table 3. *Resistance patterns of donor and transconjugant strains*

Resistance pattern of donor*	Resistances transferred	No. of trans-conjugants with resistance pattern
TMP SMX Tc	TMP SMX Tc	3
	TMP Tc	2
	TMP only	2
	TMP SMX	1
TMP SMX Tc Fur Nal	TMP SMX Tc	1
TMP SMX Tc Gm	TMP SMX Tc	1
TMP SMX Ap Tic Pip	TMP SMX Ap Tic Pip	5
TMP SMX Ap Tic Pip Cm	TMP SMX	1
	TMP Cm	1
TMP SMX Ap Tic Pip Cm Ma	TMP SMX Ap Tic Pip Ma	3
TMP SMX Ap Tic Pip Cm Fur	TMP SMX Ap Tic Pip	1
TMP SMX Ap Tic Pip Tc	TMP SMX Ap Tic Pip Tc	4
	TMP SMX Ap Tic Pip	1
	TMP Ap Tic Pip	1
	[TMP SMX Ap Tic Pip Tc and TMP SMX Tc]	1
TMP SMX Ap Tic Pip Tc Ma Nal	TMP SMX Ap Tic Pip Tc Ma	1
TMP SMX Ap Tic Pip Tc Gm	[TMP SMX Ap Tic Pip Tc Gm and TMP SMX Ap Tic Pip]	1
TMP SMX Ap Tic Pip Tc Gm Tb	TMP SMX Ap Tic Pip Gm Tb	1
TMP SMX Ap Tic Pip Tc Gm Tb Nal	TMP SMX Ap Tic Pip Gm Tb	1
TMP SMX Ap Tic Pip Tc Cm	TMP only	1
	TMP SMX Ap Tic Pip	2
	[TMP SMX Ap Tic Pip Tc and TMP only]	1
	TMP SMX Ap Tic Pip Tc Cm	3
	TMP Ap Tic Pip	1
TMP SMX Ap Tic Pip Tc Cm Ma	TMP SMX Ap Tic Pip	1
	TMP SMX Ap Tic Pip Tc Cm Ma	1
	[TMP SMX Ap Tic Pip Tc Cm Ma and TMP only]	1

* Ap, ampicillin; Cm, chloramphenicol; Fur, nitrofurantoin; Gm, gentamicin; Ma, cefamandole; Nal, nalidixic acid; SMX, sulfamethoxazole, Tc, tetracycline; Tb, tobramycin; Tic, ticarcillin.

one of 64 mg/L and one of 32 mg/L. High level resistance was also evident among other antimicrobial agents. The MIC for sulfamethoxazole for all isolates was > 1024 mg/L and for cotrimoxazole > 2·0/38·0 mg/L. Of the β-lactam antimicrobial agents piperacillin MICs ranged from 32 mg/L to > 256 mg/L (MIC_{90} of 256 mg/L); all but one resistant isolate had MICs of > 256 mg/L for ampicillin and all but three had MICs of > 256 mg/L for ticarcillin. Cefamandole MIC ranged from 16 mg/L to > 128 mg/L. The MICs of both tetracycline and chloramphenicol ranged from 16 mg/L to > 256 mg/L (MIC_{90}s of 256 mg/L and 128 mg/L

respectively). The MIC_{90}s of nalidixic acid, nitrofurantoin, cefamandole, ciprofloxacin, norfloxacin, gentamicin and tobramycin were > 50, > 128, 32, 64, 128, 32 and 64 mg/L respectively.

Incidence of haemolytic property

The 120 *E. coli* isolates were screened for their haemolytic property. A total of 49 (40·8%) haemolytic strains were detected.

Transfer of trimethoprim resistance

Forty-three of 120 strains (35·8%) successfully transferred TMP resistance to *E. coli* K-12. Resistances co-transferred with TMP are shown in Table 3. Co-transfer of SMX with TMP ocurred in 86% of transconjugants while both SMX and the β-lactams were present with TMP in 70% of transconjugants. The majority of donor strains that transferred TMP resistance to *E. coli* K-12 exhibited high level resistance (MIC > 1000 mg/L). Only 2 of the 10 strains with low-level TMP resistance had transferable resistance, the MIC of TMP for these strains was > 256 < 1000 mg/L. The frequency of transfer of TMP resistance varied from 1×10^{-1} to 2×10^{-10} transconjugants per donor with 53% of isolates showing a transfer frequency of between 10^{-2} and 10^{-4}. None of the strains was able to transfer their haemolytic property by conjugation to *E. coli* K-12.

DISCUSSION

The results of our study show that between May 1990 and December 1991, 10·3% of community *E. coli* isolates from urinary tract infections were resistant to TMP, SMX and cotrimoxazole. This proportion of resistant isolates is comparable to other studies in Europe and the United States [3, 13] but lower than most studies from Greece [2], Finland [1] and developing countries [14, 15]. In many instances the reported increased levels of TMP resistance were among hospital populations [1, 7, 15]. Although the incidence of resistance to TMP in this investigation is lower in comparison to some studies, the proportion of isolates that exhibited high-level resistance (MIC > 1000 mg/L) is similar to other surveys [2, 13]. The percentage of *E. coli* isolates demonstrating high-level resistance to TMP in this study was 91·7%.

Our results also show that TMP resistance was transferable in 35·8% of the resistant *E. coli* isolates. This high incidence of transferability, after direct transfer in broth matings, coupled with the fact that 91·7% of the organisms were highly resistant to the drug emphasizes the potential for spread of resistance plasmids throughout the community. A number of reports in the literature have revealed that high-level TMP resistance is transferred much more frequently than low-level TMP resistance [16, 17]. In this study only two isolates with low-level TMP resistance transferred their resistance to *E. coli* K-12, demonstrating that this level of resistance may also be plasmid-mediated as reported by other investigators [2, 16]. Non-transferable high-level TMP-resistance (64·2% demonstrated in this investigation) may be due to the presence of resistance genes on the chromosome. It is also significant that 98% of isolates that did not transfer TMP-resistance conjugally were able to transfer resistance to ampicillin (unpublished observa-

tions) thereby, providing another mechanism for transfer of TMP-resistance residing on transposons.

All TMP-resistant strains were associated with resistance to SMX and cotrimoxazole and approximately 70% with β-lactams (Table 2). Analysis of transconjugants showed that resistance to SMX was cotransferred in 86% of transconjugants while both SMX and β-lactam resistances were cotransferred in 70%. On the other hand resistance to tetracycline and chloramphenicol cotransferred with TMP at a much lower level. The newer β-lactamase stable cephalosporins have broad spectrum activity against Gram-negative organisms. Transferable resistance to these cephalosporins in clinical isolates of Gram-negative bacteria has been reported [18, 19]. In this study 13 TMP-resistant isolates (10·8%) were resistant to cefamandole, 6 of these transferred TMP conjugally and 5 of 6 cotransferred cefamandole resistance with selection for TMP-resistance. The transconjugants were also resistant to SMX, ampicillin, ticarcillin and piperacillin and those resistances in addition to tetracycline or tetracycline and chloramphenicol. These resistance phenotypes appear similar to some reported in transconjugants from *E. coli* in South India [19]. Further studies are underway to determine the types of β-lactamase(s) involved in these resistances after transfer experiments with selection for cefamandole. None of the isolates was resistant to third-generation cephalosporins.

Resistance to aminoglycosides, nitrofurantoin, and 4-fluoroquinolones was uncommon (Tables 1, 2). Two of four isolates resistant to both gentamicin and tobramycin and one of the two resistant to gentamicin but susceptible to tobramycin conjugally transferred their resistances along with TMP to *E. coli* K-12. Fluoroquinolone resistance was not transferred, these resistances have been reported to be located on the chromosome [20]. Nitrofurantoin resistance was not transferred to *E. coli* K-12.

Another important observation was the high proportion of isolates resistant to nalidixic acid (Table 1). These results are in sharp contrast to those seen among *Shigella* sp. in Ontario. All isolates of *Shigella* sp. were susceptible to nalidixic acid [8, 21]. Rapid development of resistance to nalidixic acid has been reported after widespread use of the drug for urinary tract infection [5]. It is not known whether the results observed in this study are a reflection of the usage of nalidixic acid for urinary tract infections. None of the isolates transferred resistance to nalidixic acid. It is also noteworthy that three isolates resistant to ciprofloxacin and norfloxacin were also resistant to nalidixic acid.

Fewer than half (40·8%) of the isolates in this study displayed haemolytic property and none transferred haemolysin by conjugation along with selection for TMP-resistance. Haemolytic *E. coli* are often isolated from patients with extraintestinal infections such as urinary tract infections. Haemolysin is an important virulence factor and several studies have shown a relationship between the occurrence of haemolytic *E. coli* and urinary tract infections [22, 23], production of haemolysin provides *E. coli* with a selective advantage by destroying phagocytic cells [24]. The genes responsible for production of haemolysin usually found on the bacterial chromosome are sometimes located on a plasmid [25]. In this study it appears that haemolysin was not plasmid-mediated. An interesting observation, however, was that of the 33 TMP-resistant/AMP-susceptible isolates,

only 5 (15·2%) were haemolytic. It is not known how significant this finding is. Further studies to investigate molecular properties of trimethoprim resistance plasmids are currently going on.

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