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The prevalence of colistin-resistant Gram-negative bacteria isolated from hospitalized patients with bacteremia

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ABSTRACT

This study sought to investigate the prevalence of colistin-resistant Gram-negative bacteria (CoR-GNB) among *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Escherichia coli*, and *Klebsiella pneumoniae* isolated from patients with bacteremia and to identify other antimicrobials as a potential therapy for CoR-GNB infections. We retrospectively reviewed the data of non-repeated clinical bacterial isolates from patients admitted to Phramongkutklao Hospital during May 2017–April 2018. We obtained the minimum inhibitory concentrations (MICs) of the studied isolates and interpreted the MIC values followed by the Clinical and Laboratory Standards Institute (CLSI) criteria. Out of 623 bacterial isolates, the prevalence of *E. coli* was predominantly high (349), followed by *K. pneumoniae* (150), *P. aeruginosa* (64), and *A. baumannii* (60). The CoR-GNB rates among *E. coli, K. pneumoniae*, *A. baumannii,* and *P. aeruginosa* were 2.9%, 17.3%, 5.0%, and 1.6%, respectively. Seven out of 26 colistin-resistant *K. pneumoniae* isolates and seven out of 10 colistin-resistant *E. coli* isolates were still susceptible to carbapenems (the MICs for imipenem and meropenem were ≤1 μg/ml). Tigecycline and aminoglycosides might be the best therapeutic choices against CoR-GNB. In conclusion, our findings confirmed a CoR-GNB prevalence of approximately 1.6%–17.3%, depending on the bacterial species. Certain available antimicrobials remain effective against CoR-GNB.

INTRODUCTION

Colistin, also called polymixin E, plays an important role as the last line of anti-Gram-negative defense against multi-drug resistant (MDR) pathogens, particularly carbapenem-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and Enterobacteriaceae. Colistin is not a new agent; it has been used since the early 1950s. Colistin prescriptions declined in the 1970s mainly due to its nephrotoxicity. However, the increase of MDR Gram-negative bacteria has led to the revitalized use of colistin during the last decade (Gregoire *et al*., 2017).

Naturally, some organisms have intrinsic colistin resistance, including *Serratia marcescens*, *Proteus* spp., *Providencia* spp., *Morganella morganii*, *Burkholderia cepacia*, and *Vibrio cholera* (Sherry and Howden, 2018). Unfortunately, because of increasing colistin consumption, organisms that have acquired colistin resistance via chromosomal genes or plasmids have been globally reported. Overall, colistin resistance among non-inherent resistant bacteria in Enterobacteriaceae has a prevalence of approximately 0.67%–1.6%, with high rates in *Enterobacter* spp. (13.9%–20.1%), followed by *K. pneumoniae* (1.5%–6.8%) and *E. coli* (0.2%–0.6%) (Sherry and Howden, 2018).

For non-fermentative Gram-negative bacteria, the rates of colistin-resistant *A. baumannii* in the global SENTRY

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Antimicrobial Surveillance database from 2001 to 2011 ranged from 0.9% to 3.3% (Gales *et al*., 2011). Recently, in data from the China Surveillance of Antimicrobial Resistance Program during 2009–2014, the rate of non-susceptible *A. baumannii* increased slightly from 0.9% to 3.0% over a 6-year period (Gao *et al*., 2017). Conversely, the rates of colistin resistance in *P. aeruginosa* in SENTRY from US and European hospitals during 2009–2012 remained at approximately 0.3% (Sader *et al*., 2014). However, the results of the Canadian Ward surveillance study reported colistin resistance in up to 5.1% of *P. aeruginosa* isolates obtained from 2008 to 2015 (Walkty *et al*., 2017).

In one study from the largest national tertiary referral center in Thailand, Eiamphungporn *et al*. (2018) found that among 317 study isolates, colistin resistance [minimum inhibitory concentration (MIC) > 2 mg/l] was observed in 226 isolates (71.3%), including 13 *E. coli* and 213 *K. pneumoniae* isolates.

The impact of colistin-resistant Gram-negative bacteria (CoR-GNB) on clinical outcomes is evident. Infection due to colistin-resistant *K. pneumoniae* was significantly associated with an increased risk for in-hospital mortality (Rojas *et al*., 2017). Thus, an appropriate regimen against CoR-GNB is urgently required.

To date, data on salvage therapy for CoR-GNB infections are limited. In addition, CoR-GNB has been reported sporadically at our institute, and there is an absence of new agents effective against such organisms. Therefore, this study sought to study the prevalence of CoR-GNB among *P. aeruginosa*, *A. baumannii, E. coli*, and *K. pneumoniae* isolated from patients with bacteremia and to determine the remaining antibacterial agents displaying activity against CoR-GNB isolates.

MATERIALS AND METHODS

Study design

This study retrospectively reviewed the data of non-repeated clinical bacterial isolates from patients admitted to Phramongkutklao Hospital, a 1,200-bed tertiary care center in Bangkok, Thailand during May 2017–April 2018. This study was approved by the ethical review committee of the Royal Thai Army Medical Department (approval no. Q005h/61_Exp) and was permitted by the Director of the Phramongkutklao Hospital prior to data collection.

Bacterial isolates

All clinical isolates obtained from patients with bacteremia were included in this study. We obtained the results for the first isolate of *P. aeruginosa*, *A. baumannii, E. coli*, or *K. pneumoniae* from each patient. We excluded all isolates if the follow-up isolates with different resistance patterns less than two antibiotic agents during 14 days after isolation of the first strain.

Definitions

MDR Gram-negative bacteria were those bacteria non-susceptible to at least three antimicrobial groups among aminoglycosides, antipseudomonal penicillins, third- or fourth-generation cephalosporins, carbapenems, trimethoprim-sulfamethoxazole, and fluoroquinolones. *Third-generation cephalosporin non-susceptible Gram-negative bacteria* included the studied isolates of *E. coli* and *K. pneumoniae* that were non-susceptible to cefotaxime, ceftriaxone, or ceftazidime. *Carbapenem-resistant (CR) Gram-negative bacteria* included the studied isolates of *E. coli* and *K. pneumoniae* that were non-susceptible to any carbapenems or the *P. aeruginosa* and *A. baumannii* isolates that were non-susceptible to imipenem, meropenem, or doripenem. *CoR-GNB* included the studied isolates with an MIC ≥4 g/ml to colistin in the broth microdilution method according to the Clinical and Laboratory Standards Institute guidelines, version 2019, which provide antimicrobial susceptibility information for methods, condition testing, validation, and interpretation (CLSI, 2019).

Data collection

We determined the MIC of the non-repeated clinical isolates. The MIC of the antimicrobial agents was determined using automated susceptibility testing (Thermo Scientific™ Sensititre™ ARIS™ 2X Instrument) based on the broth microdilution method (Chew *et al*., 2017). Growth was determined by fluorescence measurement after 18–24 hours of incubation depending on the species. The MIC value of the antimicrobials in each strain was interpreted as susceptible, intermediate, or resistant using the CLSI breakpoint.

Descriptive statistics were used for the MIC distribution. Our study investigated the MIC values of antimicrobial agents against the studied isolates. The MIC50 and MIC90 values were defined as the lowest concentration of antimicrobials at which 50% and 90% of the isolates were inhibited, respectively.

RESULTS

During the 1-year study period, 623 studied bacterial isolates were obtained from blood cultures including 349 *E. coli* isolates, 150 *K. pneumoniae* isolates, 60 *A. baumannii* isolates, and 64 *P. aeruginosa* isolates. Forty out of 623 isolates (6.4%) were CoR-GNB. The CoR-GNB prevalence rates among *E. coli, K. pneumoniae A. baumannii,* and *P. aeruginosa* were 2.9%, 17.3%, 5.0%, and 1.6%, respectively (Fig. 1).

Seven out of the 26 clinical colistin-resistant *K. pneumoniae* isolates were still susceptible to carbapenems (imipenem ≤ 2 µg/ml and meropenem ≤ 2 µg/ml). Conversely, the remaining 19 colistin-resistant *K. pneumoniae* isolates were susceptible to amikacin, gentamicin, and tigecycline with MIC 50/90 (MIC range) values of 16/16 (≤8–32) µg/ml, 2/4 (≤2–>8) µg/ml,

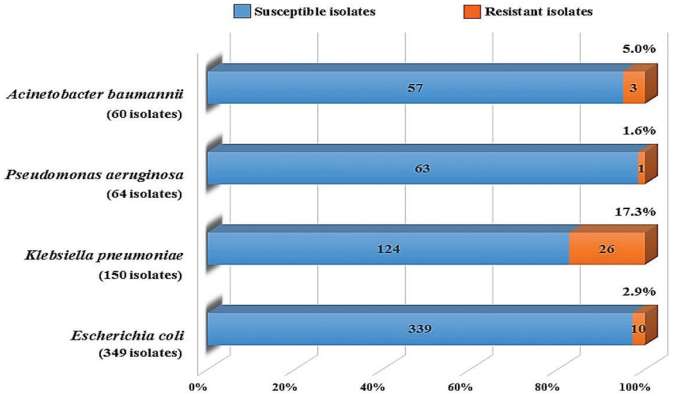

Figure 1. Colistin resistance was identified in 2.9%, 17.3%, 1.6%, and 5.0% of *E. coli* (*n* = 349), *K. pneumoniae* (*n* = 150), *P. aeruginosa* (*n* = 64), and *A. baumannii* (*n* = 60) isolates, respectively.

Table 1. MIC distribution and susceptible rate based on species-susceptible breakpoint among colistin-resistant Gram-negative bacteria ($N = 40$ isolates).

Tigecycline	**MIC (µg/ml)**						**Susceptible rate (%) ≤ 2 µg/ml/≤ 1 µg/ml**
	≤0.25	**0.5**	**1**	**2**	**4**	**8**	
K. pneumoniae ($n = 26$)	2	17	5	1	1	-	96.2/92.3
E. coli ($n = 10$)	4	3	2	1	-	-	100/90
A. baumannii ($n = 3$)	2	-	-	-	1	-	66/66
P. aeruginosa ($n = 1$)	N/A						N/A
Imipenem	**MIC (µg/ml)**						**Susceptible rate (%)**
	≤0.5	**1**	**2**	**4**	**8**	**>8**	
K. pneumoniae ($n = 26$)	5	2	-	-	-	19	26.9; (≤1 ug/ml)
E. coli ($n = 10$)	7	-	-	-	-	3	70; (≤1 ug/ml)
A. baumannii ($n = 3$)	1	-	-	-	-	2	33.3; (≤2 µg/ml)
P. aeruginosa ($n = 1$)	-	1	-	-	-	-	100; (≤2 µg/ml)
Meropenem	**MIC (µg/ml)**						**Susceptible rate (%)**
	≤0.5	**1**	**2**	**4**	**8**	**>8**	
K. pneumoniae ($n = 26$)	7	-	-	-	-	19	26.9; (≤1 ug/ml)
E. coli ($n = 10$)	6	1	-	-	-	3	70; (≤1 ug/ml)
A. baumannii ($n = 3$)	1	-	-	-	-	2	33.3; (≤2 µg/ml)
P. aeruginosa ($n = 1$)	1	-	-	-	-	-	100; (≤2 µg/ml)
Ciprofloxacin	**MIC (µg/ml)**						**Susceptible rate (%)**
	≤0.06	**0.25**	**0.5**	**1**	**2**	**>2**	
K. pneumoniae ($n = 26$)	-	-	2	-	-	24	0; (≤0.25 ug/ml)
E. coli ($n = 10$)	1	3	-	1	1	4	40; (≤0.25 ug/ml)
A. baumannii ($n = 3$)	1	-	-	-	1	1	33.3; (≤1 ug/ml)
P. aeruginosa ($n = 1$)	-	-	-	-	-	1	0; (≤0.5 ug/ml)
Amikacin	**MIC (µg/ml)**						**Susceptible rate (%) ≤ 16 µg/ml**
	≤8		**16**	**32**		**>32**	
K. pneumoniae ($n = 26$)	9		13	3		1	84.6
E. coli ($n = 10$)	7		1	2		-	80
A. baumannii ($n = 3$)	1		-	1		1	33.3
P. aeruginosa ($n = 1$)	1		-	-		-	100
Gentamicin	**MIC (µg/ml)**						**Susceptible rate (%) ≤ 4 µg/ml**
	≤2		**4**	**8**		**>8**	
K. pneumoniae ($n = 26$)	21		1	1		3	84.6
E. coli ($n = 10$)	5		-	-		5	50
A. baumannii ($n = 3$)	1		-	-		2	33.3
P. aeruginosa ($n = 1$)	-		-	-		1	0

N/A = not applicable

and 0.5/1.0 (0.5–2) µg/ml, respectively. Of the 10 colistin-resistant *E. coli* isolates, seven were susceptible to carbapenems (imipenem ≤ 1 µg/ml and meropenem ≤ 1 µg/ml), whereas the remaining three carbapenem non-susceptible isolates were universally susceptible to tigecycline with an MIC range of 0.5–1 µg/ml (Table 1).

One of the three clinical colistin-resistant *A. baumannii* isolates and one of the colistin-resistant *P. aeruginosa* isolates were carbapenem (imipenem and meropenem) susceptible. One of the two colistin-resistant *A. baumannii* isolates was susceptible to tigecycline. The MIC distribution of the antimicrobials is shown in Table 1.

DISCUSSION

Polymyxins are used as last-resort antibiotics in many areas with MDR Gram-negative occurrence; however, this has currently resulted in polymyxin-resistant bacteria. For this reason, the prevalence of colistin resistance among Gram-negative clinical isolates in Thailand was evaluated; however, only *E. coli, K. pneumoniae, P. aeruginosa*, and *A. baumannii* were included. At our setting, a prevalence of 6.4% was reported for non-repeated CoR-GNB isolates obtained from blood cultures. Among them, *K. pneumoniae* was the most common genus associated with polymyxin resistance (17.3%), followed by *E. coli* with a prevalence rate of 2.9%. Overall, the high rate of colistin resistance observed in *K. pneumoniae* was consistent with a previous study that showed higher rates of colistin resistance in *K. pneumoniae* than in *E. coli* (Sherry and Howden, 2018). However, the prevalence of colistin-resistant *K. pneumoniae* in our setting was much higher than rates reported in previous studies. This may have occurred because our studied isolates were largely CR strains. A high proportion of colistin resistance among CR Enterobacteriaceae strains has been reported worldwide (Sader *et al*., 2015).

Not surprisingly, the prevalence of colistin resistance among *A. baumannii* and *P. aeruginosa* isolates in this study remained relatively low, similar to the reported rates from other studies (*A. baumannii* and 2.4%–4.8% and *P. aeruginosa* 0%–1.4%). Additionally, even meropenem-non-susceptible, MDR, and extensively drug resistant (XDR) *Pseudomonas aeruginosa* and *A. baumannii* isolates have maintained low colistin resistance rates (Sherry and Howden, 2018).

Until now, CoR-GNB infections have been extremely associated with high mortality (Capone *et al*., 2013; Lertsrisatit *et al*., 2017). The therapeutic choices against such pathogens require investigation. The results of this study showed that overall characteristics of colistin-resistant Gram-negative bacteria include susceptibility rates of 92.5%, 80.0%, and 70.0% to tigecycline (MIC susceptible breakpoint ≤ 2 µg/ml), amikacin (MIC ≤ 16 µg/ml), and gentamicin (MIC ≤ 4 µg/ml), respectively. These results are consistent with previous reports showing that colistin-resistant strains displayed susceptibility rates of 75.00% and 29.17% to tigecycline and amikacin, respectively (Arjun *et al*., 2017). This finding was consistent with our previous study on colistin-resistant *A. baumannii*, which revealed that all strains were sensitive to tigecycline (Lertsrisatit *et al*., 2017). Thus, tigecycline and aminoglycosides might be options for CoR-GNB. Moreover, Prawang *et al*. (2019) showed that a tigecycline-gentamicin combination could suppress clinical isolates of colistin-resistant *K. pneumoniae* at low concentrations. In some cases of serious infection due to CoR-GNB infection, this combination might be useful in clinical therapy. However, some beta-lactams are able to cover certain strains of *A. bamannii* or *P. aeruginosa*. They could be an alternative choice for patients with bloodstream infections because tigecycline has a very low concentration in the serum.

CONCLUSION

Our finding confirmed the presence of CoR-GNB in Thailand. The CoR-GNB prevalence rates among *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* were 2.9%, 17.3%, 5.0%, and 1.6%, respectively. The highest rate of colistin-resistant pathogens was predominately found in *K. pneumoniae*. Therefore, the appropriate colistin use accompanied by the shortest duration of treatment may minimize the troublesome situation of colistin resistance. As a last resort, tigecycline showed the best *in vitro* activity against CoR-GNB.

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CONFLICT OF INTEREST

Authors declare that they have no conflicts of interest.

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