ORIGINAL ARTICLE

Prevalence of Acquired Colistin Resistance among Gram Negative Bacilli Isolated from Patients Admitted at Cairo University Hospitals

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ABSTRACT

Background: *Polymyxins were mostly ignored few decades ago because of their toxicity. Now are considered as a last-line therapy to treat infections caused by multidrug resistant Gram-negative bacilli. Colistin resistance mediated by chromosomal mutations and more recently by plasmid-borne mcr genes, is increasingly being reported in different countries.* ***Objective:*** *The aim of the present study is to determine the prevalence of acquired colistin resistance among gram negative bacilli isolates as well as to compare colistin resistance among different Gram-negative bacilli spp. It also aimed to study the antibiotic susceptibility of different Gram-negative bacilli isolates.* ***Methodology:*** *A total of 180 different clinical specimens were collected from the patients admitted at different Departments of Cairo University Hospitals until 115-Gram negative bacilli isolates were isolated.* ***Results:*** *The prevalence of acquired colistin resistance among gram negative bacilli isolates was found to be 10.4% (12/115). The prevalence of colistin resistance among different Gram-negative bacilli isolates was as follows: 12.5% among E. coli (3/24), 9.5% among Klebsiella spp. (4/42), 13.8% among Pseudomonas spp. (4/29) and 5.0% among Acinetobacter spp. (1/20). The overall colistin resistance rate among Enterobacteriaceae was 10.6% (7/66) while the overall colistin resistance rate among gram negative bacilli was 10.4% (12/115).* ***Conclusions:*** *Two-thirds (66.7%) of colistin resistant Gram-negative bacilli isolates were isolated from ICUs compared to other departments. All colistin resistant Gram-negative bacilli isolates were isolated from hospital acquired infections.*

INTRODUCTION

The emergence of multidrug resistant Gram-negative bacilli particularly *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and carbapenem resistant *Enterobacteriaceae* that are resistant to all β-lactams, fluoroquinolones, and aminoglycosides has led to renewed interest in polymyxin antibiotics as therapeutic agents. Polymyxins have become last-resort antibiotics in many medical centers[1,2]. However, as the use of these agents is increasing, bacterial resistance has emerged in many parts of the world which was first thought to be chromosomally mediated only until ***Liu et al***[2] reported the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in *Enterobacteriaceae*[3]. Broth microdilution (BMD) has been recommended by the Clinical and Laboratory Standards Institute to be the only approved method for testing colistin susceptibility through determining its minimum inhibitory concentration (MIC). It has also noted that The MICs obtained from testing colistin predict MICs for polymyxin B[4].

METHODOLOGY

This observational cross-sectional study was conducted over the period from April 2019 to November 2019 on patients admitted at different Departments of Cairo University Hospitals after the ethical committee approval was taken. A total of 180 clinical specimens including sputum, endotracheal aspirates, urine, pus, and blood were collected from the patients after taking their written informed consents until 115 Gram-negative bacilli isolates were collected with the exclusion of intrinsically colistin resistant bacteria.

Identification of the isolates[5]:

Identification of the isolates was done up to the genus level according to colony morphology and the conventional microbiological standard tests.

Antibiotic susceptibility testing:

The antibiotic susceptibility testing was done for all the bacterial isolates using Kirby-Bauer modified disc diffusion technique on Mueller-Hinton agar (Oxoid, UK) using commercially available discs (Himedia, India) according to the CLSI guidelines. The results

were interpreted as susceptible, intermediate, or resistant according to the CLSI guidelines[4].

Determination of colistin MIC using broth microdilution method:

- All the bacterial isolates were subjected to the broth microdilution method for colistin MIC determination which is the only approved method for testing colistin susceptibility. Colistin sulphate powder (5 million I.U. /gm) (ADWIA Pharmaceuticals Co., Egypt) was used for determination of MIC as recommended by CLSI. *E. coli* ATCC 25922 was taken as a quality control with colistin MIC ranging from 0.25 µg/ml to 2 µg/ml[4,6]. For *Pseudomonas spp.* and *Acinetobacter spp.,* Interpretation of the results was performed according to the CLSI breakpoints as susceptible or resistant (table 1)[4]. For *Enterobacteriaceae,* Interpretation of the results was performed according to the CLSI epidemiological cutoff value (ECV) as wild or non-wild type (table 2)[4].

Table 1: Colistin MIC breakpoints for *Pseudomonas spp.* and *Acinetobacter spp.*

Antimicrobial agent	Colistin MIC breakpoints (µg/ml)	
	Resistant (R)	Susceptible (S)
Colistin	≥ 4	≤ 2

Table 2: Colistin MIC ECV for *Enterobacteriaceae*

Antimicrobial agent	Colistin MIC ECV (µg/ml)	
	Non-wild type (NWT)	Wild type (WT)
Colistin	≥ 4	≤ 2

Statistical methods

Data were coded and entered using the Statistical Package for the Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY, USA). Data were summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5[7]. *P*-values less than 0.05 were considered as statistically significant.

RESULTS

Out of 115 Gram-negative bacilli isolates; 12 isolates (10.4%) were resistant to colistin while 103 isolates (89.6%) were colistin susceptible. The 12 colistin resistant gram negative bacilli isolates were; 4 isolates (33.3%) *Klebsiella spp*., 4 isolates (33.3%) *Pseudomonas spp.*, 3 isolates (25.0%) *E.coli* and 1 isolate (8.4%) *Acinetobacter spp*. Out of the 12 colistin resistant Gram-negative bacilli isolates; 5 isolates (41.5%) were isolated from pus specimens, 3 isolates (25.0%) were isolated from urine specimens, 2 isolates (16.7%) were isolated from endotracheal aspirate specimens and 2 isolates (16.7%) were isolated from blood specimens. The 12 colistin resistant Gram-negative bacilli isolates were distributed as follows; 8 isolates (66.7%) from ICUs, 1 isolate (8.3%) from urology department, 1 isolate (8.3%) from general surgery department and 2 isolates (16.7%) from orthopedic surgery department. All the 12 colistin resistant Gram-negative bacilli isolates were isolated from hospital acquired infections, but this was statistically insignificant.

The prevalence of colistin resistance showed a statistically insignificant difference among different members of the 115 Gram-negative bacilli isolates: 12.5% among *E. coli* (3/24), 9.5% among *Klebsiella spp.* (4/42), 13.8% among *Pseudomonas spp.* (4/29) and 5.0% among *Acinetobacter spp.* (1/20). The overall colistin resistance rate among *Enterobacteriaceae* was 10.6% (7/66) (table 3).

Table 3: Comparison of the prevalence of colistin resistance among different members of the 115 Gram-negative bacilli isolates

	Susceptibility to colistin				*P* value
	Resistant (R)		Susceptible (S)		
	Count	Percentage (%)	Count	Percentage (%)	
***E.coli* (24)**	3	12.5%	21	87.5%	0.809
***Klebsiella spp.* (42)**	4	9.5%	38	90.5%	
***Pseudomonas spp.* (29)**	4	13.8%	25	86.2%	
***Acinetobacter spp.* (20)**	1	5.0%	19	95.0%	

The prevalence of colistin resistance among different clinical specimens from which the 115 Gram-negative bacilli isolates were isolated was statistically insignificant; 0% among sputum specimens (0/13), 18.2% among endotracheal aspirate specimens (2/11), 8.1% among urine specimens (3/37), 13.9% among pus specimens (5/36) and11.1% among blood specimens (2/18) (table 4).

Table 4: Comparison of the prevalence of colistin resistance among different clinical specimens

	Susceptibility to colistin				*P* value
	Resistant (R)		Susceptible (S)		
	Count	Percentage (%)	Count	Percentage (%)	
Sputum (13)	0	0%	13	100.0%	0.562
Endotracheal aspirate (11)	2	18.2%	9	81.8%	
Urine (37)	3	8.1%	34	91.9%	
Pus (36)	5	13.9%	31	86.1%	
Blood (18)	2	11.1%	16	88.9%	

The prevalence of colistin resistance among different departments from which the 115 clinical specimens were collected: 20.0% in orthopedic surgery department (2/10), 12.7% in ICUs (8/63), 10.0% in general surgery department (1/10), 5.0% in urology department (1/20), 0% in chest department (0/9) and 0% in plastic surgery department (0/3). These results were statistically insignificant (table 5).

Table 5: Comparison of the prevalence of colistin resistance among different departments

	Susceptibility to colistin				*P* value
	Resistant (R)		Susceptible (S)		
	Count	Percentage (%)	Count	Percentage (%)	
ICU (63)	8	12.7%	55	87.3%	0.750
Chest (9)	0	0%	9	100.0%	
Urology (20)	1	5.0%	19	95.0%	
General Surgery (10)	1	10.0%	9	90.0%	
Orthopedic Surgery (10)	2	20.0%	8	80.0%	
Plastic Surgery (3)	0	0%	3	100.0%	

Antibiotic susceptibility profile of the 12 colistin resistant bacteria:

- ***Enterobacteriaceae:***

Colistin resistant *E. coli* isolates were mostly resistant to pipercillin, amoxicillin-clavulanate, ampicillin-sulbactam, ceftazidime, ceftriaxone, cefotaxime, cefoperazone and trimethoprim-sulfamethoxazole. Colistin resistant *E. coli* isolates were mostly susceptible to pipercillin-tazobactam, cefoxitin, meropenem, ertapenem, gentamicin, tobramycin, amikacin, doxycycline, and nitrofurantoin. Colistin resistant *Klebsiella spp.* isolates were mostly resistant to pipercillin, amoxicillin-clavulanate, ampicillin-sulbactam, pipercillin-tazobactam, cefoxitin, ceftazidime, ceftriaxone, cefotaxime, cefoperazone, cefepime, imipenem, meropenem, ertapenem, amikacin, ciprofloxacin, levofloxacin and ofloxacin. Colistin resistant *Klebsiella spp.* isolates were mostly susceptible to gentamicin, doxycycline, and trimethoprim-sulfamethoxazole. The overall rates of carbapenem resistance among colistin resistant *Enterobacteriaceae* were 71.4%, 57.1% and 57.1% for imipenem, meropenem and ertapenem, respectively. Results of antibiotic susceptibility tests performed on colistin resistant *E. coli* isolates are presented in table (6-a). Results of antibiotic susceptibility tests performed on colistin resistant *Klebsiella spp.* isolates are presented in table (6-b).

Table 6-a: Antibiotic susceptibility profile of colistin resistant *E. coli* isolates (n= 3)

	Resistant (R)		Intermediate (I)		Susceptible (S)	
	Count	Percentage (%)	Count	Percentage (%)	Count	Percentage (%)
Pipercillin	**3**	**100%**	0	0%	0	0%
Amoxicillin-clavulanate	**3**	**100%**	0	0%	0	0%
Ampicillin-sulbactam	**3**	**100%**	0	0%	0	0%
Pipercillin-tazobactam	0	0%	0	0%	**3**	**100%**
Cefoxitin	0	0%	0	0%	**3**	**100%**
Ceftazidime	**3**	**100%**	0	0%	0	0%
Ceftriaxone	**3**	**100%**	0	0%	0	0%
Cefotaxime	**3**	**100%**	0	0%	0	0%
Cefoperazone	**3**	**100%**	0	0%	0	0%
Cefepime	2	66.7%	1	33.3%	0	0%
Imipenem	1	33.3%	1	33.3%	1	33.3%
Meropenem	0	0%	0	0%	**3**	**100%**
Ertapenem	0	0%	0	0%	**3**	**100%**
Gentamicin	0	0%	0	0%	**3**	**100%**
Tobramycin	0	0%	0	0%	**3**	**100%**
Amikacin	0	0%	0	0%	**3**	**100%**
Ciprofloxacin	2	66.7%	0	0%	1	33.3%
Levofloxacin	2	66.7%	0	0%	1	33.3%
Ofloxacin	2	66.7%	0	0%	1	33.3%
Doxycycline	0	0%	0	0%	**3**	**100%**
Trimethoprim-sulfamethoxazole	**3**	**100%**	0	0%	0	0%
Nalidixic acid	2	66.7%	0	0%	1	33.3%
Nitrofurantoin	0	0%	0	0%	**3**	**100%**

Table 6-b: Antibiotic susceptibility profile of colistin resistant *Klebsiella spp.* isolates (n= 4)

	Resistant (R)		Intermediate (I)		Susceptible (S)	
	Count	Percentage (%)	Count	Percentage (%)	Count	Percentage (%)
Pipercillin	**4**	**100%**	0	0%	0	0%
Amoxicillin-clavulanate	**4**	**100%**	0	0%	0	0%
Ampicillin-sulbactam	**4**	**100%**	0	0%	0	0%
Pipercillin-tazobactam	**4**	**100%**	0	0%	0	0%
Cefoxitin	**4**	**100%**	0	0%	0	0%
Ceftazidime	**4**	**100%**	0	0%	0	0%
Ceftriaxone	**4**	**100%**	0	0%	0	0%
Cefotaxime	**4**	**100%**	0	0%	0	0%
Cefoperazone	**4**	**100%**	0	0%	0	0%
Cefepime	**4**	**100%**	0	0%	0	0%
Imipenem	**4**	**100%**	0	0%	0	0%
Meropenem	**4**	**100%**	0	0%	0	0%
Ertapenem	**4**	**100%**	0	0%	0	0%
Gentamicin	3	75%	0	0%	**1**	**25%**
Tobramycin	3	75%	1	25%	0	0%
Amikacin	**4**	**100%**	0	0%	0	0%
Ciprofloxacin	**4**	**100%**	0	0%	0	0%
Levofloxacin	**4**	**100%**	0	0%	0	0%
Ofloxacin	**4**	**100%**	0	0%	0	0%
Doxycycline	2	50%	1	25%	**1**	**25%**
Trimethoprim-sulfamethoxazole	3	75%	0	0%	**1**	**25%**

- ***Pseudomonas spp.*:**

Colistin resistant *Pseudomonas spp.* isolates were mostly resistant to pipercillin, pipercillin-tazobactam, ceftazidime, cefepime, levofloxacin and ofloxacin. Colistin resistant *Pseudomonas spp.* isolates were mostly susceptible to tobramycin, gentamicin, amikacin, and ciprofloxacin. Results of antibiotic susceptibility tests performed on colistin resistant *Pseudomonas spp.* isolates are presented in table (7).

Table 7: Antibiotic susceptibility profile of colistin resistant *Pseudomonas spp.* isolates (n= 4)

	Resistant (R)		Intermediate (I)		Susceptible (S)	
	Count	Percentage (%)	Count	Percentage (%)	Count	Percentage (%)
Pipercillin	**3**	**75.0%**	0	0%	1	25.0%
Pipercillin-tazobactam	**3**	**75.0%**	0	0%	1	25.0%
Ceftazidime	**3**	**75.0%**	0	0%	1	25.0%
Cefepime	**3**	**75.0%**	1	25.0%	0	0%
Imipenem	2	50.0%	1	25.0%	1	25.0%
Meropenem	2	50.0%	1	25.0%	1	25.0%
Gentamicin	2	50.0%	0	0%	**2**	**50.0%**
Tobramycin	1	25.0%	0	0%	**3**	**75.0%**
Amikacin	2	50.0%	0	0%	**2**	**50.0%**
Ciprofloxacin	2	50.0%	0	0%	**2**	**50.0%**
Levofloxacin	**3**	**75.0%**	0	0%	1	25.0%
Ofloxacin	**3**	**75.0%**	0	0%	1	25.0%

- ***Acinetobacter spp.*:**

Colistin resistant Acinetobacter spp. isolate was of intermediate susceptibility to meropenem and resistant to the rest of antibiotics used. Results of antibiotic susceptibility test performed on colistin resistant Acinetobacter spp. isolate are presented in table (8).

Table 8: Antibiotic susceptibility profile of colistin resistant *Acinetobacter spp.* isolate (n= 1)

	Resistant (R)		Intermediate (I)		Susceptible (S)	
	Count	Percentage (%)	Count	Percentage (%)	Count	Percentage (%)
Pipercillin	1	100.0%	0	0%	0	0%
Ampicillin-sulbactam	1	100.0%	0	0%	0	0%
Pipercillin-tazobactam	1	100.0%	0	0%	0	0%
Ceftazidime	1	100.0%	0	0%	0	0%
Ceftriaxone	1	100.0%	0	0%	0	0%
Cefotaxime	1	100.0%	0	0%	0	0%
Cefepime	1	100.0%	0	0%	0	0%
Imipenem	1	100.0%	0	0%	0	0%
Meropenem	0	0%	**1**	**100.0%**	0	0%
Gentamicin	1	100.0%	0	0%	0	0%
Tobramycin	1	100.0%	0	0%	0	0%
Amikacin	1	100.0%	0	0%	0	0%
Ciprofloxacin	1	100.0%	0	0%	0	0%
Levofloxacin	1	100.0%	0	0%	0	0%
Doxycycline	1	100.0%	0	0%	0	0%
Trimethoprim-sulfamethoxazole	1	100.0%	0	0%	0	0%

DISCUSSION

The results obtained from this study showed that the overall colistin resistance rate among gram negative bacilli was 10.4%. This result was in line with another study which reported that 13.5% Gram-negative bacterial isolates were resistant to colistin[8]. In disagreement with our study, a higher resistance rate was reported by Matuschek et al.[9] who stated that the colistin resistance rate was 48% among Gram-negative bacterial isolates[9]. On the other hand, a lower resistance rate was reported by Albur et al.[10] who stated that the colistin resistance rate was 1.8% out among Gram-negative bacterial isolates[10].

In our study, the prevalence of colistin resistance among *E. coli* isolates was 12.5% and it was 9.5% among *Klebsiella spp.* isolates with overall resistance rate of 10.6% among *Enterobacteriaceae* isolates. This result agreed with another Egyptian study conducted by Mohammed et al.[11] who stated that 8.6% *E. coli* isolates and 9.1% *Klebsiella spp.* isolates were resistant to colistin with overall resistance rate of 8.8% among *Enterobacteriaceae* isolates[11]. In disagreement with our study, a higher colistin resistance rate among *Enterobacteriaceae* isolates was reported by ***Lutgring et al.***[12] who stated that 25.4% *Enterobacteriaceae* isolates were colistin resistant[12]. On the other hand, a lower colistin resistance rate was reported in Taiwan by Lai et al.[13] who stated that the prevalence of colistin resistance was 0% among *E. coli* isolates and 2.2% among *K. pneumoniae* isolates. Another study conducted in Hungary by Juhász et al.[14] reported a colistin resistance rate of 0.6% among *Enterobacteriaceae* isolates.

In our study, the prevalence of colistin resistance among *Pseudomonas spp.* isolates was 13.8%. This result was in line with another study conducted in Istanbul, Turkey by Doymaz and Karaaslan[15] who stated that 12.9% *Pseudomonas aeruginosa* isolates were colistin resistant[15]. In disagreement with our study, a higher colistin resistance rate was reported by Matuschek et al.[9] who stated that 42.8% *P. aeruginosa* isolates were colistin resistant[9]. On the other hand, a lower colistin resistance rate was reported by Asar et al.[16] who stated that the prevalence of colistin resistance among *P. aeruginosa* isolates was 6.8%[16].

In our study, the prevalence of colistin resistance among *Acinetobacter spp.* isolates was 5%. This result was in line with another Egyptian study conducted by Al-Agamy et al.[17] who reported a colistin resistance rate of 5% among *Acinetobacter baumannii* isolates[17]. In disagreement with our study, a higher colistin resistance rate was reported by Matuschek et al.[9] who stated that the prevalence of colistin resistance among *A. baumannii* isolates was 36.4%[9]. On the other hand, a lower colistin resistance rate was reported by Juhász et al.[14] who stated that that the prevalence of colistin resistance among *Acinetobacter spp.* isolates was 2.6%[14]. The prevalence of colistin resistance among gram negative bacilli may vary among different studies due to geographical and/or chronological variations. In addition, differences in sample size and methodology can contribute to these variations in colistin resistance[18].

In our study, we reported a higher prevalence of colistin resistant Gram-negative bacilli among endotracheal aspirate, pus, and blood specimens (18.2%, 13.9% and 11.1%) respectively compared to urine and sputum specimens (8.1% and 0%) respectively. In our study, 66.7% of colistin resistant Gram-negative bacilli isolates were isolated from ICUs compared to other departments. Urine was the most common source of colistin resistant Gram-negative bacilli, followed by blood and respiratory samples in a study conducted by Arjun et al.[19] In addition, 100% of colistin resistant Gram-negative bacilli isolates were isolated from hospital acquired infections. According to Prim et al.[20] only 77% of colistin resistant Gram-negative bacilli isolates were isolated from hospital acquired infections[20].

In our study, the rates of carbapenem resistance among *Enterobacteriaceae* isolates were 56.1%, 36.4% and 37.9% for imipenem, meropenem and ertapenem, respectively. These rates were found to be higher among colistin resistant *Enterobacteriaceae* isolates (71.4%, 57.1% and 57.1% respectively). The rate of carbapenem resistance among *Pseudomonas spp.* isolates was 75.9% for both imipenem and meropenem while it was 65% for imipenem and 35% for meropenem among *Acinetobacter spp.* isolates. The high rates of carbapenem resistance in this study could be attributed to the fact that the clinical specimens were collected from patients admitted at a tertiary care hospital and more than 50% of them were collected from ICUs. Moreover, prolonged hospitalization, critical illness, surgery, comorbid conditions, the presence of a wound, the use of invasive devices or mechanical ventilation and previous use of antimicrobials (including cephalosporins, carbapenems, and fluoroquinolones) are all considered risk factors for infection with carbapenem resistant bacteria. This result was in line with another study conducted by Kostyanev et al.[21] who reported carbapenem non-susceptibility rates of 53%, 65% and 48% among *Enterobacteriaceae* isolates for imipenem, meropenem and ertapenem, respectively. The study also reported non-susceptibility rates of 61% and 65% among *P. aeruginosa* isolates for imipenem and meropenem, respectively. Similarly, the non-susceptibility rates among *Acinetobacter spp.* isolates were 59% and 63% for imipenem and meropenem, respectively[21]. Similarly, the frequency of carbapenem resistance among Gram-negative bacilli was calculated to be 30.9% in the study conducted by Haji et al[22]. In

disagreement with our study, a lower carbapenem resistance rate was reported by Garg et al.[23] who stated that 9.2% Gram-negative bacterial isolates were carbapenem resistant[23].

CONCLUSION

Healthcare-associated infections caused by multidrug-resistant (MDR) Gram-negative bacilli represent a problem worldwide. This has led to the widespread use of colistin combination therapy. However, colistin resistance is beginning to emerge, resulting in narrow alternative antibiotic choices. In the current study, two-thirds (66.7%) of colistin resistant Gram-negative bacilli isolates were isolated from ICUs compared to other departments. All colistin resistant Gram-negative bacilli isolates were isolated from hospital acquired infections. The rates of carbapenem resistance among colistin resistant *Enterobacteriaceae* isolates were 71.4%, 57.1% and 57.1% for imipenem, meropenem and ertapenem, respectively. Colistin resistant *Acinetobacter spp.* isolate was of intermediate susceptibility to meropenem and resistant to the rest of antibiotics used.

Recommendation:

The presence of colistin resistance among Gram-negative bacilli isolates causing healthcare-associated infections emphasizes the necessity for early detection of colistin resistance. In addition, reporting to infection control staff should be done to overcome their spread.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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