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Original research article

Study of *in vitro* efficacy of colistin on clinical isolates of multidrug resistant gram negative bacilli

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Abstract

Reports of nephrotoxicity and neurotoxicity, however, deterred physicians from using the antibiotic, especially with the emergence of other antibiotics (e.g., aminoglycosides) that were less toxic. Between the 1970s and 1990s, Colistin was not used often, and the number of studies analyzing its use and pharmacology was minimal. The study included multidrug resistant Gram negative bacilli isolated from various clinical samples from patients from all the hospitals. Sample size was calculated as 133, rounded off to 150, assuming 1% alpha error, and 15% relative precision, 69% to 95% sensitivity of Colistin among MDR gram negative bacteria. No major or minor errors were noted in isolates of *Pseudomonas* spp. *Acinetobacter* spp. showed minor and major errors of 34.8% and 39.1% respectively. *Citrobacter* spp. showed predominantly minor error – 60% and major error of 30%. *E.coli* showed minor and major errors of 48.1% and 14.8% respectively. *Enterobacter* spp. showed minor and major errors of 50% and 33.3% respectively. *Klebsiella* spp. showed minor and major errors of 46.2% and 35.9% respectively.

Keywords: Efficacy, Colistin, Multidrug Resistant Gram Negative Bacilli

Introduction

Rapidly increasing antibiotic resistance and lack of new antibiotics in the development pipeline present a major global medical challenge. Unfortunately, the past two decades have seen a marked decline in the discovery and development of novel antibiotics and a remarkable increase in resistance to those currently available. This led to looking at older class of drugs which have not been used for a long time [1].

Polymyxins, were discovered in 1947 and were used extensively until the 1980s. Various polymyxins, A to E were discovered, of which only polymyxin B and polymyxin E (Colistin sulphate/colistimethate sodium) had clinical implications. Colistin (also called polymyxin E) was first isolated in Japan in 1949 from *Bacillus polymyxa* var. *colistinus* and became available for clinical use in 1959 [2].

Reports of nephrotoxicity and neurotoxicity, however, deterred physicians from using the antibiotic, especially with the emergence of other antibiotics (e.g., aminoglycosides) that were less toxic. Between the 1970s and 1990s, Colistin was not used often, and the number of studies analyzing its use and pharmacology was minimal.

Recently, the lack of treatment options for MDR bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, has led to the reemergence of colistin as an antimicrobial therapy. Because such a large gap exists between the years that Colistin was used clinically, available pharmacokinetic and pharmacodynamic data are very limited [3].

Hence, in recent years it has attracted considerable interest as an antibiotic for use against an increasing number of serious infections due to resistant Gram-negative pathogens and will be used increasingly in the future as highly resistant organisms continue to be clinically important and as therapeutic options remain limited [4].

Various international studies have emerged proving the efficacy of Colistin in MDR and XDR GNB. But studies from India about the efficacy of Colistin in the Indian scenario are lacking though the use of Colistin in clinical settings is fast gaining acceptance.

Hence the need for a study to evaluate the efficacy of Colistin in our setting in MDR GNB was required. The present study was undertaken to evaluate the *in vitro* efficacy of Colistin against multidrug resistant Gram negative bacilli isolated from various clinical samples

Methodology

Source of Data

The study included multidrug resistant Gram negative bacilli isolated from various clinical samples from patients from all the hospitals. Sample size was calculated as 133, rounded off to 150, assuming 1%

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alpha error, and 15% relative precision, 69% to 95% sensitivity of Colistin among MDR gram negative bacteria.

Inclusion criteria
Multidrug resistant Gram negative clinical isolates

Exclusion criteria
1. Non multidrug resistant Gram negative organisms
2. All Gram positive organisms
3. All Gram negative cocci
4. All organisms showing inherent resistance to Colistin such as *Proteus species*, *Vibrio species*, *Burkholderia species.*

Processing of specimens
Gram negative organisms were identified as per standard protocol by Gram stain, catalase, oxidase, motility, Oxidation-Fermentation test, nitrate reduction, indole, Methyl Red, Voges–Proskauer, citrate, urease, Triple Sugar Iron agar, sugar fermentation and amino acid decarboxylation tests.
Antibiotic susceptibility testing was done on Mueller Hinton agar using Kirby-Bauer disk diffusion method as per CLSI. Gram negative isolates were tested against 9 groups of antibiotics.

Results

Table 1: Colistin susceptibility by disc diffusion method

	Number	Percentage
Sensitive	53	35.3%
Intermediate sensitive	61	40.7%
Resistant	36	24.0%
Total	150	100%

Of the 150 isolates tested against Colistin, and interpreted as per the CLSI guidelines and Galani *et al*., 53(35.3%) were sensitive, 61(40.7%) were intermediate sensitive and 36 (24%) were resistant by disc diffusion method.
Majority of the isolates fell into the intermediate sensitive category.

Table 2: Colistin susceptibility by disc diffusion method organism wise

	Sensitive		Intermediate sensitive		Resistant	
	Number	Percentage	Number	Percentage	Number	Percentage
Acinetobacter spp.	6	26.1%	8	34.8%	9	39.1%
Citrobacter spp.	1	10.0%	6	60.0%	3	30%
E.coli	20	37.0%	26	48.1%	8	14.8%
Enterobacter spp.	1	16.7%	3	50.0%	2	33.3%
Klebsiella spp.	7	17.9%	18	46.2%	14	35.9%
Pseudomonas spp.	18	100%	0	.0	0	.0
Total	53		61		36	

When tested by the disc diffusion method, 100% of *Pseudomonas* spp., 37% of *E.coli*, 26.1% of *Acinetobacter* spp., 17.9% of *Klebsiella* spp., 16.7% of *Enterobacter* spp. and 10% of *Citrobacter* spp. were sensitive.
60% of *Citrobacter* spp., 50% of *Enterobacter* spp. 48.1% of *E.coli*, 46.2% of *Klebsiella* spp. and 34.8% of *Acinetobacter* spp. were intermediate sensitive.
39.1% of *Acinetobacter* spp., 35.9% of *Klebsiella* spp., 33.3% of *Enterobacter* spp., 30% of *Citrobacter* spp. and 14.8% of *E.coli*, were resistant.

Table 3: Number of isolates showing MIC

Organisms	No.	MIC_{50} (µg/dl)	MIC_{90} (µg/dl)	Number of isolates showing MIC (µg/dl)			
				0.5	1	1.5	2

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 08, 2023

Acinetobacter spp.	23	1.5	1.5	0	0	22	1
Citrobacter spp.	10	1.5	1.5	0	0	10	0
E.coli	54	1.5	1.5	2	4	48	0
Enterobacter spp.	6	1.5	1.5	0	0	6	0
Klebsiella spp.	39	1.5	1.5	0	2	37	0
Pseudomonas spp.	18	1.5	1.5	0	0	15	3

All the 150 isolates (100%) were sensitive to Colistin when tested by the E-test.

Table 4: Susceptibility comparison by disc diffusion test and E-test

	Sensitive	Intermediate sensitive	Resistant
Disc diffusion test	53(35.3%)	61(40.7%)	36(24%)
E-test	150(100%)	-	-

Colistin exhibited excellent activity against all isolates. MIC for 90% of the organisms (MIC90) = 1.5 µg/dl and MIC for 50% of the organisms (MIC50) = 1.5 µg/dl.

Table 5: Mean and median values along with maximum and minimum values of MIC

Number of isolates	Mean	Std. Deviation	Minimum	Maximum
150	1.4800	0.17262	0.50	2.00

Among the 150 isolates (100%) sensitive to Colistin by E-test, the minimum MIC value noted was 0.50 µg/l and the maximum MIC value was 2.00µg/l. The mean was 1.48 µg/l with standard deviation of 0.172.

Table 6: Mean and standard deviation values along with maximum and minimum values of MICs organism wise

Growth organism	Number	Mean	Standard deviation	Minimum	Maximum
Acinetobacter spp.	23	1.5217	0.10426	1.50	2.00
Citrobacter spp.	10	1.5000	0.00000	1.50	1.50
Escherichia coli	54	1.4259	0.22586	0.50	1.50
Enterobacter spp	6	1.5000	0.00000	1.50	1.50
Klebsiella spp	39	1.4744	0.11173	1.00	1.50
Pseudomonas spp	18	1.5833	0.19174	1.50	2.00

ANOVA, P=0.02

Among the 23 isolates of *Acinetobacter* spp. tested, the minimum and maximum MIC values were 1.5µg/l and 2µg/l respectively with a mean of 1.52µg/l.
All the 10 isolates of *Citrobacter* spp. and 6 isolates of *Enterobacter* spp. showed MIC values of 1.5µg/l.
Of the 54 isolates of *E.coli*, the minimum and maximum MIC values were 0.5µg/l and 1.5µg/l respectively with a mean of 1.42µg/l.
Of the 39 isolates of *Klebsiella* spp., the minimum and maximum MIC values were 1µg/l and 1.5µg/l respectively with a mean of 1.47µg/l.
Of the 18 isolates of *Pseudomonas* spp., the minimum and maximum MIC values were 1.5µg/l and 2µg/l respectively with a mean of 1.58µg/l.

Table 7: Error between disc diffusion test and E-test

	Number	Percentage
Minor error	61	40.7%
Major error	36	24.0%

No very major error (susceptible by disc diffusion test and resistant by E-test) was noted.
36 isolates (24%) showed major error (resistant by disc diffusion and susceptible by E-test), unacceptable levels were >3% by CLSI.
Minor error (intermediate by disc diffusion test and susceptible by E-test) was noted among 61 isolates (40.7%). As per the CLSI unacceptable levels were >10 %.

Table 8: Error between disc diffusion and E-strip organism wise

Organism	Error	Number	Percentage
Acinetobacter spp.	Minor	8	34.8%
	Major	9	39.1%

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 08, 2023

Citrobacter spp.	Minor	6	60.0%
	Major	3	30.0%
E.coli	Minor	26	48.1%
	Major	8	14.8%
Enterobacter spp.	Minor	3	50.0%
	Major	2	33.3%
Klebsiella spp.	Minor	18	46.2%
	Major	14	35.9%
Pseudomonas spp.	Minor	0	0
	Major	0	0

No major or minor errors were noted in isolates of *Pseudomonas* spp. *Acinetobacter* spp. showed minor and major errors of 34.8% and 39.1% respectively. *Citrobacter* spp. showed predominantly minor error – 60% and major error of 30%. *E.coli* showed minor and major errors of 48.1% and 14.8% respectively. *Enterobacter* spp. showed minor and major errors of 50% and 33.3% respectively. *Klebsiella* spp. showed minor and major errors of 46.2% and 35.9% respectively.

Discussion

Studies by Samant *et al*., [5] Wattal *et al*. [6] and Rajput & Naik [7] showed 100%, Behera *et al*. [8] 99.2 % and Somily *et al*. [9] 98.8% sensitivity to Colistin by disc diffusion test. These studies have considered ≥11 mm zone as sensitive and ≤10 mm zone as resistant.

Studies by Tan *et al*. [10] and Lo-Ten-Foe *et al.* [11] have shown 70% and 60.7% susceptibility respectively and have considered resistant ≤ 10 mm zone and sensitive ≥ 14mm zone.

In the present study, using the CLSI criteria and criteria by Galani *et al*., a low sensitivity of 35.3% with a large intermediate sensitive number of 40.7% was noted.

There is no uniformity in the interpretation provided by different countries across the world, making it difficult to choose a suitable criteria for interpretation. Further, the E-test in present study showed all the isolates to be sensitive to Colistin thereby suggesting that screening by disc diffusion test was unreliable. Colistin, due to high molecular weight, diffuses poorly in agar, resulting in relatively small zones of inhibition.

In the present study, all the 150 isolates (100%) were sensitive to Colistin by E-test method which was in good correlation with other studies as shown in the above table.

Although agar dilution and broth micro dilution methods are frequently recommended for the investigation of Colistin susceptibility, there are difficulties in the routine application of these techniques. Lo-Ten-Foe *et al*., [11] Tan *et al*. [10] and Behera *et al.* [8] have recommended E-test as an accurate alternative for dilution methods.

Very major error (sensitive by disc diffusion test and resistant by E-test) was not noted in the present study and was concordant with the result of van der Heijden *et al*. [13] and Behera *et al*. [8]

Major error (resistant by disc diffusion and sensitive by E-test) was high in the present study – 24 % whereas other studies reported 0-0.7%.In the study by Behera *et al*. [8] and Somily *et al*., [9] a low rate of errors were noted as they considered zone ≥ 11mm as sensitive and zone ≤ 10 mm as resistant. Nicodemo *et al*. [14] have considered zone ≥ 11mm as sensitive and zone ≤ 8 mm as resistant.

Gales *et al*. have proposed sensitive zone >14 mm and resistant zone < 11mm could reduce the rate of very major errors but resulted in high minor errors [15].

As majority of the isolates fell into intermediate sensitive category in present study, a large rate of minor errors of 40.7% was noted. This was again higher than those noted in other studies due to different interpretation criteria as discussed above

The above table shows MIC_{50} values from various studies. Present study showed MIC_{50} as 1.5 µg/dl for *Acinetobacter* spp., *Citrobacter* spp., *E.coli*, *Enterobacter* spp., *Klebsiella* spp. and *Pseudomonas* spp.

Table 9: MIC_{90} (µg/dl) in various studies

Study	Place	Acineto.	Citro.	E.coli	Entero.	Kleb.	Pseudo.
Tan *et al.* [10]	Singapore	2	-	1	16	1	4
Galani *et al.* [15]	Athens	0.5	-	0.5	16	16	2
Rajenderan *et al.* [16]	Vellore	64	-	0.5	-	1	2
Present study	MMCRI, Mysore	1.5	1.5	1.5	1.5	1.5	1.5

(Kleb –*Klebsiella* spp., Acineto- *Acinetobacter* spp., Pseud- *Pseudomonas* spp., Citro-*Citrobacter* spp., Entero – *Enterobacter* spp.)

The above table shows MIC_{90} values from various studies. Present study showed MIC_{90} as 1.5 µg/dl for *Acinetobacter* spp., *Citrobacter* spp., *E.coli*, *Enterobacter* spp., *Klebsiella* spp. and *Pseudomonas* spp.

Colistin is not a part of routine antibiotic sensitivity testing panel in the department and is very sparingly used in the hospital which probably contributes to high sensitivity. Its TID dosing, price and fear of

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nephrotoxicity along with the knowledge of being a last resort drug have restricted its use in the hospital although significant multidrug resistance is noted here.

However, considering the increasing use of Colistin for the treatment of serious infections and the emergence of resistance to this antibiotic in some countries, accurate susceptibility test results are essential.

Susceptibility testing for Colistin is plagued by different factors, such as the lack of consensus regarding breakpoints for resistance between the CLSI, the EUCAST, the CA-SFM and the BSAC; the poor diffusion of Colistin in the agar; and the absence of correlation between different methods for the investigation of Colistin susceptibility.

Conclusion

- Of the 150 isolates tested against Colistin, 53(35.3%) were sensitive, 61(40.7%) were intermediate sensitive and 36 (24%) were resistant by disc diffusion method.
- All the 150 isolates (100%) were sensitive to Colistin when tested by the E-test.
- Among the 150 isolates, the minimum MIC value noted was 0.50 µg/l and the maximum MIC value was 2.00µg/l. The mean was 1.48 µg/dl with standard deviation of 0.172.
- MIC_{50} and MIC_{90} of the isolates were 1.5 µg/dl.

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