In Vitro Activity of Colistin (Polymyxin E) against 3,480 Isolates of Gram-Negative Bacilli Obtained from Patients in Canadian Hospitals in the CANWARD Study, 2007-2008[∇]

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The in vitro activity of colistin was evaluated versus 3,480 isolates of gram-negative bacilli using CLSI broth microdilution methods. The MIC₉₀ of colistin was ≤ 2 $\mu\text{g/ml}$ against a variety of clinically important gram-negative bacilli, including *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. All multidrug-resistant ($n = 76$) *P. aeruginosa* isolates were susceptible to colistin (MIC, ≤ 2 $\mu\text{g/ml}$). These data support a role for colistin in the treatment of infections caused by multidrug-resistant *P. aeruginosa*.

Colistin (polymyxin E) is a polypeptide antimicrobial originally discovered in 1949 that targets the bacterial cell membrane (4). The use of colistin was largely abandoned by the early 1980s due to toxicity concerns (nephrotoxicity and neurotoxicity) (4, 9). However, there has recently been renewed interest in using polymyxins, including colistin, for the treatment of infections caused by gram-negative bacilli (especially *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) due to their activity versus multidrug-resistant (MDR) isolates (4, 9). There have been no current national surveillance studies published evaluating the in vitro activity of colistin versus a variety of nosocomial gram-negative bacterial isolates. The purpose of this study was to evaluate the in vitro activity of colistin against gram-negative bacilli obtained from patients in Canadian hospitals (CANWARD).

(These data were presented at the 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy-Infectious Diseases Society of America 46th Annual Meeting, Washington, DC, 25 to 28 October 2008 [13].)

Twelve sentinel hospital sites (10 in 2008) located in major population centers in 7 of the 10 provinces in Canada participated in the CANWARD study. These sites were geographically distributed in a population-based fashion. From January 2007 through December 2008, inclusive, each study site was asked to submit clinical isolates (consecutive, one per patient, per infection site) from inpatients and outpatients with respiratory ($n = 200$ per year), urine ($n = 100$ per year), wound/intravenous ($n = 50$ per year), and bloodstream ($n = 360$ per year) infections. Only isolates that were deemed clinically significant were submitted. Isolate identification was performed by the submitting site using local criteria. Where indicated, identification was confirmed at the reference site. Isolates were shipped on Amies semisolid

transport medium to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), where they were then subcultured on appropriate media and stocked in skim milk at -80°C .

Following two subcultures from frozen stock, the in vitro activity of colistin (colistin sulfate) was determined by broth microdilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (2, 3). Colistin MIC interpretive standards were defined according to CLSI breakpoints (2). At present, colistin susceptibility breakpoints (CLSI) only exist for *P. aeruginosa* and *A. baumannii*.

Extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* and *K. pneumoniae* isolates were defined as isolates having a positive ESBL confirmatory test, as described by CLSI (2). MDR *P. aeruginosa* isolates were defined as isolates demonstrating resistance to antimicrobials from three or more different classes. The number that appears in the following tables after the MDR designation indicates the number of different classes to which isolates were resistant (e.g., MDR3 indicates *P. aeruginosa* isolates that were resistant to at least one antimicrobial agent from three different classes). For the purpose of this report, the four antimicrobial classes considered were aminoglycosides (amikacin and gentamicin), fluoroquinolones (ciprofloxacin and levofloxacin), cefepime and piperacillin-tazobactam (considered together as one class), and carbapenems (meropenem). Colistin was not used in the classification of MDR isolates.

The in vitro activity of colistin was evaluated against 3,480 isolates of gram-negative bacilli from the specimen sources blood (51.6%), urine (25.8%), respiratory (18.1%), and wound (4.5%) and ward types emergency room (31.6%), medical (31.4%), intensive care unit (15.2%), clinic/office (14.4%), and surgical (7.4%). The MIC distribution of colistin, stratified by bacteria species, is presented in Table 1. Over 90% of *E. coli* and *K. pneumoniae* isolates (including 163 ESBL producers) had a MIC of ≤ 1 $\mu\text{g/ml}$. Twenty-nine of 31 *A. baumannii* isolates (93.5%) were susceptible to colistin (MIC, ≤ 2 $\mu\text{g/ml}$). In contrast, colistin demonstrated minimal activity (MIC₅₀, ≥ 8 $\mu\text{g/ml}$) versus *Proteus mirabilis*,

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TABLE 1. MIC distribution of colistin versus 3,480 isolates of gram-negative bacilli obtained from patients in Canadian hospitals

Organism (no. of isolates)	No. (%) of isolates susceptible at MIC ($\mu\text{g/ml}$) ^a :										Total susceptible
	≤ 0.06	0.12	0.25	0.5	1	2	4	8	16	>16	
<i>E. coli</i> (1,732)	1 (0.1)	19 (1.2)	432 (26.1)	975 (82.4)	258 (97.3)	36 (99.4)	3 (99.5)	0 (99.5)	2 (99.7)	6 (100.0)	1,732
<i>P. aeruginosa</i> (561)	0 (0.0)	0 (0.0)	0 (0.0)	14 (2.5)	149 (29.1)	351 (91.6)	41 (98.9)	1 (99.1)	0 (99.1)	5 (100.0)	561
<i>K. pneumoniae</i> (515)	0 (0.0)	4 (0.8)	50 (10.5)	347 (77.9)	88 (95.0)	11 (97.1)	3 (97.7)	1 (97.9)	1 (98.1)	10 (100.0)	515
<i>E. cloacae</i> (186)	0 (0.0)	3 (1.6)	23 (14.0)	104 (69.9)	23 (82.3)	3 (83.9)	2 (84.9)	1 (85.5)	5 (88.2)	22 (100.0)	186
<i>P. mirabilis</i> (119)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	118 (100.0)	119
<i>S. marcescens</i> (108)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.9)	0 (1.9)	0 (1.9)	0 (1.9)	0 (1.9)	106 (100.0)	108
<i>K. oxytoca</i> (108)	0 (0.0)	0 (0.0)	20 (18.5)	60 (74.1)	19 (91.7)	4 (95.4)	2 (97.2)	1 (98.1)	0 (98.1)	2 (100.0)	108
<i>S. maltophilia</i> (83)	0 (0.0)	0 (0.0)	1 (1.2)	3 (4.8)	5 (10.8)	4 (15.7)	15 (33.7)	14 (50.6)	5 (56.6)	36 (100.0)	83
<i>E. aerogenes</i> (37)	0 (0.0)	0 (0.0)	7 (18.9)	20 (73.0)	9 (97.3)	0 (97.3)	1 (100.0)				37
<i>A. baumannii</i> (31)	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.5)	21 (74.2)	6 (93.5)	0 (93.5)	0 (93.5)	1 (96.8)	1 (100.0)	31

^a As determined by the colistin broth microdilution value. Cumulative percentages of all isolates tested are shown in parentheses.

Serratia marcescens, and *Stenotrophomonas maltophilia* (Table 1).

The in vitro activity of colistin was evaluated against 561 *P. aeruginosa* isolates (including 76 MDR isolates). The percentages of *P. aeruginosa* isolates susceptible (MIC, ≤ 2 $\mu\text{g/ml}$), intermediately susceptible (MIC, 4 $\mu\text{g/ml}$), and resistant (MIC, ≥ 8 $\mu\text{g/ml}$) to colistin were 91.6%, 7.3%, and 1.1%, respectively. All MDR isolates were susceptible to colistin. The MIC distribution of colistin versus *P. aeruginosa* isolates, stratified by antimicrobial resistance, is presented in Table 2. Between 91 and 100% of antimicrobial-resistant *P. aeruginosa* isolates remained susceptible to colistin (Table 2).

The data presented here demonstrate that colistin is active in vitro (MIC₉₀, ≤ 2 $\mu\text{g/ml}$) versus a variety of clinically important gram-negative bacilli. These results are in agreement with colistin susceptibility data described in two other small surveillance studies (1, 12). They are also consistent with susceptibility results for polymyxin B, a related polypeptide antimicrobial that was recently evaluated versus over 50,000 gram-negative bacterial isolates collected as part of the SENTRY surveillance study (5).

All MDR *P. aeruginosa* isolates evaluated in this study remained susceptible to colistin. These data suggest that colistin

may be a viable therapeutic option for the treatment of infections caused by MDR *P. aeruginosa*. A number of cohort and observational clinical studies have been published that support the efficacy of colistin in treating patients with infections caused by MDR gram-negative bacilli (*A. baumannii* and *P. aeruginosa*) (6–11).

There are several limitations to the data described in this report. Isolates were determined to be clinically significant by the submitting microbiology laboratory based on local criteria. It is possible that some of the included isolates were actually colonizers as opposed to pathogens. Few *A. baumannii* isolates were collected as a part of CANWARD, and those that were obtained tended to be susceptible to antimicrobials from a number of different classes (data not shown). Hence, no conclusions can be drawn from these data on the in vitro activity of colistin versus MDR *A. baumannii*. Finally, the number of MDR *P. aeruginosa* isolates evaluated in this study was also relatively small.

In summary, colistin was active in vitro (MIC₉₀, ≤ 2 $\mu\text{g/ml}$) against a variety of clinically important gram-negative bacilli, including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *A. baumannii*, and *P. aeruginosa*. All 76 MDR *P. aeruginosa* clinical isolates evaluated remained susceptible (MIC, ≤ 2 $\mu\text{g/ml}$) to colistin. These data support a role for colistin in

TABLE 2. MIC distribution of colistin versus *P. aeruginosa* isolates obtained from patients in Canadian hospitals

<i>P. aeruginosa</i> category (no. of isolates)	No. (%) of isolates susceptible at MIC ($\mu\text{g/ml}$) ^a :							Total susceptible
	0.5	1	2	4	8	16	>16	
All isolates (561)	14 (2.5)	149 (29.1)	351 (91.6)	41 (98.9)	1 (99.1)	0 (99.1)	5 (100.0)	561
Amikacin resistant (38)	2 (5.3)	14 (42.1)	21 (97.4)	0 (97.4)	0 (97.4)	0 (97.4)	1 (100.0)	38
Cefepime resistant (73)	5 (6.8)	24 (39.7)	42 (97.3)	2 (100.0)				73
Ciprofloxacin resistant (147)	6 (4.1)	40 (31.3)	90 (92.5)	9 (98.6)	0 (98.6)	0 (98.6)	2 (100.0)	147
Gentamicin resistant (104)	4 (3.8)	33 (35.6)	66 (99.0)	0 (99.0)	0 (99.0)	0 (99.0)	1 (100.0)	104
Levofloxacin resistant (166)	3 (1.8)	48 (30.7)	102 (92.2)	10 (98.2)	0 (98.2)	0 (98.2)	3 (100.0)	166
Meropenem resistant (57)	2 (3.5)	17 (33.3)	37 (98.2)	1 (100.0)				57
Piperacillin-tazobactam resistant (56)	1 (1.8)	20 (37.5)	31 (92.9)	4 (100.0)				56
MDR3 isolates (50)	2 (4.0)	16 (36.0)	32 (100.0)					50
MDR4 isolates (26)	2 (7.7)	9 (42.3)	15 (100.0)					26
All MDR isolates (76)	4 (5.3)	25 (38.2)	47 (100.0)					76

^a As determined by the colistin broth microdilution value. Cumulative percentages of all isolates tested are shown in parentheses. The percentage of isolates susceptible at a colistin MIC of ≤ 2 $\mu\text{g/ml}$ is in boldface, as this represents the colistin susceptibility breakpoint for *P. aeruginosa*.

the treatment of patients with infections caused by MDR *P. aeruginosa*.

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