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Prognostication of Resistance Pattern of Colistin Resistant Gram-Negative Bacilli at Intensive Care Units

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KEYWORDS

Enterobacteriaceae, Mikrolatest MIC, Gram-negative bacilli, Colistin, antibiotics

ABSTRACT:

Colistin, or polymyxin E, is an antibiotic effective against Gram-negative bacteria, especially Enterobacteriaceae. Due to its potential for horizontal transmission, it is used as a last resort for treating Enterobacteriaceae infections. This study evaluates colistin resistance in Gram-negative bacilli from clinical samples and analyzes their antibiotic resistance patterns. We identified 1175 colistin-resistant Gram-negative bacilli isolates from various ICU sources, with only 1.44% (n=17) classified as colistin-resistant. These included one isolate from the Neonatal ICU, three from the Pediatric ICU, seven from the ICU, three from Obstetrics and Gynecology, and one from the Male Medical Ward. The resistant isolates comprised 6 (35.2%) Escherichia coli, 3 (17.6%) Enterobacter cloacae, and 8 (47%) Klebsiella pneumoniae. Despite more Klebsiella spp. being present, E. coli showed greater sensitivity to colistin. Pseudomonas spp. did not show colistin resistance. The resistance levels indicated that 47% of the colistin-resistant isolates had MIC values of ≥16µg/ml. A related study found p-values for Pseudomonas ranging from 0.001 to 0.052, for E. coli from 0.001 to 1, for Klebsiella spp. from 0.001 to 0.8, for Acinetobacter spp. from 0.1 to 1, and for Enterobacter spp. from 0.177 to 1 when compared to colistin and other antibiotics. Pseudomonas spp shows reduced effectiveness against clinical isolates, especially with antibiotics like Ampicillin, Amoxyclav, Cefuroxime, Ceftriaxone, Etrapenem, and Nalidixic acid. In contrast, Enterobacter spp exhibited diminished efficacy against Piperacillin/Tazobactam, Etrapenem, Tigecycline, Amikacin, Ceftazidime, and Doripenem. Additionally, antibiotics such as triclocarban, ceftazidime, aztreonam, doripenem, levofloxacin, and minocycline had reduced bactericidal inhibition zones against Klebsiella spp. Resistance in E. coli and Klebsiella spp was particularly significant for Colistin, while other species showed less resistance. Further research should include additional pathogenic microorganisms to better understand colistin susceptibility.

INTRODUCTION

In the ICU, the risk of infection is 5 to 10 times higher than in other hospital areas. Multi-drug resistance is a significant factor in life-threatening nosocomial infections [1]. Many hospitals focus on treating long-term and high-risk patients [2,3]. Nosocomial infections can be caused by various pathogens, with bacteria being the most common. Key bacteria include Staphylococcus aureus, Enterobacter, E. coli, enterococci, and Pseudomonas aeruginosa, each with unique resistance mechanisms [4,5]. The use of antibiotics, dating back to penicillin, has led to global resistance development. Currently, several antibiotic classes are available, including sulfones, diarylquinolines, sulphonamides, and carbapenems like Clindamycin [6]. Carbapenems target both Gram-positive and Gram-negative bacteria,

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with each class having a specific action mechanism [7]. For example, sulfamethoxazole inhibits dihydropteroate synthase, while Vancomycin disrupts cell wall synthesis by binding to D-alanyl-D-alanine. Studies in India have shown that certain bacterial isolates, such as E. coli, K. pneumoniae, A. baumannii, and Citrobacter, have higher resistance levels compared to others like P. aeruginosa, P. mirabilis, and Streptococcus, which show lower resistance [8,9]. Colistin, or polymyxin E, is a polypeptide antibiotic effective against gram-negative bacteria like E. coli, Enterobacter, Salmonella, and Shigella [10,11]. It includes five types: A, B, C, D, and E, with only B and E used clinically [12]. Both have similar antimicrobial effects. Resistance mechanisms can vary among bacteria, depending on the type of resistance [13,14]. Colistin targets gram-negative bacteria through five mechanisms, the most notable being membrane lysis, which occurs due to the attraction between negatively charged lipid A and positively charged diaminobutyric acid (Dab) in colistin [15,16]. Another mechanism is the vesicle-vesicle contact pathway, where colistin disrupts osmotic balance by facilitating interactions between the cytoplasmic and outer membranes, leading to cell lysis [17,18]. Colistin also activates the hydroxyl radical death pathway by generating reactive oxygen species (ROS) that damage bacterial DNA, lipids, and proteins [19,20]. Additionally, it inhibits NDH-2 activity in the inner membrane, enhancing its antimicrobial action. Lastly, colistin binds to lipid A, targeting endotoxins and promoting cytokine release, including IL-8 and TNF-alpha [21,22,23]. Colistin is available as colistin sulfate and colistimethate sodium [24]. It has a basic pH of 2 to 6 and its salts are stable [25]. The half-life of colistin sulfate is about 251 ± 79 minutes, while colistimethate sodium's half-life ranges from 0.026 to 2.2 hours.

MATERIALS AND METHODS:

Sample Collection:

This research collected samples from various intensive care units, including Neonatal, Pediatric, general ICUs, Obstetrics and Gynecology, Tubulin Folding Cofactor D, and Male Medical Ward.

A total of 1,175 Gram-negative bacilli isolates were gathered and analyzed from September 2023 to July 2024, with ethics committee approval. Isolates were sourced from a clinical microbiology lab.

Inclusion criteria included patients of all ages, including pediatrics, admitted to the ICU for over 48 hours. Key species identified were Enterobacteriaceae (e.g., E. coli, Klebsiella), Pseudomonas aeruginosa, and Acinetobacter baumannii.

Exclusion criteria ruled out specific colistin-resistant organisms like Proteus spp., Providencia spp., Serratia spp., and Morganella morganii. A total of 1,175 Gram-negative isolates were tested for colistin susceptibility using the Broth Microdilution (BMD) method and Mikrolatest MIC Colistin kit, following manufacturer guidelines.

Antimicrobial susceptibility was assessed using BMD and Mikrolatest MIC, with specimens processed according to EUCAST breakpoints. The minimum inhibitory concentration (MIC) was determined using the broth micro-dilution technique.

Test for detection of minimum inhibitory concentration (MIC) of Colistin: `

The broth microdilution (BMD) method is a standardized technique for evaluating bacterial susceptibility to Polymyxin, using Escherichia coli ATCC 25922 as the quality control organism. Colistin powder, stored at 4°C, was used to prepare the antibiotic stock solution according to specific guidelines. An inoculum was created by suspending isolated colonies from a 24-hour blood agar plate into broth, adjusting to a turbidity of 0.5 on the McFarland scale. 96-well microtiter plates were filled with Mueller Hinton I broth containing serial two-fold dilutions of the antimicrobial agents and the bacteria at the McFarland standard. The plates were incubated at 35°C for 24 to 48 hours, after which bacterial growth was assessed for turbidity. This method accurately determines the MIC, quantifying the antibiotic concentration (µg/mL) needed to inhibit bacterial growth. The BMD MIC range was found to be between 0.25 µg/mL and 128 µg/mL, serving as a reference for comparison with other studies. The MIKROLATEST MIC is derived from the broth microdilution method to assess the in vitro effectiveness of antimicrobial agents, determined through a 2X dilution process across seven

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concentrations, indicating the lowest concentration that stops visible bacterial growth. The MIC of colistin was assessed using the MIKROLATEST® MIC colistin kit (ErbaLachema).

Results were visually assessed on the plate, with E. coli ATCC25922 serving as a control. This CE=IVD approved broth microdilution test for Colistin has cut-off values of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 mcg/mL.

Statistical analysis: Analysis involved a chi-square test to explore relationships between qualitative variables. Results from various antimicrobial susceptibility tests were compared to those from the broth microdilution (BMD) method. Categorical variables were presented as proportions, while continuous variables were shown as means with standard deviations (SD). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each method in detecting colistin resistance were calculated to evaluate effectiveness, with a p-value < 0.05 considered significant. All analyses were conducted using SPSS version 24 (SPSS Inc, Chicago, IL, USA).

Results

The research examined colistin resistance in gram-negative bacteria using two methods for antimicrobial susceptibility testing: Mikrolatest MIC & the Broth Microdilution Method (BMD). The findings indicated that 1.44% of the bacterial isolates showed colistin resistance (Table 1). The Mikrolatest MIC method exhibited a high sensitivity of 82.35% and a specificity of 89.21% for detecting colistin resistance in gram-negative bacteria; however, it had a low positive predictive value (PPV) of 10% (Table 2). Among the colistin-resistant isolates, the lowest minimum inhibitory concentration (MIC) recorded was 4 µg/ml in four isolates (23.5%) for both BMD and Mikrolatest MIC, while the highest MIC was ≥16 µg/ml in eight isolates (47%) for BMD and seven isolates (41.1%) for Mikrolatest MIC. The difference in the number of isolates identified as colistin-resistant by the two methods was statistically significant (P=1) (Table 3). The distribution of colistin-resistant gram-negative bacterial isolates included eight from Klebsiella spp., with six (75%) obtained from blood and two (25%) from other sources. In contrast, E. coli had no isolates from blood but six from different sources. For Enterobacter cloacae, three isolates were collected from other sources, with none coming from blood samples (Table 4). The antibiotic susceptibility profile of 17 colistin-resistant bacterial isolates, evaluated using both the Mikrolatest kit and the Broth Microdilution method, showed a 100% resistance rate to colistin and cefuroxime compared to other antibiotics (Table 5).

The study on Pseudomonas indicated that the susceptibility p-values mainly ranged from 0.001 to 0.052, highlighting significant differences when compared to various antibacterial agents and Colistin. For Klebsiella spp., the susceptibility p-values generally fell between 0.001 and 0.8. In the case of E. coli, the susceptibility p-values were particularly striking, spanning from 0.001 to 1.0. Acinetobacter spp. showed susceptibility p-values ranging from 0.1 to 1.0, while Enterobacter spp. had p-values between 0.177 and 1.0 (Table 6). Colistin proved to be more effective against Pseudomonas species than antibiotics like Piperacillin/Tazobactam, Cefoperazone/Sulbactam, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Tigecycline, Trimethoprim/Sulfamethoxazole, and Doripenem. Likewise, Colistin outperformed E. coli compared to antibiotics such as Amoxicillin/Clavulanate, Piperacillin/Tazobactam, Cefoperazone/Sulbactam, Cefepime, Ertapenem, Nitrofurantoin, Tigecycline, Gentamicin, Amikacin, Meropenem, Trimethoprim/Sulfamethoxazole, and Imipenem. Additionally, Colistin showed greater effectiveness against Klebsiella spp. than antibiotics including Amoxicillin/Clavulanate, Piperacillin/Tazobactam, Ceftriaxone, Cefoperazone/Sulbactam, Cefepime, Ertapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Nalidixic acid, Ciprofloxacin, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole. It is noteworthy that in this research, Klebsiella spp. exhibited lower sensitivity to Colistin compared to *Escherichia coli*. Furthermore, resistance was observed with antibiotics such as Triclocarban, Ceftazidime, Aztreonam, Doripenem, Levofloxacin, and Minocycline, as indicated by the lack of any zone of inhibition **(Table-6).**

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Table 1: Details of MIC values by different methods

Isolates(total16)	MICby BMD	MIKROLATEST MIC
E. coli- Bronchoalveolar lavage fluid	16µg/ml	16µg/ml
E. coli-Pus	8µg/ml	8µg/ml
E. coli- Pus	16µg/ml	16µg/ml
Klebsiella-Blood	16µg/ml	16µg/ml
Klebsiella-Blood	4µg/ml	4µg/ml
Klebsiella-Blood	4µg/ml	16µg/ml
Klebsiella-Pus	16µg/ml	16µg/ml
Enterobacter cloacae-Urine	16µg/ml	16µg/ml
E. coli-Pus	8 µg/ml	4 µg/ml
Enterobacter cloacae-pus	4µg/ml	8µg/ml
E. coli-Pus	16µg/ml	8 µg/ml
E. coli-Urine	16 µg/ml	8 µg/ml
Klebsiella-Pus	16µg/ml	16µg/ml
Klebsiella-Blood	8µg/ml	8 µg/ml
Klebsiella-Blood	8 µg/ml	16 µg/ml
Klebsiella-Blood	8 µg/ml	8 µg/ml
Enterobacter cloacae- Bronchoalveolar lavage fluid	4µg/ml	8µg/ml
N=17 Spearman's rank correlation; BMD vs Micro latest, r=0.049S		

Table-2: The performance for the detection of colistin resistance in Enterobacteriaceae of different antimicrobial susceptibility testing methods

Testing method	BMD no. of Isolates		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	R	NR				
Mikrolatest MIC Colistin	14	125	(82.35)	(89.21)	(10.0)	(99.7)

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	3	1033				
Broth microdilution (BMD)	17	1158	NA	NA	NA	NA
	0	0				
BMD= broth microdilution; NR= Not resistant R= resistant. NA=not applicable: all of the isolates were detected colistin resistance by the Broth microdilution system in this study; therefore, sensitivity, specificity and NPV were not calculated. Mcnemer test Micro latest x^2 _____ 124.187 ; P<0.001 BMD x^2——— 1440.001; P<0.001						

Table 3: Colistin resistant isolates with MIC value

MIC Value µg/ml	Mikrolatest MIC -n (%)	BMD-n(%)	P value
4	4(23.5))	4 (23.5)	1
8	6(35.2)	5(29.4)	
≥16	7(41.1)	8(47.0)	
Mikrolatest test, Colistin MIC was>16 µg/ml in 7 isolates (41.1%), whare as BMD has >16 µg/ml is 8 isolates(47%), the lowest MIC value is 4 µg/ml in 4 isolates (23.5%) in both BMD and microlatest MIC. and the least value Is 4 µg/ml.colistin resistant by the two methods was found to be statistically significant(P= 1)			

Table 4: Distribution of colistin resistant Gram-negative bacilli in various clinical samples

Specimen	*Klebsiella spp*	*E. coli*	*Enterobacter Cloacae*
Blood	6	0	0
Others	2	6	3
*Klebsiella spp*isolated only in Blood. Others in equal distribution. x^2=9.600 P=0.75 Inference- Statically significant.			

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Table 5:Antimicrobial sensitivity pattern of colistin resistant Gram-negative Bacilli

Antibiotics	**Sensitive**	**Resistant**	**Sensitive%**	**Resistant%**
Ampicillin/Clavulonicacid	3	14	17.6	82.4
Piperacillin/Tazobactam	6	11	35.2	64.8
Cefuroxime	0	17	0	100
Ceftriaxone	2	15	11.7	88.3
Cefoperazone/Sulbactam	6	11	35.2	64.3
Cefepime	3	14	17.6	82.4
Imipepnem	7	10	41.1	58.9
Meropenem	5	12	29.4	70.6
Amikacin	10	7	58.8	41.2
Gentamicin	6	11	35.2	64.8
Nalidixic Acid	10	7	58.8	41.2
Ciprofloxacin	11	6	64.7	35.3
Nitrofurantoin	11	6	64.7	35.3
Colistin	0	17	0	100
Trimethoprim/Sulfamethoxa Zole	6	11	35.2	64.8
Tigecycline	4	13	23.5	76.5
High resistance rates to aminoglycosides, fluoroquinolones, penicillin's, β- lactam/β-lactamase inhibitor combinations, cephalosporins, carbapenems and folate pathway inhibitors respectively were observed among all test isolates. Among these isolates 62.5% sensitivity was seen with ciprofloxacin, nitrofuranton.				

Table 6. Results of susceptibility analysis reports of Different spp to colistin and other antibiotics

S. No.	Antibiotic	Antibiotic response	Pseudomonas spp			Acinrtobacter spp			E.coli spp			Enterobacter spp			Klebsiella spp		
			Colistin response		P - Values	Colistin response		P - Values	Colistin response		P- Values	Colistin response		P - Values	Colistin response		P - Values
			Resista	Sensitiv		Resista	Sensitiv		Resista	Sensitiv		Resista	Sensitiv		Resista	Sensitiv	

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			nt	e		nt	e		nt	e		nt	e		nt	e	
1	AMP	Resistant	0	1	-	-	-	-	10	288	0.7	1	2	1	26	377	0.62
		Sensitive	0	0		-	-		0	27		1	0		0	4	
2	AC	Resistant	0	0	-	-	-	-	10	199		7	29	1	23	247	
		Sensitive	0	1		-	-		0	115	0	0	1		3	134	0.02
3	PIT	Resistant	20	51		2	29	1	5	124		0	10	0.177	16	173	
		Sensitive	19	104	0.05	1	29		6	217	0.8	7	27		10	215	0.14
4	CXM	Resistant	0	0	-	-	-	-	9	256		7	29	1	20	291	
		Sensitive	0	0		-	-		1	59	0.8	0	2		6	88	0.82
5	CXM-A	Resistant	0	0	-	-	-	-	9	266		7	29	0.508	20	294	
		Sensitive	0	0		-	-		1	49	1	1	2		6	86	0.85
6	CTR	Resistant	0	0	-	0	27	-	4	229		2	10	1	18	253	
		Sensitive	0	0		0	8		6	86	0.1	5	21		8	127	0.95
7	CFS	Resistant	27	58		1	20	1	4	96		1	11	0.66	15	150	
		Sensitive	22	107	0.02	2	41		8	245	0.9	7	27		11	241	0.08
8	CPM	Resistant	28	57		1	23	1	3	112		1	10	0.658	12	164	
		Sensitive	21	106	0.01	2	36		9	228	0.8	7	28		14	227	0.83
9	ETP	Resistant	0	0	-	-	-	-	2	64		0	8	0.307	12	148	

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		Sensitive	0	2		-	-		8	251	0.7	7	23		14	233	0.6
10	IPM	Resistant	25	43		1	21	1	8	57		2	12	1	13	137	
		Sensitive	24	123	0	1	39		3	284	< 0.001	6	25		13	254	0.18
11	MRP	Resistant	19	41		2	22	0.6	4	53		0	10	0.171	11	121	
		Sensitive	29	125	0.07	1	36		8	288	0.2	8	28		15	270	0.32
12	AK	Resistant	30	50		1	3	1	1	38		0	5	0.569	8	82	
		Sensitive	19	116	< 0.001	2	6		10	303	0.8	8	33		18	307	0.36
13	GEN	Resistant	33	56		2	20	0.3	4	85		2	3	0.203	9	117	
		Sensitive	16	108	< 0.001	1	40		8	256	0.7	6	35		17	274	0.78
14	NA	Resistant	0	0	-	0	0	-	6	270		2	8	1	12	215	
		Sensitive	0	1		0	2		3	45	0.3	5	23		14	166	0.41
15	CIP	Resistant	31	65		1	30	1	8	257		3	11	0.684	14	248	
		Sensitive	18	101	0.01	2	30		4	84	0.7	5	27		12	142	0.43
16	TGC	Resistant	38	157		1	2	0.1	7	15		2	6	0.623	314	75	
		Sensitive	9	3	0	2	58		2	326	< 0.001	6	30		11	15	< 0.001
17	NIT	Resistant	4	1	-	-	-	-	10	66		6	21	0.648	18	233	
		Sens	0	0		-	-		0	249	< 0.0	1	10		7	144	0.4

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18	TRS	Resistant	13	4	0.24	1	21	1	7	171		3	25	0.232	11	163	
		Sensitive	6	6		2	39		5	170	0.8	5	13		14	224	0.98
19	TCC	Resistant	31	83		1	7	1	2	9	0.2	1	4	1	0	7	-
		Sensitive	14	68	0.14	1	15		0	16		0	3		0	2	
20	CAZ	Resistant	26	54		0	8	0.5	2	12	0.5	0	3	1	0	6	-
		Sensitive	19	98	0.01	2	14		0	14		1	4		0	3	
21	AT	Resistant	5	5	0.03	0	0	-	2	14	0.5	1	4	1	0	8	-
		Sensitive	0	10		0	0		0	12		0	3		0	0	
22	DRP	Resistant	21	39		2	6	0.1	2	2	0	0	4	1	0	3	-
		Sensitive	12	105	0	0	15		0	23		1	3		0	6	
23	LE	Resistant	27	63		1	8	1	2	24	1	1	2	0.375	0	7	-
		Sensitive	18	89	0.04	1	14		0	2		0	5		0	2	
24	MI	Resistant	9	5		2	4	0.1	2	8	0.1	1	5	1	0	5	-
		Sensitive	9	6	0.89	0	19		0	18		0	2		0	3	

N=1852. AMP – Ampicillin; AC – Amoxyclav; AK – Amikacin; AT – Aztreonam; CXM – Cefuroxime; CXM-A - Cefuroxime Axetil; CTR – Ceftriaxone; CFS – Cefoperazone sulbactum; CPM – Cefepime; CIP – Ciprofloxacin; CAZ – Ceftazidime; DRP - Doropenem; ETP – Etrapenem; GEN – Gentamycin IPM – Imipenem; LE – Levofloxacin; MRP – Meropenem; NA – Nalidixicacid; NIT – Nitrofurantoin; PIT- Piperacillin/Tazobactum; TGC – Tigicycline;; TRS - Trimethoprim/ Sulfamethoxazole; MI- Minocycline MI;Ticarcillin/clavulanic Acid; TCC- Triclocarban.

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DISCUSSION

This study used the microlatest method to evaluate colistin susceptibility against the broth microdilution technique. A total of 1175 Gram-negative bacilli isolates from various ICU clinical samples were analyzed, revealing a colistin resistance rate of only 1.44% (n=17). These resistant isolates came from the Neonatal Intensive Care Unit (n=1), Pediatric Intensive Care Unit (n=3), Intensive Care Unit (n=7), Obstetrics and Gynecology (n=3), Tubulin Folding Cofactor D (n=2), and Male Medical Ward (n=1), with an average patient age of 42.3 years. Among them, 6 (35.2%) were Escherichia coli, 3 (17.6%) were Enterobacter cloacae, and 8 (47%) were Klebsiella pneumoniae. The Mikrolatest MIC colistin test showed high sensitivity (82.35%) and specificity (89.21%) for detecting colistin resistance, but had a low positive predictive value (10%). The lowest MIC recorded was 4 µg/ml in 4 isolates (23.5%) for both BMD and Mikrolatest MIC, while the highest was ≥16 µg/ml in 8 isolates (47%) for BMD and 7 isolates (41.1%) for Mikrolatest MIC. Distribution of colistin-resistant Gram-negative bacteria showed Klebsiella spp. accounted for 6 (75%) isolates from blood and 2 (25%) from pus, while E. coli had 4 (66.6%) from pus, 1 (16.6%) from bronchoalveolar lavage fluid, and 1 (16.6%) from urine. Enterobacter cloacae had 1 (33.3%) isolate each from urine, pus, and bronchoalveolar lavage fluid. The antibiotic sensitivity profile of the 17 colistin-resistant isolates indicated high resistance to ampicillin, piperacillin, cefuroxime, ceftriaxone, cefepime, imipenem, meropenem, gentamicin, colistin, trimethoprim, and tigecycline, with 100% resistance to colistin and cefuroxime.

This study compares the susceptibility of Colistin and other antibiotics against various gram-negative bacteria, including Pseudomonas, Acinetobacter, E. coli, Enterobacter, and Klebsiella spp. The prevalence of pathogens and antibiotic susceptibility results align with previous research. While some resistance patterns are consistent across regions, others are geographically specific. Colistin is highly effective against severe infections. The study aims to evaluate the susceptibility profiles of gram-negative bacteria in clinical isolates. This research examines the susceptibility of Colistin and other antibiotics to gram-negative bacteria like Pseudomonas, Acinetobacter, E. coli, Enterobacter, and Klebsiella spp. Findings on pathogen prevalence and antibiotic susceptibility are consistent with earlier studies. Some resistance patterns are universal, while others vary by region. Colistin is effective against critical infections. The goal is to assess the susceptibility profiles of these bacteria in clinical samples.

Colistin is more effective against Pseudomonas species than antibiotics like Piperacillin/Tazobactam and others. It also shows greater sensitivity to E. coli compared to Amoxicillin/Clavulanate and similar antibiotics. Additionally, colistin is more effective against Klebsiella spp than antibiotics such as Amoxicillin/Clavulanate and others. However, Klebsiella spp showed lower sensitivity to colistin compared to E. coli. Some antibiotics, including Triclocarban and Ceftazidime, showed resistance with no inhibition zones. Among the tested clinical isolates, antibiotics like Ampicillin and Ceftriaxone were less effective against Pseudomonas spp, while Piperacillin/Tazobactam and others were less effective against Enterobacter spp. Similarly, Triclocarban and others were less effective against Klebsiella spp.

Certain antibiotics, such as Ampicillin, Amoxyclav, Trimethoprim/Sulfamethoxazole, and Gentamycin, show significant effectiveness against Klebsiella spp. Both E. coli and Klebsiella spp. are highly sensitive to antibiotics like Piperacillin/Tazobactam, Amikacin, Meropenem, Cefepime, and Cefoperazone sulbactam. E. coli is especially responsive to Meropenem, Tigecycline, Imipenem, Nitrofurantoin, and Ertapenem.

A study [26] indicated that 11.8% (4/34) of tested isolates showed resistance to colistin, with 100% (2/2) of Acinetobacter junii, 10% (1/10) of E. coli, and 14.3% (1/7) of Pseudomonas aeruginosa resistant, as determined by the BMD method. Most reports suggest resistance rates below 10%, with variations likely due to different methodologies [27].

The antimicrobial susceptibility test for colistin was assessed using the MicroScan Walkaway system on 327 carbapenemase-producing Enterobacteriaceae isolates [28]. A reference method (Manual BMD) reported a high major error rate (26.9%) for Mikrolatest. Another

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study involving 325 isolates found 16 major errors and 13 very major errors with Mikrolatest. The Mikrolatest MIC for colistin showed high sensitivity (87.5%) and specificity (90.8%), with a positive predictive value of 9.6%, demonstrating good concordance with BMD [29,30].

Mikrolatest systems are commonly used in clinical microbiology for antimicrobial susceptibility testing, but their low positive predictive value can lead to unreliable results in this study.

Variations in methodologies for measuring minimum inhibitory concentrations (MICs) may explain the results, with Mikrolatest showing greater consistency [30]. In this study, Mikrolatest outperformed other testing methods, reporting no false resistance or susceptibility, and correlating well with broth microdilution (r=0.498), which is the gold standard for colistin susceptibility.

Antibiotic resistance (AR) has become a major concern globally due to rising healthcare costs and increased morbidity and mortality from infectious diseases, particularly in developing countries. Reports indicate a troubling trend in antibiotic susceptibility among bacterial isolates. While drug resistance is primarily a medical issue, its spread is influenced by environmental, epidemiological, cultural, communal, and commercial factors. In both developing and developed nations, AR is often deprioritized compared to other infectious diseases [31,32].

This has led to the use of older antibiotics like colistin and polymyxin B, which have shown effectiveness against these infections. Colistin is now considered a 'last-line' treatment for multidrug-resistant gram-negative pathogens. It plays a vital role in treating severe chronic and nosocomial infections caused by resistant bacteria, underscoring the importance of plasmid-mediated colistin resistance in Enterobacteriaceae. The study on colistin resistance rates in multidrug-resistant K. pneumoniae and E. coli from cancer patients further supports these findings [33,34].

This research reports that the resistance odds ratio for Pseudomonas against carbapenems like DRP and IPM in the presence of colistin is 4.7 and 2.9 times higher. Coexistence of carbapenem and colistin resistance has been noted. The combination of colistin with aminoglycosides shows an odds ratio over 3.5, while pairing colistin with cephalosporins (CFS, CPM, CAZ) yields odds ratios of 2.2, 2.4, and 2.4, indicating significant multiple resistance [35,36]. The odds of Pseudomonas showing multiple resistance (colistin and ciprofloxacin) is 2.6 times higher. In contrast, the lowest odds ratio for Pseudomonas occurs with colistin and tigecycline at 0.008, suggesting both are broad-spectrum antibiotics, with tigecycline showing good susceptibility.

Conclusion

The study finds that resistance in Pseudomonas and E. coli to colistin is significant, while it is less so for other examined bacterial species. Other bacteria, like Enterobacter and Klebsiella spp., may have genetic changes, particularly mcr gene mutations. It is suggested that future studies include more pathogenic microbial species to better understand colistin susceptibility.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Study procedure was approved by the Institutional Ethics Committee, Department of Lifesciences, Adikavi Nannayya University, Rajahmahendravaram, India.

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REFERENCES

1. Etemad M, Khani Y, Hashemi-Nazari SS, Izadi N, Eshrati B, Mehrabi Y. Survival rate in patients with ICU-acquired infections and its related factors in Iran's hospitals. BMC Public Health. 2021 Apr 24;21(1):787.
2. Teng J, Imani S, Zhou A, Zhao Y, Du L, Deng S, Li J, Wang Q. Combatting resistance: understanding multi-drug-resistant pathogens in intensive care units. Biomedicine & Pharmacotherapy. 2023 Nov 1; 167:115564. Sun et al, 2018)
3. Giraldi G, Montesano M, Sandorfi F, Iachini M, Orsi GB. Excess length of hospital stay due to healthcare acquired infections. Methodologies evaluation. Annali di Igiene Medicina Preventiva e di Comunita. 2019;31(5):507-16.
4. Du Q, Zhang D, Hu W, Li X, Xia Q, Wen T, Jia H. Nosocomial infection of COVID-19: A new challenge for healthcare professionals. International journal of molecular medicine. 2021 Apr 1;47(4):1-.
5. Jeannot K, Bolard A, Plesiat P. Resistance to polymyxins in Gram-negative organisms. International journal of antimicrobial agents. 2017 May 1;49(5):526-35.
6. Ghassani A. Study of the activity of murepavadin on Pseudomonas aeruginosa cystic fibrosis clinical strains and identification of resistance mechanisms (Doctoral dissertation, Université Bourgogne Franche-Comté).
7. Hutchings, M., Truman, A., & Wilkinson, B. (2019). Antibiotics: past, present and future. Current Opinion in Microbiology, 51(Figure 1), 72–80.
8. Eyler RF, Shvets K. Clinical pharmacology of antibiotics. Clinical Journal of the American Society of Nephrology. 2019 Jul 1;14(7):1080-90.
9. Trojan R, Razdan L, Singh N. Antibiotic susceptibility patterns of bacterial isolates from pus samples in a tertiary care hospital of Punjab, India. International journal of microbiology. 2016;2016(1):9302692.
10. Bialvaei AZ, Samadi Kafil H. Colistin, mechanisms and prevalence of resistance. Current medical research and opinion. 2015 Apr 3;31(4):707-21.
11. Dijkmans AC, Wilms EB, Kamerling IM, Birkhoff W, Ortiz-Zacarías NV, Van Nieuwkoop C, Verbrugh HA, Touw DJ. Colistin: revival of an old polymyxin antibiotic. Therapeutic drug monitoring. 2015 Aug 1;37(4):419-27.
12. Chen X, Zhao X, Che J, Xiong Y, Xu Y, Zhang L, Lan R, Xia L, Walsh TR, Xu J, Lu J. Detection and dissemination of the colistin resistance gene, mcr-1, from isolates and faecal samples in China. Journal of medical microbiology. 2017 Feb;66(2):119-25.
13. Baeuerlein A, Ackermann S, Parlesak A. Transepithelial activation of human leukocytes by probiotics and commensal bacteria: Role of Enterobacteriaceae-type endotoxin. Microbiology and immunology.2009 Apr;53(4):241-51
14. Anyanwu MU, Okpala CO, Chah KF, Shoyinka VS. Prevalence and traits of mobile colistin resistance gene harbouring isolates from different ecosystems in Africa. BioMed research international. 2021;2021(1):6630379.
15. El-Sayed Ahmed MA, Zhong LL, Shen C, Yang Y, Doi Y, Tian GB. Colistin and its role in the Era of antibiotic resistance: an extended review (2000–2019). Emerging microbes & infections. 2020 Jan 1;9(1):868-85.
16. Karaiskos I, Souli M, Galani I, Giamarellou H. Colistin: still a lifesaver for the 21st century?. Expert opinion on drug metabolism & toxicology. 2017 Jan 2;13(1):59-71.
17. Velkov T, Thompson PE, Nation RL, Li J. Structure-activity relationships of polymyxin antibiotics. Journal of medicinal chemistry. 2010 Mar 11;53(5):1898-916.

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www.jchr.org

JCHR (2025) 15(5), 2905-2918 | ISSN:2251-6727

18. Yu Z, Qin W, Lin J, Fang S, Qiu J. Antibacterial mechanisms of polymyxin and bacterial resistance. BioMed research was international. 2015;2015(1):679109.

19. Sharma VK, Johnson N, Cizmas L, McDonald TJ, Kim H. A review of the influence of treatment strategies on antibiotic-resistant bacteria and antibiotic-resistance genes. Chemosphere. 2016 May 1;150:702-14.

20. Deris ZZ, Swarbrick JD, Roberts KD, Azad MA, Akter J, Horne AS, Nation RL, Rogers KL, Thompson PE, Velkov T, Li J. Probing the penetration of antimicrobial polymyxin lipopeptides into gram-negative bacteria. Bioconjugate chemistry. 2014 Apr 16;25(4):750-60.

21. Matsushita K, Ohnishi T, Kaback HR. NADH-ubiquinone oxidoreductases of the Escherichia coli aerobic respiratory chain. Biochemistry. 1987 Dec 1;26(24):7732-7.

22. Yu Z, Zhu Y, Fu J, Qiu J, Yin J. Enhanced NADH metabolism involves colistin-induced killing of Bacillus subtilis and Paenibacillus polymyxa. Molecules. 2019 Jan 22;24(3):387.

23. Şentürk S. Evaluation of the anti-endotoxic effects of polymyxin-E (colistin) in dogs with naturally occurring endotoxic shock. Journal of veterinary pharmacology and therapeutics. 2005 Feb;28(1):57-63.

24. Falagas ME, Kasiakou SK, Saravolatz LD. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clinical infectious diseases. 2005 May 1;40(9):1333-41.

25. Bialvaei AZ, Samadi Kafil H. Colistin, mechanisms and prevalence of resistance. Current medical research and opinion. 2015 Apr 3;31(4):707-21.

26. Singh RI, Bhatia M, Anusha KR, Singh V, Omar BJ, Gupta P. Comparative evaluation of microscan walkaway 96 plus ID/AST system and mikrolatest broth microdilution kit in assessing In vitro colistin susceptibility of carbapenem-resistant clinical gram-negative bacterial isolates: Experience from a tertiary care teaching hospital in Rishikesh, Uttarakhand. Indian journal of medical microbiology. 2019 Oct 1;37(4):502-8.

27. Falagas ME, Rafailidis PI, Matthaiou DK. Resistance to polymyxins: mechanisms, frequency and treatment options. Drug resistance updates. 2010 Aug 1;13(4-5):132-8.

28. Pfennigwerth N, Kaminski A, Korte-Berwanger M, Pfeifer Y, Simon M, Werner G, Jantsch J, Marlinghaus L, Gatermann SG. Evaluation of six commercial products for colistin susceptibility testing in Enterobacterales. Clinical Microbiology and Infection. 2019 Nov 1;25(11):1385-9.

29. Jayol A, Nordmann P, André C, Poirel L, Dubois V. Evaluation of three broth microdilution systems to determine colistin susceptibility of Gram-negative bacilli. Journal of Antimicrobial Chemotherapy. 2018 May 1;73(5):1272-8.

30. Garcia LS, editor. Clinical microbiology procedures handbook. American Society for Microbiology Press; 2010.

31. Gupta S, Govil D, Kakar PN, Prakash O, Arora D, Das S, Govil P, Malhotra A. Colistin and polymyxin B: a re-emergence. Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine. 2009 Apr;13(2):49.

32. Bialvaei AZ, Samadi Kafil H. Colistin, mechanisms and prevalence of resistance. Current medical research and opinion. 2015 Apr 3;31(4):707-21.

33. Lim LM, Ly N, Anderson D, Yang JC, Macander L, Jarkowski III A, Forrest A, Bulitta JB, Tsuji BT. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2010 Dec;30(12):1279-91.

34. Mlynarcik P, Kolar M. Molecular mechanisms

of polymyxin resistance and detection of mcr genes. Biomedical Papers of the Medical Faculty of Palacky University in Olomouc. 2019 Mar 1;163(1).

35. Garbati MA, Abdulhak AB, Baba K, Sakkijha H. Infection due to colistin-resistant Enterobacteriacae in critically-ill patients. The Journal of Infection in Developing Countries. 2013 Oct 15;7(10):713-9.

36. Marchaim D, Kaye D, Kaye KS. Use of colistin in critically Ill patients. Polymyxin Antibiotics: From Laboratory Bench to Bedside. 2019:155-79.