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Emergence of Quinolone Resistance among Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* in Ontario, Canada

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One hundred two isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) randomly selected from across the Canadian province of Ontario were tested for their susceptibility to ciprofloxacin, norfloxacin, and nalidixic acid by the agar dilution method. Forty-nine percent (50 of 102) had high levels of resistance to these quinolone compounds. For the 50 resistant isolates, ciprofloxacin and norfloxacin had high MICs for 90% of isolates (MIC_{90}s) of 128 μg/ml and >128 μg/ml, respectively; for these isolates, the nalidixic acid MIC_{90} was >640 μg/ml. The majority (98%) of the 50 isolates were also resistant to tobramycin (MIC_{90}, >128 μg/ml), while 42% of the isolates were resistant to gentamicin (MIC_{90}, 64 μg/ml). Quinolone-resistant MRSA isolates were susceptible to bacteriophages from several groups, indicating independent selection of resistant strains. These results suggest that a reappraisal of the use of fluoroquinolones against MRSA in Canada is necessary.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are important nosocomial pathogens, and outbreaks of MRSA have been reported worldwide (6, 16). Since the first reports of gentamicin resistance among staphylococci (11, 12), strains resistant to both methicillin and gentamicin have been the cause of serious infections and extensive outbreaks (3, 4, 15). Many of these organisms are often resistant to a number of other antibiotics; thus, the treatment of infections and the control of outbreaks have become highly desirable.

In the past few years, the fluoroquinolones, extremely active quinolone antimicrobial agents, have been among the relatively small number of drugs used in the therapy of infections caused by MRSA (1, 8, 17). However, since the introduction of fluoroquinolones, few reports have appeared in the literature regarding the resistance of MRSA to ciprofloxacin, norfloxacin, and ofloxacin (6, 9, 10, 14).

Two of these antimicrobial agents, norfloxacin and ciprofloxacin, were introduced into routine clinical use in Canada during 1988 and 1989, respectively (5), but the potential of these quinolone derivatives in eradicating Canadian MRSA isolates is still unknown. The following in vitro study was conducted to evaluate this potential.

MATERIALS AND METHODS

Bacterial strains. One hundred two strains of MRSA recently isolated from clinical specimens from across the Canadian province of Ontario and submitted to our clinical bacteriology laboratory for bacteriophage susceptibility were investigated. These isolates were chosen randomly from our collection over a 7-month period between July 1989 and February 1990. Clinical sources included blood; surgical wounds; nasal, throat, and eye swabs; and other, unnamed sources. The origins of the isolates included neighboring hospitals and regional public health laboratories across Ontario. Duplicates were not included in this study, and none of the patients were transferred from one institution to another.

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All isolates were screened for resistance to oxacillin and methicillin at 6 and 10 μg/ml, respectively, before further testing. Isolates were examined for resistance to ciprofloxacin, norfloxacin, and nalidixic acid at concentrations of 1, 4, and 8 μg/ml, respectively. Fifty of the isolates were found to be resistant to the quinolones and were further investigated.

Antimicrobial agents. The drugs used in this investigation were obtained from their respective distributors as follows: ciprofloxacin (Miles Inc., West Haven, Conn.), norfloxacin (Merck Frost Canada Inc., Kirkland, Quebec, Canada), methicillin and oxacillin (Sigma Chemical Co., St. Louis, Mo.), vancomycin and tobramycin (Eli Lilly Canada Inc., Toronto, Ontario, Canada), gentamicin (Schering Canada Inc., Pointe Claire, Quebec, Canada), nalidixic acid (Sterling Drug, Aurora, Ontario, Canada), and novobiocin (The Upjohn Co. of Canada, Don Mills, Ontario, Canada).

Antimicrobial susceptibility tests. The susceptibilities of all strains were determined by an agar dilution technique with Mueller-Hinton agar (BBL). Inocula were prepared by culturing in brain heart infusion broth at 35°C in air for 2 h. The cultures were further diluted in brain heart infusion broth to match the turbidity of a 1:10 dilution of a 0.5 McFarland standard (7) and inoculated with a Steers replicator (13), delivering 2 μl, to obtain a final inoculum of 10^4 to 10^5 CFU. Control drug-free plates were similarly inoculated. Plates were read after incubation at 35°C in air for 24 h.

Measurement of MICs. The MICs of ciprofloxacin, norfloxacin, nalidixic acid, gentamicin, and tobramycin were determined by the agar dilution method. Basically, serial twofold dilutions of antibiotic powders were incorporated into duplicate Mueller-Hinton agar plates. Fresh plates were seeded with inocula of 10^4 CFU by use of a Steers replicator. 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.

Bacteriophage typing. Bacteriophage susceptibility was determined by use of the international set of *Staphylococcus* typing phages at the routine test dilution, and strains showing no significant lysis were tested at 100 times the routine

TABLE 1. In vitro susceptibilities of 50 clinical isolates of MRSA to ciprofloxacin, norfloxacin, nalidixic acid, gentamicin, and tobramycin

Antimicrobial agent	MIC (μg/ml)[a]		
	50	90	Range
Ciprofloxacin	64	128	4.0–>128
Norfloxacin	128	>128	2.0–>128
Nalidixic acid	>640	>640	40.0–>640
Gentamicin	1.0	64	0.5–128
Tobramycin	>128	>128	1.0–>128

[a] 50 and 90, MIC for 50 and 90% of isolates, respectively.

test dilution (2). The propagating strains for the international and experimental phages were used as indicators.

RESULTS

Antimicrobial activity. The MICs for 50 and 90% of isolates and the ranges of MICs of ciprofloxacin, norfloxacin, nalidixic acid, gentamicin, and tobramycin for 50 isolates of MRSA are shown in Table 1. The majority (98%) of the quinolone-resistant MRSA showed high-level resistance to tobramycin. The one isolate that was susceptible to tobramycin was also susceptible to gentamicin (MICs, 1.0 and 0.5 μg/ml, respectively). Twenty-eight isolates (56%) were susceptible to gentamicin but resistant to tobramycin. Many of the quinolone-susceptible isolates were also resistant to gentamicin and tobramycin, 48 and 82%, respectively; the MIC_{90} of gentamicin was 32 μg/ml, and that of tobramycin was >128 μg/ml. All isolates were susceptible to novobiocin and vancomycin (MICs, ≤1.0 and ≤2.0 μg/ml, respectively).

Bacteriophage susceptibility. The results of testing of susceptibility to standard bacteriophages are shown in Table 2.

TABLE 2. Bacteriophage susceptibilities of 50 quinolone-resistant MRSA isolates

Phage type[a]	Phage group[b]	No. of isolates
29/52/79/80/6/42E/54/92/93/95/82+	I, III, P	1
29/52/52A/79/80/54/93/82+	I, III, P	2
29/52/52A/79/80/54/85/92/93/82+	I, III, P	3
29/52/52A/79/80/42E+	I, III	1
47	III	2
47/54+	III	3
47/54/75/83A/92/93+	III, P	1
52A/83A/95+	I, III, NA	1
54/83A/95+	III, NA	4
54/95+	III, NA	1
75+	III	3
77/93	III, P	2
77/92/93	III, P	2
77/92/93+	III, P	5
77/83A/92/93+	III, P	3
83A/89/90/92/93	III, P	1
92/93+	P	3
93	P	3
93+	P	5
94/96	P	1
95+	NA	1
None (not typeable)[c]		2

[a] Tested at the routine test dilution, unless otherwise indicated.
[b] P, provisional; NA, not allocated.
[c] Tested at 100 times the routine test dilution.

Fluoroquinolone resistance was distributed among 22 different phage patterns. The predominant phage group associated with resistance was group III; in this group, 70% of isolates were resistant. Sixty-four percent of isolates were susceptible to phages belonging to the provisional group, while susceptibilities to group I phages and to other experimental phages which have not been allocated to a particular group were 16 and 14%, respectively. Only two isolates (4%) were non-phage typeable. A number of isolates were susceptible to phages belonging to more than one group.

DISCUSSION

The development and spread of multiple-antibiotic-resistant MRSA have gained much attention over the years (6, 15, 16). Traditionally, quinolones such as nalidixic acid had poor activity for gram-positive organisms (17). Fluoroquinolone compounds such as ciprofloxacin and norfloxacin, first synthesized in the 1980s, were found to have extended antimicrobial spectra which included gram-positive bacteria (8, 17), and there was a great deal of hope that these compounds would be useful in eradicating MRSA (1, 5, 17).

However, since these compounds became available for clinical use, resistance among MRSA has been observed in different parts of the world (6, 9, 10, 14). Ciprofloxacin and norfloxacin were released for routine clinical use in Canada between 1988 and 1989 (7). Nevertheless, 49% of the MRSA isolates (50 of 102) chosen at random from various centers across the province of Ontario over a 7-month period beginning in July 1989 were found to have resistance to ciprofloxacin and norfloxacin. The characteristic features of the quinolone-resistant MRSA isolates from Ontario, Canada, are their high level of resistance, their occurrence in separate institutions in different parts of the province, and their distribution among several different phage types. The resistance of the majority (98%) of the isolates to tobramycin and of a high percentage (42%) to gentamicin (Table 1) makes the emergence of these isolates a potentially serious public health problem.

The phage group determination performed in our study showed that the ciprofloxacin- and norfloxacin-resistant isolates belonged to multiple phage types from several phage groups. The predominant phage group associated with resistance was group III; 70% of isolates were associated with this group. The response to phages from the provisional group was a close second; in this group, 64% of isolates were susceptible (Table 2). The finding in this study of multiple phage types among the ciprofloxacin- and norfloxacin-resistant MRSA isolates suggests that resistance developed independently across the province of Ontario rather than that transmission of one isolate occurred between several different institutions. It is not presently known whether group III is the most common phage group associated with MRSA in Ontario, and this is currently being investigated. The quinolone-susceptible MRSA isolates also showed resistance to gentamicin and tobramycin, 48 and 82%, respectively. Resistance to these aminoglycosides appears to be a problem among MRSA isolates in Ontario (4).

The data presented here demonstrate the development of high-level resistance to norfloxacin and ciprofloxacin among MRSA isolates in the province of Ontario. High-level resistance to the aminoglycosides tobramycin and gentamicin was also encountered among the isolates investigated. Isolates from a number of neighboring hospitals and regional public health laboratories across Ontario were examined. The high percentage of resistance to quinolone compounds

(49%) in this test population, although not necessarily a reflection of the percentage to be found in every hospital in Ontario, is alarming. In view of these results, we feel that there is a need for reevaluation of the use of the 4-fluoroquinolones described in this report for treating MRSA infections. To our knowledge, this is the first report of 4-fluoroquinolone-resistant MRSA in Canada.

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