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Prevalence of Polymyxin E (Colistin) Resistance among Gram-Negative Microorganisms Isolated from Fayoum University Hospital

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Abstract:

Introduction: Antibiotic resistance is viewed as a major danger to human health in the 21st century.

Aim of the study: To evaluate the dominance of colistin tolerance in Gram-negative microorganisms collected from patients hospitalized at Fayoum University Hospital.

Subjects and Methods: The current research, which was observational and cross-sectional, took place for a duration of six months in 2023. 115 isolates were collected in total. Complete identification was achieved through traditional microbiological techniques.

Results: Out of 115 Gram-negative *bacilli* isolates, 14 showed resistance to colistin, while 101 were sensitive to colistin.

Conclusions: There is an increasing fascination with Polymyxins because of the worldwide rise of multidrug-resistant Gram-negative bacteria and the absence of novel antibiotics available.

Keywords: Colistin; Antimicrobial Resistance; Polymyxins.

1. Introduction

Antibiotic insensitivity is currently recognized as a significant worldwide danger to human health in the 21st century. Evidence of a worldwide crisis and a looming catastrophe of reverting to the pre-antibiotic era has surfaced [1].

The lipopeptide antibiotics called polymyxins are made by fermenting the Gram-positive bacteria *Paenibacillus polymyxa*. They kill a variety of Gram-negative *bacilli* by messing up the bacterial cell membrane through electric and water-hating interaction [2].

Nations exhibiting a significant occurrence of carbapenem-resistant Gram-negative bacteria and widespread colistin application in medical environments often demonstrate heightened colistin resistance in human specimens [3].

In most European nations, food-producing animals used more polymyxins than humans, and a significant link was found between the usage and resistance to colistin in these animals [3].

2. Subjects and Methods

2.1 Subjects

The present study, carried out in 2023, lasted for six months and was observational with a cross-sectional design. A sum of 115 isolates were collected and categorized into 6 groups.

Inclusion criteria

The isolates were categorized into six distinct groups:

Group 1: 65 isolates from the intensive care unit (ICU) department.

Group 2: 20 isolates from the urology department.

Group 3: 9 isolates from the orthopedic department.

Group 4: 8 isolates from the chest department.

Group 5: 8 isolates from the general surgery department.

Group 6: 5 isolates from the Children's ICU (PICU) department.

Exclusion criteria

Cases with the proliferation of organisms apart from Gram-negative bacteria.

2.2 Study design

A cross-sectional observational study was conducted.

2.3 Methods

Samples from each patient after proper collection were sent as soon as possible to the microbiology laboratory for culture and sensitivity testing. The isolates were recognized up to the genus level through colony morphology and conventional microbiological tests. (Gram stain, oxidase test, Triple Sugar Iron test (TSI), citrate test, urease test, Lysine Decarboxylase test (LDC), and Motility Indole Ornithine test (MIO)) [4]. *E. coli* isolates are identified by being lactose fermenters producing smooth pink colonies on MacConkey's agar, Gram-negative *bacilli*, oxidase negative and the following pattern of biochemical reactions:

TSI: (acid/acid+gas), Citrate: (-ve), Urease: (-ve), LDC: (+ve), Ornithine: (+ve), Indole: (+ve).

Data collection

Klebsiella spp. isolates are identified by being lactose fermenters producing large mucoid pink colonies on MacConkey's agar, Gram-negative *bacilli*, oxidase negative and the following pattern of biochemical reactions:

Exclusion of systemic isolates

TSI: (acid/acid+gas), Citrate: (+ve), Urease: (+ve), LDC: (+ve), Ornithine: (-ve), Indole: (-ve).

Pseudomonas spp. isolates are identified by being non-fermenters producing pale pigmented colonies on MacConkey's agar, Gram-negative *bacilli*, and oxidase positive. *Acinetobacter* spp. isolates are identified by being non-fermenters producing pale colonies on MacConkey's agar, Gram-negative *coccobacilli*, and oxidase negative.

All bacterial isolates were subjected to antibiotic susceptibility testing [5]. Antibiotic panels evaluated for noteworthy Gram-negative isolates comprised (Piperacillin, Amoxicillin-Clavulanic, Ampicillin-Sulbactam, Piperacillin-tazobactam, Gentamicin, Tobramycin, Trimethoprim-sulfamethoxazole, Nitrofurantoin). The organisms were classified as susceptible or resistant to any of these antibiotics according to CLSI guidelines (**Table 1**).

Table 1: Inhibition zone diameter breakpoints for Enterobacteriaceae.

Antimicrobial agent	Disk content (µg)	Zone diameter (mm)		
		Resistant (R)	Intermediate (I)	Susceptible (S)
Pipercillin	100	≤ 17	18-20	≥ 21
Ampicillin-sulbactam	10/10	≤ 11	12-14	≥ 15
Pipercillin-tazobactam	100/10	≤ 17	18-20	≥ 21
Cefoxitin	30	≤ 14	15-17	≥ 18
Ceftazidime	30	≤ 17	18-20	≥ 21
Ceftriaxone	30	≤ 19	20-22	≥ 23
Cefotaxime	30	≤ 22	23-25	≥ 26
Cefoperazone	75	≤ 15	16-20	≥ 21
Cefepime	30	≤ 18	19-24	≥ 25
Imipenem	10	≤ 19	20-22	≥ 23
Meropenem	10	≤ 19	20-22	≥ 23
Ertapenem	10	≤ 18	19-21	≥ 22
Gentamicin	10	≤ 12	13-14	≥ 15
Tobramycin	10	≤ 12	13-14	≥ 15
Amikacin	30	≤ 14	15-16	≥ 17
Ciprofloxacin	5	≤ 21	22-25	≥ 26
Levofloxacin	5	≤ 16	17-20	≥ 21
Ofloxacin	5	≤ 12	13-15	≥ 16
Doxycycline	30	≤ 10	11-13	≥ 14
Trimethoprim-sulfamethoxazole	1.25/23.75	≤ 10	11-15	≥ 16

3. Results

Regarding the demographic characteristics of enrolled patients, the mean age was 54.93 years old ranging from 6 to 78 years.70 patients were males (60.9%) while 45 patients were females (31.9%) **(Table 2).**

Table 1: Demographic characteristics of enrolled patients.

Variables		Clinical specimens	
		Count	(%)
Age	<50 years	44	38.3%
	>50 years	71	61.7%
Gender	Male	70	61%
	Female	45	39%

Out of the 115 clinical specimens, four sputum samples were collected from the ICU department, and eight samples were collected from the chest department. Eleven endotracheal aspirate samples were collected from the ICU department. 18 urine samples were collected from the ICU department, and 20 samples were collected from the urology department. Thirteen pus samples were collected from the ICU department, eight from the general surgery department, nine from the orthopedic department and five from the PICU department. Nineteen blood culture samples were collected from the ICU department (**Table 3**).

Table 3: Distribution of clinical specimens among different departments.

	Department					
	ICU	**Chest**	**Urology**	**General Surgery**	**Orthopedic Surgery**	**PICU**
Sputum	4	8	0	0	0	0
Endotracheal Aspirate	11	0	0	0	0	0
Urine	18	0	20	0	0	0
Pus	13	0	0	8	9	5
Blood	19	0	0	0	0	0

Regarding types of bacterial isolates isolated from different specimens, sputum samples were as follows: one was *E. coli* spp., seven were *Klebsiella* spp., three were *Pseudomonas* spp., and one was *Acinetobacter* spp. Endotracheal Aspirate samples were as follows: one was *E. coli* spp., six were *Klebsiella* spp., two were Pseudomonas spp., and two were *Acinetobacter* spp. Urine samples were as follows: 14 were *E. coli* spp., 12 were Klebsiella spp., nine were Pseudomonas spp., and three were Acinetobacter spp. Pus samples were as follows: six were *E. coli* spp., ten were *Klebsiella* spp., ten were *Pseudomonas* spp., and nine were *Acinetobacter* spp. Blood samples were as follows: three were *E. coli* spp., nine were *Klebsiella* spp., two were Pseudomonas spp., and five were *Acinetobacter* spp. (**Table 4**).

Table 4: Types of Gram-negative bacilli isolates isolated from different specimens.

	Isolated bacteria			
	Escherichia coli	***Klebsiella species***	***Pseudomonas species***	***Acinetobacter species***
Sputum	1	7	3	1
Endotracheal Aspirate	1	6	2	2
Urine	14	12	9	3
Pus	6	10	10	9
Blood	3	9	2	5

Of 115 Gram-negative bacterial isolates,14 were resistant to colistin, whereas 101 were susceptible (**Table 5, Figure 1**).

Table 5: Occurrence of colistin resistance in the Gram-negative bacilli isolates.

	N
Resistant	14
Susceptible	101

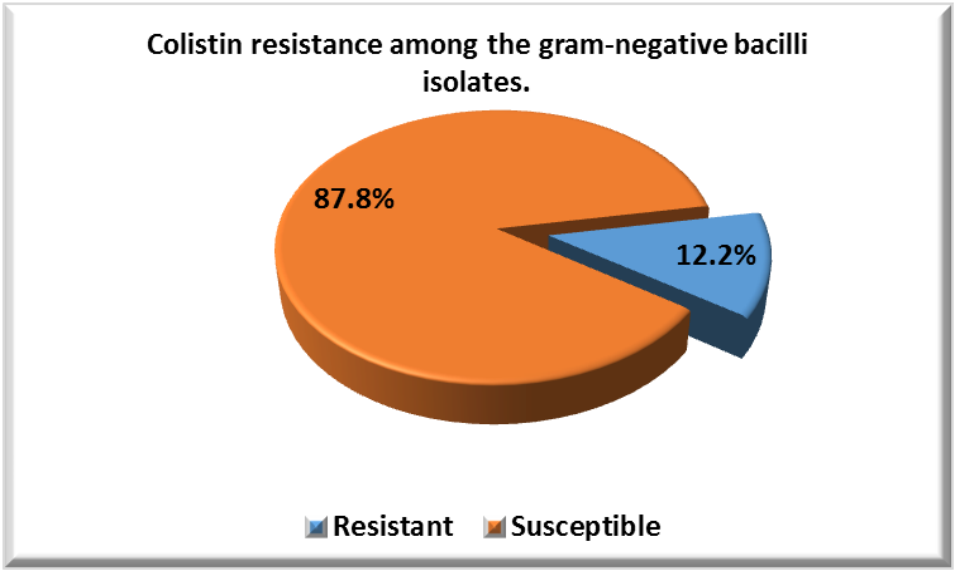

Figure 1: Dominance of colistin resistance in strains of Gram-negative bacilli

4. Discussion

Widespread and frequently unsuitable antibiotic use imposes selective pressure, leading to the quick emergence and dissemination of multidrug-resistant Gram-negative bacteria [6].

Colistin (or polymyxin E) is among the limited treatment choices available for serious infections caused by MDR bacteria. In 1947, Koyama and colleagues discovered it in Japan from Bacillus polymyxa subsp. colistinus, a spore-forming soil bacterium, was first used as an intravenous formulation during the 1950s [7]. Certain species have innate resistance to polymyxin, including *Providencia* species, *Burkholderia* species, *Proteus* species, *Morganella morgani* species, *Vibrios* and *Campylobacter* species. Gram-negative *cocci*, Gram-positive *cocci* and anaerobic bacteria don't show efficacy to polymyxin [8].

In this study, we sought to examine the dominance of polymyxin E (colistin) insensitivity between Gram-negative bacteria. This result is consistent with research conducted by Lellouche et al., revealing that 13.5% of Gram-negative bacterial isolates from a total of 364 were resistant to colistin [9]. A different research carried out by Asar et al. at the University Medical Center Hamburg-Eppendorf, Germany, revealed that 6 out of 68 (8.8%) Gram-negative bacterial isolates showed resistance to colistin [10]. Aydın et al. performed a second multicenter study across 20 tertiary care centers in different areas of Turkey, discovering that 106 out of 1556 (6.8%) Gram-negative bacterial isolates exhibited resistance to colistin [11]. Matuschek et al. contradicted our findings by reporting a higher resistance rate of 48% (36 out of 75) for colistin among Gram-negative bacterial isolates [12]. Another research conducted in Tamil Nadu, India by Ramesh et al found that 27 out of 94 (28.7%) Gram-negative bacterial isolates showed resistance to colistin [13]. In contrast, Albur et al. reported a lower resistance rate, stating that 1.8% (8 out of 438) of Gram-negative bacterial isolates were resistant to colistin [14].

5. Conclusion

The growing global dominance of multidrug-resistant Gram-negative bacteria, combined with a shortage of new antimicrobial agents, has led to increased use of polymixin. Continued monitoring and expanded studies involving larger, diverse populations. Integration of molecular tools

to identify specific resistance genes and mechanisms. Promotion of antimicrobial stewardship programs to mitigate resistance development.

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Ethical committee approval: Before its commencement, the research received permission from the ethics committee of Fayoum University Hospital in December 2022, under the reference number M635. The participants were provided with information regarding the study's aims, examinations, investigations, confidentiality of their information, and their freedom to choose not to participate.

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