Incidence and susceptibility of aerobic Gram-negative bacilli from 20 Canadian intensive care units: 1989-1993

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OBJECTIVE: To assess the prevalence of antibiotic resistance in Canadian intensive care units.

DESIGN: The antimicrobial profiles of 1939 Gram-negative bacilli isolated in 20 Canadian intensive care units were analyzed using a custom designed MicroScan panel.

SETTING: The majority of the hospitals were tertiary care institutions, but some community hospitals were included.

PATIENTS: Adult intensive care unit patients were the sources of isolates.

MAIN RESULTS: *Pseudomonas aeruginosa* was the most frequently isolated microorganism overall, with *Escherichia coli* the most common initial isolate. Comparison of initial and repeat isolates showed that *P aeruginosa* readily acquired resistance to all antibiotic classes except the aminoglycosides. *Enterobacter aerogenes* developed resistance to ciprofloxacin and *Enterobacter cloacae* demonstrated resistance to all beta-lactam antibiotics except for imipenem on repeat isolation. Other Enterobacteriaceae remained susceptible. Historical comparison with data derived four years previously from 15 of the centres showed increased resistance of *P aeruginosa* and *Acinetobacter* species to ciprofloxacin while other susceptibility patterns remained stable.

CONCLUSIONS: The prevalence of Gram-negative resistance in Canadian hospitals is less than that reported in surveys done in some other countries, and was relatively stable over four years.

Key Words: Antibiotics, Intensive care units, Resistance

Incidence et sensibilité des bacilles gram-négatifs aérobies provenant de 20 unités de soins intensifs canadiens – 1989-1993

OBJECTIF : Évaluer la prévalence de la résistance aux antibiotiques dans les unités de soins intensifs au Canada.

MODÈLE : Profil antimicrobien de 1 939 bacilles gram-négatifs isolés dans 20 unités de soins intensifs au Canada qui ont été analysés à l'aide d'une plaque MicroScan faite sur commande.

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CONTEXTE : La majorité des hôpitaux étaient des établissements de soins tertiaires, mais certains hôpitaux communautaires ont été inclus.

PATIENTS : Les sources des isolats ont été des patients d'unités de soins intensifs pour adultes.

PRINCIPAUX RÉSULTATS : *Pseudomonas aeruginosa* a été le microorganisme le plus souvent isolé de façon globale, *Escherichia coli* étant l'isolat initial le plus commun. La comparaison des isolats de départ et des isolats lors d'infections répétées a montré que *P. aeruginosa* pouvait acquérir facilement une résistance à toutes les classes d'antibiotiques à l'exception des aminosides. *Enterobacter aerogenes* a développé une résistance à la ciprofloxacine et *Enterobacter cloacae* a démontré une résistance à toutes les bêta-lactamines à l'exception de l'imipénem lors de prélèvements répétés. D'autres *Enterobacteriaceae* sont demeurés sensibles. La comparaison historique avec des données dérivées quatre ans auparavant de 15 des centres a démontré une résistance accrue de *P. aeruginosa* et des *Acinetobacter* à la ciprofloxacine, alors que les autres modes de sensibilité sont demeurés stables.

CONCLUSION : La prévalence de la résistance des organismes gram-négatifs dans les hôpitaux canadiens est moindre que ce que l'on avait rapporté dans les enquêtes épidémiologiques effectuées dans les autres pays et ont été relativement stables pendant quatre ans.

Nosocomial infections are a significant cause of mortality and morbidity in patients hospitalized in intensive care units (ICUs), with infection rates disproportionately higher than in general wards (1). Gram-negative pathogens predominate, with *Pseudomonas aeruginosa* and *Enterobacter* species accounting for 21% of isolates in a survey of adult and pediatric ICUs in the United States (2). The choice of appropriate antimicrobial regimens in such critically ill patients is crucial. Therapeutic decisions are influenced by antibiotic resistance patterns, which change in response to the pressure of antimicrobial use, changes in patient population, prevalence of microorganisms and the emergence of new resistance mechanisms. Hospital-wide surveys of resistance can serve as guides in antibiotic selection, but the epidemiology of ICU resistance may not be discerned by that approach.

This study examined the antimicrobial profiles of 1939 Gram-negative bacilli isolated from patients hospitalized in 20 Canadian ICUs over a six-month period in 1993. Resistance in Gram-negative bacilli was studied because of their predominance as ICU pathogens and because of the selective pressure exerted upon them by the new beta-lactam antibiotics. The purpose of the survey was to determine the prevalence of isolates and their patterns of resistance and to compare these with the patterns previously noted in the 15 centres that participated in a similar study in 1989.

PATIENTS AND METHODS

Microorganisms: A total of 1939 isolates of aerobic Gram-negative bacilli were obtained from blood, urine, lower respiratory tract, wound, peritoneal cavity, abscess and indwelling line specimens of ICU patients in 20 Canadian teaching hospitals. Each participating hospital laboratory collected approximately 100 consecutive isolates, which were identified by standard methods. Once identified, isolates were stored frozen until susceptibility tests were performed. Repeat isolates of the same species from the same patient site were excluded unless they were collected at least 48 h apart. No attempts were made to differentiate strains of the same species. Standard quality control strains of *Escherichia coli* and *P. aeruginosa* from the American Type Culture Collection were used according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (3).

Patients: There were no patient exclusion criteria and 852 adult patients contributed one or more isolates to the study. Patient demographic data were not recorded. Patients were prescribed treatment by their attending physicians without regard to the study.

Antimicrobial susceptibility tests: Broth microdilution minimum inhibitory concentration (MIC) tests were performed using custom MicroScan CAN91 panels (Baxter Diagnostics Inc, California) with 16 antimicrobials at four doubling dilutions above and below the susceptibility breakpoints. Dilutions were performed using brain-heart infusion broth according to the manufacturer's recommendations. The antibiotics tested were amikacin, ampicillin, ampicillin/sulbactam, cefoperazone, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin, ticarcillin, ticarcillin/clavulanate and tobramycin. The plates were read at 18 to 24 h and interpreted according to the criteria described in the NCCLS document M100-S4:(M7-A2) (3).

Study design and analysis: Each of the 20 participating laboratories received the study protocol, broth microdilution plates and reagents, and standardized data recording forms. The results were entered into a computer database at Merck Frosst Canada Inc, Kirkland, Quebec. The prevalence of bacterial species and overall percentage of resistance to the antibiotics tested were separately analyzed for initial and repeat isolates. Historical comparison was undertaken in 15 of the 20 centres that had participated in a similar study of 33 Canadian ICUs done in 1989-90 as a Merck Frosst local surveillance project (4). This previous study used MicroScan MIC Plus Type 2 panels (Baxter Diagnostics Inc) and identical patient selection and microorganism sampling criteria. A two-tailed Fisher's exact test was used to calculate P values with 0.05 as the threshold of significance.

RESULTS

1993 data analysis: Twenty centres contributed 1939 isolates from 852 patients in 1993. Initial isolates comprised 66.7% of the total (1293) and 33.3% (646) were repeat specimens. The majority were from respiratory specimens (1113) followed by urine (249), wounds (221), blood and other sterile sites (226), catheter tip cultures (117) and miscellaneous other sites (13). The frequency and percentage susceptibility of species with

TABLE 1

Frequency and percentage of susceptibility of common isolates (20 centres, 1993, initial and repeat isolates)

Species	Number of isolates	Antibiotic													
		IPM 4*	CAZ 8	CFT 8	CAX 8	CFP 16	TIM 16	T 16	PIP 16	AMP 8	AS 8	GM 4	TOB 4	AMK 16	CP 1
<i>Pseudomonas aeruginosa</i>	433	81	85	18	23	80	83	88	92	0	0	73	96	92	83
<i>Escherichia coli</i>	372	100	98	98	98	90	88	73	75	70	73	95	96	99	98
<i>Enterobacter cloacae</i>	222	99	61	61	59	63	53	53	60	9	29	96	96	99	95
<i>Klebsiella pneumoniae</i>	196	98	96	95	94	94	93	5	69	3	87	95	95	98	96
<i>Stenotrophomonas maltophilia</i> [†]	122	2	59	10	4	40	56	28	33	4	8	9	5	13	9
<i>Serratia marcescens</i>	115	97	100	96	96	93	90	81	93	6	11	98	80	98	91
<i>Klebsiella oxytoca</i>	74	98	97	98	94	93	94	6	66	1	86	100	100	100	100
<i>Enterobacter aerogenes</i>	72	100	65	76	69	63	52	50	61	13	37	98	98	98	91
<i>Acinetobacter</i> species [‡]	65	100	89	60	69	18	92	90	66	26	93	66	69	76	67
<i>Proteus mirabilis</i>	65	96	100	100	100	96	100	95	96	89	90	96	98	98	96
<i>Morganella morganii</i>	40	85	87	87	95	87	77	75	82	5	27	95	100	90	100
<i>Citrobacter freundii</i>	35	100	57	60	57	57	54	48	54	17	45	85	88	88	85
<i>Acinetobacter anitratus</i>	23	100	82	47	52	8	82	82	52	8	95	78	86	91	82
<i>Proteus vulgaris</i>	16	93	100	68	43	62	100	43	62	0	56	100	100	100	100

*Breakpoint (µg/mL); [†]Formerly *Xanthomonas maltophilia*; [‡]Excluding *A. anitratus*. AMK Amikacin; AMP Ampicillin; AS Ampicillin/sulbactam; CAX Ceftriazone; CAZ Ceftazidime; CFP Cefoperzone; CFT Cefotaxime; CP Ciprofloxacin; GM Getamycin; IPM Imipenem; P Piperacillin; T Ticarcillin; TIM Timentin; TOB Tobramycin

greater than 10 isolates is presented in Table 1. Fifty species were represented in the survey, with 14 species comprising more than 95% of all isolates.

P. aeruginosa was the most frequently isolated organism overall, followed by *E. coli*, which was the most common initial isolate.

E. coli, *Klebsiella* species and *Proteus* species were susceptible to beta-lactam drugs, aminoglycosides and ciprofloxacin. *P. aeruginosa* and *Xanthomonas maltophilia* (since renamed *Stenotrophomonas maltophilia*) accounted for the greatest proportion of resistance overall, except to ceftazidime and ticarcillin/clavulanate for which the inducible Enterobacteriaceae (ie, *Enterobacter* species, *Citrobacter freundii*, *Morganella morganii*) and *Acinetobacter* species dominated.

Comparison of the susceptibility patterns of initial and repeat isolates of the 11 most common species (Table 2) showed that *P. aeruginosa* readily acquired resistance to all the agents except to the aminoglycosides and ticarcillin/clavulanate. *Enterobacter aerogenes* developed resistance to ciprofloxacin and *Enterobacter cloacae* developed resistance to the cephalosporins, ticarcillin/clavulanate and to ciprofloxacin but re-

mained susceptible to imipenem and the aminoglycosides. The noninducible Enterobacteriaceae (ie, *Klebsiella* species and *E. coli*) remained susceptible on repeat isolation except for an increase in resistance to tobramycin in *Klebsiella pneumoniae*.

Historical data analysis: Fifteen of the 20 centres in the 1993 study also participated in a similar survey in 1989-90 (4). Susceptibilities to nine of the antibiotics were determined in both of studies, and the NCCLS breakpoints remained the same. The frequency of distribution of the microorganisms (as a percentage of all initial isolates) was stable over time, except for *Acinetobacter* species, which was relatively less frequent in 1993. Table 3 demonstrates changes that occurred since 1989-90 in susceptibility to antibiotics common to both studies. The only increase in resistance noted was to ciprofloxacin in *P. aeruginosa* and *Acinetobacter* species.

DISCUSSION

The participating ICUs in this susceptibility survey represented a broad cross-section of Canadian hospitals, from tertiary care teaching centres to community hospitals, ranging

TABLE 2
Percentage susceptibility of selected antibiotics for the 11 most common microorganisms (initial versus repeat isolates)

Species	Number of isolates		Antibiotic																	
			Imipenem		Ceftazidime		Cefotaxime		Ceftriaxone		Timentin		Gentamicin		Tobramycin		Amikacin		Ciprofloxacin	
	Initial	Repeat	Initial	Repeat	Initial	Repeat	Initial	Repeat	Initial	Repeat	Initial	Repeat	Initial	Repeat	Initial	Repeat	Initial	Repeat	Initial	Repeat
<i>Escherichia coli</i>	275	97	100	100	98	98	97	98	97	98	89	85	95	93	96	96	99	98	98	100
<i>Pseudomonas aeruginosa</i>	230	203	87	73*	89	80*	23	12*	26	21	86	79	74	71	96	96	93	91	87	78*
<i>Enterobacter cloacae</i>	143	79	100	98	67	49*	68	48*	66	46*	59	43*	97	96	97	96	99	98	97	93
<i>Klebsiella pneumoniae</i>	141	55	97	100	97	92	97	92	95	92	94	90	96	94	97	90*	99	98	95	98
<i>Serratia marcescens</i>	70	45	97	97	100	100	97	95	97	95	92	86	97	100	75	88	98	97	91	91
<i>Klebsiella oxytoca</i>	56	18	98	100	96	100	98	100	94	94	92	100	100	100	100	100	100	100	100	100
<i>Enterobacter aerogenes</i>	48	24	100	100	64	66	77	75	68	70	54	50	100	95	100	95	100	95	97	79*
<i>Proteus mirabilis</i>	48	17	95	100	100	100	100	100	100	100	100	100	95	100	97	100	97	100	95	100
<i>Acinetobacter species</i>	44	21	100	100	90	85	59	61	75	57	90	95	70	57	75	57	75	80	72	57
<i>Citrobacter freundii</i>	26	9	100	100	61	44	65	44	61	44	57	44	84	88	88	88	92	77	92	66
<i>Morganella morganii</i>	27	13	81	92	92	76	92	76	100	84	85	61	100	84	100	100	96	76	100	100

*P<0.05

TABLE 3
Historical comparison in percentage of susceptibility of initial isolates (same 15 centres, 1989 versus 1993)

Species	Number of isolates		Antibiotic													
			Imipenem		Ceftazidime		Cefotaxime		Ceftriaxone		Timentin		Ticarcillin		Ciprofloxacin	
	1989	1993	1989	1993	1989	1993	1989	1993	1989	1993	1989	1993	1989	1993	1989	1993
<i>Escherichia coli</i>	270	211	98	100	98	99	99	97	99	97	93	88	74	74	100	99
<i>Pseudomonas aeruginosa</i>	201	173	83	91*	83	89	15	22	20	24	89	85	87	86	95	84*
<i>Enterobacter cloacae</i>	140	114	97	100	75	68	72	69	77	67	61	60	62	60	100	97
<i>Klebsiella pneumoniae</i>	110	98	99	97	98	97	97	96	97	95	95	94	4	5	99	95
<i>Stenotrophomonas maltophilia</i> [†]	53	53	3	0	30	56*	3	7	1	3	56	62	13	26	18	9
<i>Serratia marcescens</i>	47	47	100	97	89	100	87	97	89	100	87	95	59	74	91	87
<i>Klebsiella oxytoca</i>	36	39	100	97	100	94	94	97	94	92	91	89	0	2	100	100
<i>Enterobacter aerogenes</i>	30	35	100	100	80	71	83	82	86	74	70	60	66	51	100	97
<i>Proteus mirabilis</i>	52	39	92	94	96	100	96	100	96	100	94	100	86	92	100	94
<i>Acinetobacter</i> species	66	17	98	100	92	88	77	64	83	82	92	82	83	82	92	58*
<i>Morganella morganii</i>	16	25	100	88	81	92	81	92	87	100	81	88	87	88	100	100
<i>Citrobacter freundii</i>	21	17	100	100	61	70	76	70	76	70	66	70	47	64	100	94
<i>Proteus vulgaris</i>	12	11	91	90	100	100	75	54	41	36	100	100	25	45	100	100

* $P < 0.05$; [†]Formerly *Xanthomonas maltophilia*

across a wide geographic area. By restricting our study to isolates from ICUs, we focused on microorganisms with the greatest potential for resistance. The proportion of isolates derived from the anatomical areas sampled in our study was similar to the frequency of ICU infections by site described in other recent surveys (5-7). The clinical significance of isolates was not assessed and no attempts were made to determine whether isolates were part of nosocomial outbreaks.

The broth microdilution method was selected as an easily reproducible standardized method for all centres. It also afforded comparison with the data collected in 1989-90. This method has recently been found to have less sensitivity than agar dilution or the E test in detecting plasmid-mediated, extended spectrum beta-lactamases (8). The data may therefore somewhat underrepresent the true prevalence of beta-lactam resistance in *E coli* and *Klebsiella* species.

The pooled resistance data for the 20 hospitals showed that *E coli*, and *Klebsiella* and *Proteus* species were susceptible to the beta-lactam drugs, aminoglycosides and ciprofloxacin, even the repeat isolates (Table 2). This suggests that the prevalence of the extended spectrum, plasmid-mediated beta-lactamases remained low in Canada in 1993.

The inducible Enterobacteriaceae (*Enterobacter* species, *C freundii* and *Morganella morganii*) and *Acinetobacter* species demonstrated considerable resistance to beta-lactam antibiotics, including third-generation cephalosporins, even on initial isolation. Resistance to imipenem was not seen in these organisms. This pattern of resistance to third-generation cephalosporins and susceptibility to imipenem is consistent with class I beta-lactamase activity (9).

Comparison of initial with repeat isolates revealed striking decreases in susceptibility to cephalosporins and ticarcillin/clavulanate in *E cloacae* isolates. In addition to beta-lactamase-mediated resistance, increased resistance to ciprofloxacin was noted in *E aerogenes*. Fluoroquinolone resistance is largely due to mutant DNA gyrases, but the emergence of such resistance in common with beta-lactam resistance is more likely due to increased drug extrusion across the cytoplasmic membrane (10). *P aeruginosa* rapidly developed resistance to beta-lactam antibiotics (including imipenem) and ciprofloxacin but not to aminoglycosides. Reduced susceptibility to imipenem can occur in *P aeruginosa* as a combination effect of chromosomal beta-lactamase and loss of the D2 porin (11). Avoiding extended courses of broad spectrum beta-lactam agents when possible and reculturing and retesting antimicrobial sensitivities during prolonged courses of therapy may be advisable.

Except for ceftazidime and ticarcillin/clavulanate resistance in initial isolates of inducible Enterobacteriaceae, *P aeruginosa* and *S maltophilia* accounted for the largest proportion of resistance overall. *S maltophilia* displayed the greatest resistance and is known to express a zinc-based beta-lactamase that endows it with broad spectrum resistance to beta-lactam drugs, including imipenem (12).

Opportunities to study changes in isolate frequencies and antibiotic resistance patterns over time, particularly with the same methods and study sites, are few. We were able to com-

pare data for 15 centres common to both study periods. Initial isolates were chosen to eliminate distortions due to excessive numbers of repeat isolates from 'jackpot' patients, resulting in a more accurate depiction of changes in susceptibility over time.

The proportion of *Acinetobacter* species isolated was significantly lower in the 1993 study than in the 1989-90 study. The isolation frequencies of the other microorganisms were remarkably similar, in contrast to other surveys in which *Enterobacter* species have been noted to be increasing (13,14). An increase in resistance to ciprofloxacin among *P aeruginosa* and *Acinetobacter* species was the only significant change in resistance noted and may reflect increased use of the drug in the community as well as in the hospital setting. The absence of increased beta-lactam resistance compared with recent reports from the United States (13,14) may be due in part to policies restricting certain antibiotics in the majority of Canadian hospitals and to the six-month to one-year lag in Canadian drug licensing compared with the United States.

The potential for rapid emergence of resistance, particularly in *P aeruginosa* and the inducible Enterobacteriaceae, is ever-present. This report provides indirect evidence for the efficacy of rational antibiotic policies and continuing education of physicians in practices that minimize the emergence of antibiotic resistance.

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REFERENCES

1. Pittet D, Herwaldt LA, Massanari RM. The intensive care unit. In: Bennett JV, Brachman PS, eds. Hospital Infections. Boston: Little, Brown and Co, 1992:405-39.
2. Jarvis WR, Edwards JR, Culver DH, et al. Nosocomial infection rates in adult and paediatric intensive care units in the United States. *Am J Med* 1991;(Suppl 3B):185-91.
3. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard M7-A2 (M100-S4). Villanova: National Committee for Clinical Laboratory Standards, 1993.
4. Incidence of multiresistance in serial Gram-negative isolates from intensive care units. Kirkland: Merck-Frosst Canada Inc, 1990.

5. Shah PM, Asanger R, Kahan FM. Incidence of multi-resistance in Gram negative aerobes from intensive care units of 10 German hospitals. *Scand J Infect Dis* 1991;78(Suppl):22-34.
 6. Verbist L. Incidence of multi-resistance in Gram negative bacterial isolates from intensive care units in Belgium: a surveillance study. *Scand J Infect Dis* 1991;78(Suppl):48-53.
 7. Snyderman DR. Clinical implications of multi-drug resistance in the intensive care unit. *Scand J Infect Dis* 1991;78(Suppl):54-63.
 8. Katsanis GP, Spargo J, Ferraro MJ, et al. Detection of *Klebsiella pneumoniae* and *Escherichia coli* strains producing extended-spectrum β -lactamases. *J Clin Microbiol* 1994;32:691-6.
 9. Richmond MH, Sykes RB. The β -lactamases of Gram-negative bacteria and their possible physiological role. *Adv Microb Physiol* 1973;9:31-88.
 10. Nikaido H. Outer membrane barrier as a mechanism of antimicrobial resistance. *Antimicrob Agents Chemother* 1989;33:1831-6.
 11. Livermore DM. Interplay of impermeability and chromosomal β -lactamase activity in imipenem-resistant *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1992;36:2046-8.
 12. Bush K. Classification of β -lactamases: groups 2c, 2d, 2e, 3 and 4. *Antimicrob Agents Chemother* 1989;33:271-6.
 13. Schaberg DR, Culver DH, Gaynes RP. Major hands in the microbial etiology of nosocomial infection. *Am J Med* 1991;(Suppl 3B):72-5.
 14. Sanders CC, Sanders WG Jr. β -lactam resistance in Gram negative bacteria: global trends and clinical impact. *Clin Infect Dis* 1992;15:824-39.
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