The in-vitro activity of colistin in gram-negative bacteria

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ABSTRACT

Introduction: Colistin is a polypeptide antibiotic belonging to the polymyxins, and has been increasingly used for the treatment of multiresistant gram-negative infections. There is little current available data on the susceptibility of gram-negative bacilli to colistin, in part because susceptibility testing for colistin remains problematic, and also because the use of colistin is not widespread. This study tested clinical isolates of gram-negative bacilli for susceptibility to colistin using the reference susceptibility testing method of agar dilution.

Methods: 102 strains of gram-negative bacilli were collected over a one-year period. Antibiotic susceptibility profiles were derived from disc susceptibility testing, and organisms were identified by standard microbiological methods. Isolates were selected for inclusion in the study using susceptibility profiles and epidemiological data. Minimum inhibitory concentrations to colistin were obtained by performing agar dilution according to a standardised method.

Results: 30 percent of tested isolates were resistant to colistin. All *Acinetobacter* spp. and *Escherichia coli* were susceptible to colistin. Colistin resistance was detected predominantly in *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*, but was also present in *Enterobacter* spp. and *Klebsiella* spp.

Conclusion: Colistin resistance is uncommon in the Enterobacteriaceae, but present in a significant proportion of *S. maltophilia* and *P. aeruginosa* isolates. From the results of this study, we recommend that susceptibility testing be performed whenever the clinical use of the polymyxins is considered.

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INTRODUCTION

Colistin and polymyxin B belong to the group of polypeptide antibiotics collectively known as polymyxins. Although parenteral formulations of the polymyxins have existed since the 1960s for the treatment of gram-negative infections, clinical use declined significantly following the introduction of broad-spectrum antibiotics with less toxicity, such as the cephalosporins. Topical, non-absorbable formulations of colistin continued to be used for selective decontamination of the gastrointestinal tract[1], while nebulised colistin was increasingly used in the treatment of cystic fibrosis patients colonised with *Pseudomonas aeruginosa (P. aeruginosa)*[2]. The predominant side effects of parenteral colistin described in early studies include neuromuscular blockade and dose-related renal toxicity[3] although more recent experience suggests that the incidence of major side-effects may be lower than previously reported[4].

The inexorable rise of antibiotic resistance and the paucity of new antimicrobials[5] have led to renewed interest in the use of colistin for the treatment of infections with multiple-resistant bacteria[6]. Colistin has successfully been used for the treatment of ventilator-associated pneumonia caused by *Acinetobacter baumanii*[4] and *P. aeruginosa*[7] and bacteraemia with *Klebsiella pneumoniae (K. pneumoniae)*[8].

Much of the data on the antimicrobial activity of the polymyxins are derived from early studies. There is very little current knowledge about the prevalence of innate or acquired resistance to colistin. Knowledge of local antibiotic susceptibility profiles[9] is an important prerequisite for the appropriate selection and use of antibiotics. This study reports on the in-vitro susceptibility of 102 clinical isolates of gram-negative bacilli to colistin, tested using the agar-dilution method.

Table I. Susceptibility of gram-negative bacilli included in the study.

		AN	SAM	CAZ	CIP	IMP	GEN	MIN	TZP	SXT
Acinetobacter spp.	n	13	12	10	5	6	7	14	6	8
	%	(72%)	(67%)	(56%)	(28%)	(33%)	(39%)	(78%)	(33%)	(44%)
Enterobacter spp.	n	7	n/a	4	4	7	5	n/a	4	8
	%	(88%)		(50%)	(50%)	(88%)	(63%)		(50%)	(50%)
E. coli	n	11	n/a	4	3	13	10	n/a	8	4
	%	(85%)		(31%)	(23%)	(100%)	(77%)		(62%)	(31%)
Klebsiella spp.	n	7	n/a	3	2	15	4	n/a	2	2
	%	(44%)		(19%)	(13%)	(94%)	(25%)		(13%)	(13%)
P. aeruginosa	n	15	n/a	9	9	13	7	n/a	16	n/a
	%	(45%)		(27%)	(27%)	(39%)	(21%)		(48%)	
S. maltophilia	n	0	n/a	n/a	n/a	0	0	14	n/a	13
	%	(0%)				(0%)	(0%)	(82%)		(76%)

n/a: not applicable; AN: amikacin; SAM: ampicillin-sulbactam; CAZ: ceftazidime; CIP: ciprofloxacin; IMP: imipenem; GEN: gentamicin; MIN: minocycline; TZP: piperacillin-tazobactam; SXT: trimethoprim-sulphamethoxazole

METHODS

18 saccharolytic *Acinetobacter* spp., 33 *P. aeruginosa*, 17 *Stenotrophomonas maltophilia* (*S. maltophilia*) and 34 Enterobacteriaceae isolates were collected over a 12-month period from clinical specimens, commencing from April 2004. Only unique isolates were included. Bacterial identification was performed using standard laboratory methods[10-12], and the following commercial identification kits: API20E, API20NE and Vitek II (bioMérieux, France). Antimicrobial disc susceptibility testing was performed for the following antibiotics: gentamicin (10mcg), piperacillin-tazobacam (110 mcg), ciprofloxacin (5 mcg), ceftazidime (30 mcg), imipenem (10 mcg), ampicillin-sulbactam (20 mcg), sulfamethoxazole-trimethoprim (23.75 mcg/1.25 mcg), minocycline (30 mcg), and amikacin (30 mcg). Susceptibility results were interpreted according to standards published by the Clinical Laboratory Standards Institute (CLSI)[13] (Table I). Isolates that were only susceptible to two or less antibiotics from the tested panel were deemed multi-resistant. Isolates belonging to the same genus were grouped by their antibiogram profile. No more than two isolates from each genus with the same antimicrobial susceptibilities were included in this study.

Minimum inhibitory concentrations (MICs) to colistin were obtained by the agar dilution method, performed according to CLSI methods[14]. Colistin sulfate powder (Sigma-Aldrich, Singapore) was dissolved in sterile ultrapure water and added to molten Mueller-Hinton II agar (Becton-Dickinson, Maryland, USA) to provide twofold concentrations ranging from 0.25 to 128 mg/L. Bacterial suspensions were prepared from fresh overnight cultures and adjusted to a turbidity density of 0.5 MacFarland using a nephelometer (bioMérieux, France). The bacterial suspension was applied to agar plates using a multipoint innoculator (Mast Diagnostics, Bootle, England) to yield a final inoculum of 10^4 colony forming units per spot. The results were read following ambient atmospheric incubation for 16-18 hours at 35°C. Sterility and growth controls were performed. American Type Culture Collection (ATCC) strains of *Escherichia coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were included as quality controls (QC). Test values obtained for the QC strains were in line with published standards[13]. The MIC value for each tested organism was defined as the lowest concentration that inhibited visible growth of the organism. Strains with MIC of ≥4 mg/L were interpreted as resistant to colistin[15].

RESULTS

102 bacterial isolates were included in the study, of which 51 were multi-resistant. 31 isolates (30%) were resistant to colistin. In general, colistin demonstrated good activity against *Acinetobacter* spp. (MIC_{90} ≤2 mg/L), *K. pneumoniae* (MIC_{90} ≤1 mg/L), and *E. coli* (MIC_{90} ≤2 mg/L). MIC values for *Enterobacter* spp. (MIC_{90} ≤16 mg/L) and *P. aeruginosa* (MIC_{90} ≤4 mg/L) isolates were much more diverse. All tested strains of *Stenotrophomonas maltophilia* were resistant to colistin (MIC_{90} ≥128 mg/L). Susceptibility to colistin was most prevalent in *Acinetobacter* spp. with no resistant isolates detected. 11 isolates (33%) of *P. aeruginosa*, one isolate (8%) of *K. pneumoniae* and two isolates (15%) of *Enterobacter spp.* were resistant to

Table II. In-vitro activity of colistin in gram-negative bacilli.

Organism (number of isolates)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	% susceptibility[a]
Acinetobacter spp. (18)	≤1	2	<1 - 2	100%
E. coli (13)	≤1	≤1	<1 - 2	100%
Enterobacter spp. (8)	≤1	16	<1 - 16	75%
K. pneumoniae (16)	≤1	≤1	<1 - 4	94%
P. aeruginosa (33)	2	4	2 - 16	67%
S. maltophilia (17)	128	>256	8 - >256	0%

[a] defined as MIC ≤2 mg/L

colistin. The distribution of colistin MICs for this study is listed in Table II.

DISCUSSION

Colistin is a cationic polypeptide that exerts its antimicrobial activity against the bacterial cell wall through anionic displacement of stabilising magnesium and calcium. This results in leakage of cell contents and eventual cell death[16]. The polymyxins have bactericidal activity against *Acinetobacter* spp., *P. aeruginosa* and most members of the Enterobacteriaceae family and have been reported to demonstrate reasonable in-vitro activity against *S. maltophilia*[15,17]. *Burkholderia pseudomallei*, *Proteus* spp., *Providencia* spp. and *Serratia* spp. are intrinsically resistant. The polymyxins demonstrate no activity against gram-negative and gram-positive cocci, gram-positive bacilli and anaerobes[18].

The evolution of multiple drug resistance among gram-negative bacilli has resulted in the development of resistance to beta-lactams, aminoglycosides and the carbapenems[19]. Polymyxins have increasingly been used in the treatment of gram-negative infections, where no other less toxic or effective antibiotic is available. The increased clinical use of parenteral polymyxins has created a pressing need for up-to-date susceptibility data and standardised susceptibility testing methods.

Few systematic surveys of antibiotic resistance have been performed on this group of antimicrobials, so reliable data on true resistance rates are lacking. Interpretation of categoric resistance is further complicated by susceptibility criteria which may vary from country to country[20]. Colistin resistance is best documented in *P. aeruginosa*[21]. A survey of cystic fibrosis patients in the United Kingdom reported that 3.1% of *P. aeruginosa* isolates were resistant to colistin, based on a susceptibility breakpoint concentration ≤4 mg/L and susceptibility testing by Etest[22]. Another study, also from the United Kingdom, tested clinical gram-negative isolates by agar dilution using the same breakpoint concentrations of 4 mg/L. This study reported similar levels of resistance in *P. aeruginosa*, but also documented unexpectedly high rates of resistance in *Enterobacter* spp. (32%) and *Klebsiella* spp. (12%). Conversely, a survey of bloodstream isolates from the United States, tested by agar dilution and using a susceptibility breakpoint concentration of ≤2 mg/L, documented low levels of polymyxin resistance in *Acinetobacter* spp. and the Enterobacteriaceae.

The results of this study reinforce the importance of local and regional susceptibility data. In our institution, over a third of *P. aeruginosa* isolates were found to have low-level in-vitro resistance to colistin. In contrast to other published reports[17], all our isolates of *S. maltophilia* were resistant to colistin with MIC's ranging from 4 mg/L to over 64 mg/L. Although the actual numbers tested were small, colistin resistance in *Klebsiella* spp. and *Enterobacter* spp. was not uncommon. All tested isolates of *Acinetobacter* spp. remained susceptible to colistin. This study was not an epidemiological survey of colistin resistance in gram-negative isolates in Singapore, as only isolates from one institution were tested. In order to minimise the possibility of testing related clonal strains, isolates were specifically selected using demographic and antibiotic susceptibility patterns. The mechanisms of resistance have best been studied in *P. aeruginosa* and primarily appear to result from changes in the outer membrane protein OprH[23], although alterations in lipopolysaccharide fatty acid composition have been detected for in-vitro adaptive resistant strains[24]. Resistance in *Salmonella* species results from changes in negatively-charged surface lipopolysaccharides[25]. Resistance to colistin appears to confer cross-resistance to other polymyxins[18].

Antibiotic susceptibility testing for the polymyxins remains problematic. More data are available for susceptibility testing of colistin sulphate than for polymyxin B. However, susceptibility testing using either agent appears to be predictive of resistance to the polymyxin class of antibiotics[13,20]. Although standardised disc susceptibility testing methods for colistin have been published in the United Kingdom[26] and France[27], equivalent data from the CLSI in the United States are lacking. Disc susceptibility testing has been documented to be inaccurate, with a high proportion of false susceptibility reports[15]. Agar dilution or broth microdilution methods show good reproducibility[15,20]. There remains no information on the accuracy of semi-automated methods such as Vitek (BioMérieux, France) or Microscan (Dade-Behring, USA). Etest methods have been shown to be accurate for testing colistin susceptibility

in *Acinetobacter* spp., with over 98% categorical agreement[28]. In contrast, the results by the Etest method for *S. maltophilia* were less satisfactory, with a very major error rate of 6%[17].

Resistance to colistin appears to be common in *P. aeruginosa* and *S. maltophilia* isolates in our institution. These results suggest that universal susceptibility to the polymyxins should not be assumed, particularly for *P. aeruginosa*. Although there remain some uncertainty regarding the most appropriate breakpoints for susceptibility testing, we recommend performing MIC susceptibility testing prior to empirical use of the polymyxins for multi-resistant gram-negative bacilli.

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