Emergence of Colistin Resistant Gram Negative Bacilli, in a Tertiary Care Rural Hospital from Western India

Satyajeet Krishnarao Pawar[1,*], Geeta Satish Karande[2], Ravindra Vasantrao Shinde[3], Vaishali Satyajeet Pawar[4]

[1,3]Associate Professor, [2]Professor & Head, Dept. of Microbiology, [4]Tutor, Dept. of Biochemistry, Krishna Institute of Medical Sciences, Karad

***Corresponding Author:**
Email: drskpawar@gmail.com

Abstract

Background: Colistin is one of the last resort of antibiotics used specifically in the treatment of multidrug resistant gram negative bacilli. Though an older class of antibiotic with no use in last four decades, resurgence of colistin use is also causing emergence of colistin resistant gram negative bacilli.

Objective: The aim of study was to find prevalence of colistin resistance and antibiotic susceptibility pattern of these colistin resistant strains isolated from different clinical specimens in a tertiary care hospital.

Methods: The study included clinical specimens received from wards and Intensive Care Units (ICUs), during January 2015 to December 2015. The specimens were processed by standard method, bacteria were identified by VITEK 2 compact (Biomerieux, France) automation system and antimicrobial susceptibility testing was done with the same system to detect MIC for penicillins, β-lactam/ β-lactamase inhibitor, cephalosporins, carbapenems, aminoglycosides, quinolones, folate inhibitors, glycicyclins and polymixins.

Results: Colistin resistance was observed in 9.98% of the clinical isolates. 51.81% of these colistin resistant strains were *Pseudomonas* spp**.** Tigecycline (41.50%) showed maximum sensitivity to colistin resistant strains.

Conclusion: Colistin resistance (9.98%) found in the study is quite alarming. A restricted and rational use of the colistin is the need of hour. A combination of colistin and tigecycline may be useful to prevent further rise of pan drug resistant bacteria.

Keywords: Colistin, MIC, MDR, Antibiotic, Antimicrobial Sensitivity Pattern

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2394-5478.2016.00066.2

Introduction

Antimicrobial resistance has become a major health issue with many clinical isolates showing limited or no susceptibility to currently available antimicrobials.[1] Lack of effective treatments and the limited number of antibiotics in development to treat infections caused by these clinical isolates especially gram-negative bacilli, is a major concern.[2,3] With no new development of antimicrobials with activity against multidrug-resistant (MDR) gram-negative bacteria, there is reemergence of older antibiotics like colistin.[1,4,5]

Though colistin was first discovered in the 1940s, it was in use against problematic gram-negative bacteria until the late 1950s.[2,3] Reports of nephrotoxicity and neurotoxicity, however, caused physicians to stop using the antibiotic, especially with the emergence of other antibiotics (e.g., aminoglycosides) that were less toxic.[4,5] The drug was almost in hibernation for last five decades or so.[5] But with emergence of MDR[6,7] and pandrug-resistant (PDR)[6,7] gram negative bacilli, there is revival of polymixin group of antibiotics especially colistin.[6]

With widespread use of colistin there are reports of rapidly rising resistance against the antibiotic.[8] There are many laboratory studies with prevalence of colistin resistance being ranging from 1.9% - 3.3%.[9] The present study was therefore carried out in a rural part of western India to find out prevalence of colistin resistance in a Tertiary Care Hospital.

Material and Methods

Study Design: Laboratory based prospective study.

Study Period: From January 2015 to December 2015.

Settings: Study was carried out at Department of Microbiology, Krishna Institute of Medical Sciences, Karad.

Inclusion criteria: Colistin resistant **c**linical isolates from various clinical specimens, were included in the study.

Exclusion criteria: Repeat isolates from same patient from repeat specimen were excluded from study to avoid duplication of isolate. Also colistin sensitive strains were excluded from study.

Methodology

The clinical specimens received in Microbiology Department from ICUs and wards during this period

were included. Institutional ethical committee clearance was taken. The specimens included were pus, endotracheal secretions, sputum, urine, stool, cerebrospinal fluid, blood, and body fluids like ascitic fluid, peritoneal fluid, pleural fluid and other specimens like catheter tips, knee aspirate, corneal scrapings etc. Processing of the specimens was done on blood agar, chocolate agar, and MacConkey's agar[10]. Bacterial colonies were identified by VITEK 2 compact (Biomerieux, France) automation system and antimicrobial susceptibility testing was done with the same system to detect minimum inhibitory concentration (MIC). For this antimicrobials used in the panel were tigecycline, meropenem, cefoperazone/ sulbactam, amikacin, minocycline, azitreonam, piperacillin/ tazobactam, doripenem, cefepime, gentamicin, trimethoprim/ sulfamethoxazole, ceftazidime, levofloxacin, ciprofloxacin, imipenem, ertapenem, ticarcillin/ clavulanicacid, nalidixicacid, amoxicillin/ clavulanicacid, ceftriaxone, cefuroxime, ampicillin, and colistin.

Interpretation of test was done as per CLSI (2015) guidelines[11]. Colistin resistance was defined as MIC of ≥ 4 µg/ml.[11,12] Quality control of the test was done by standard ATCC strain *E.coli* 25922, *P. aeruginosa* 27853[11]. Results of all colistin resistant strains, isolated during study period were included for data analysis in the study. For this, software MS Excel was used.

Result

Total 3596 gram negative bacilli were isolated in the study period. While 3237 (90.02%) were sensitive to colistin while 359 (9.98%) were resistant. (Table 1)

Table 2 shows frequency of distribution of colistin resistant isolates. Maximum 186(51.81%) were *Pseudomonas* spp., while *Proteus* spp.62 (17.27%), *Acinetobacter* spp.42 (11.69%) were next most number of isolates.

Among various clinical specimens (Table 2) pus 152(42.34%) showed maximum colistin resistant isolates, while 71(19.78%) were isolated from catheter tips and 60 (16.71%) were isolated from urine.

Pus (Table 2) was the major contributory source for isolation of *Acinetobacter* spp, *Citrobacter* spp., *Esch.coli*, *Klebsiella* spp, *Proteus* spp. and *Pseudomonas* spp. Colistin resistant *Enterobacter* spp. were most isolated from catheter tips.

253 (70.47%) off the total 359 colistin resistant strains were having MIC value of ≥ 16µg/ml (Table 3)

31% (n=110) of the bacteria were isolated from clinical specimens received from ICUs while 69% (n=249) were from different wards in the Hospital.

Table 4 showed antimicrobial sensitivity pattern of gram negative colistin resistant bacilli. Maximum sensitivity was for tigecyclin 41.50% (n = 149) followed by meropenem 34.56% (n=122) & cefoperazone/sulbactam 121 (33.70%).

Sensitivity to other antimicrobial groups like cephalosprins, quinolones, was less than 30% to other antimicrobials. All the strains were resistant to ampicillin. Other carbapenem antimicrobials like imipenem and ertapenem showed 23.51% and 22.22% of sensitivity only.

Table 1: Colistin Resistance of Bacterial Isolates

Colistin	Bacterial isolates (n)	Percentage (%)
Sensistive	3237	90.02
Resistant	359	9.98
Total	3596	100

Table 2: Distribution of frequency of colistin resistant gram negative bacilli in various clinical specimens

Specimen	*Acinetobacter* spp	*Citrobacter* spp.	*Enterobacter* spp.	*Esch. coli*	*Klebsiella* spp	*Proteus* spp.	*Pseudomonas* spp	*Serratia* spp.	Total
Blood	1	0	0	0	0	0	2	2	5
Catheter Tip	11	2	3	5	6	12	31	1	71
CSF	0	0	0	0	0	0	4	0	4
ETT	7	1	0	1	1	1	21	1	33
Peritoneal fluid	0	0	0	0	0	1	0	0	1
Pus	11	3	2	5	13	42	76	0	152
Sputum	7	0	1	2	4	0	19	0	33
Urine	5	0	0	4	12	6	33	0	60
Total	42	6	6	17	36	62	186	4	359

Table 3: Frequency of colistin resistant isolates with MIC value

MIC Value ug/ml	Number (n)	Percentage (%)
4	66	18.38
8	40	11.15
>=16	253	70.47
Total	359	100

Table 4: Antimicrobial sensitivity pattern of colistin resistant gram negative bacilli

Antibiotics	Sensitive	Resistant	Sensitive %	Resistance %
Tigecycline	149	210	41.50	58.50
Meropenem	122	231	34.56	65.44
Cefoperazone/Sulbactam	121	238	33.70	66.30
Amikacin	110	241	31.34	68.66
Minocycline	94	209	31.02	68.98
Azitreonam	40	91	30.53	69.47
Piperacillin/Tazobactam	106	248	29.94	70.06
Doripenem	72	171	29.63	70.37
Cefepime	105	253	29.33	70.67
Gentamicin	96	263	26.74	73.26
Trimethoprim/ Sulfamethoxazole	92	265	25.77	74.23
Ceftazidime	76	226	25.17	74.83
Levofloxacin	73	230	24.09	75.91
Ciprofloxacin	86	273	23.96	76.04

Imipenem	83	270	23.51	76.49
Ertapenem	10	35	22.22	77.78
Ticaicillin/ Clavulanic Acid	25	117	17.61	82.39
Nalidixic Acid	8	48	14.29	85.71
Amoxicillin/ Clavulanic Acid	6	50	10.71	89.29
Ceftriaxone	2	54	3.57	96.43
Cefuroxime	2	54	3.57	96.43
Ampicillin	0	53	0.00	100.00
Colistin	0	359	0.00	100.00

Discussion

Colistin is used as last resort of antimicrobials especially in the present worrisome therapeutic scenario of MDR and PDR (pan dug resistant) gram negative infections.[13] In fact there is resurgence in use of the colistin all over world including India.[1,14] The drug act on outer cell membrane of gram negative bacteria and releases lipopolysaccharides.[15] This in turn results in to disruption of cell membrane leading to leakage of cell content, causing cell lysis and finally cell death.[4,5]

Within years after the reuse of colistin, there have been reports of colistin resistant strains. [16] Indiscriminate antibiotic use in India is leading to cases of bacteria resistant to colistin. In the present study, prevalence of colistin resistant gram negative bacilli (n=359) was 9.98%. In a review published in 2007, percentage of colistin resistance ranged from 1.9% - 3.3%.[9] Taneja et al in a study from North India found 16% of the carbapenem resistant MDR strains were resistant to both tigecycline and colistin.[17] Chand Wattal et al from a tertiary care hospital in North India showed that 8% of the *Pseudomonas aeruginosa* were colistin resistant.[18]

In a study from Kuwait, 12% of resistance among acinetobacter baumannii was observed.[19]

A series of 13 patients with colistin resistance has been reported from South India (Gafur et al).[8]

Adaptive or mutational mechanism is the basis for development of colistin resistance in gram negative bacilli.[1,13] Mutations result in alteration in outer membrane of gram negative bacilli which is the site of colistin action.[1] Also plasmid mediated colistin resistance has been established in animal foods, and humans.[20,21] In India there is widespread veterinary use of colistin. All this may explain the present high level resistance of almost 10% to colistin in this rural agriculture area of western India.

Pseudomonas spp. (n=186) was major gram negative colistin resistant bacilli in the study. *Acinetobacter* spp.(n=42), *Klebsiella* spp.(n=36) *Esch.coli* (n=17) also showed quite a few colistin resistant strains. Infections caused by colistin resistant gram negative bacilli has been reported in many studies in recent years.[22,23] A large number of resistant strains (n=62) out of 359 colistin resistant isolates were *Proteus* spp. which shows intrinsic resistance.[23] Pus was major contributing source for colistin resistant isolate. This may be explained on the basis of polymicrobial nature of pus.[24]

Increased MIC value of ≥ 16 µg/ml of 70.47% of the colistin resistant isolates suggest of high level of resistance.[25] This also suggest of sudden emergence of colistin resistance.[25] Besides presence of this resistance in critically ill patients in ICU, what was alarming was isolation of colistin resistant bacilli (69%) from patients admitted in various wards. This points fingers towards resurgent use of the antibiotic colistin not only at tertiary reference centre but also at private health sector in this region of India.

Tigecycline do remain as one of the last resort of the antimicrobials to colistin resistant clinical isolates. But threatening situation was 210 (58.50%) clinical isolates were resistant to tigecycline which fulfills definition of pan drug resistance.[8] So whether we have already reached preantibiotic era again?

Carbapenem group showed sensitivity ranging from 22.22% to 34.56% only, in colistin resistant isolates. This coexistence of carbapenem and colistin resistance has been reported from many parts of world.[23,26]

Conclusion

Colistin still remains as one of the last resort of antimicrobials in the treatment of MDR gram negative bacilli with no new antimicrobial development in near future. But there is worrisome therapeutic scenario; with emergence of colistin resistant clinical isolates as almost 9.98% of the clinical isolates were resistant in present study. Tigecycline will be useful in colistin resistant cases. Microbiologist, consultant and hospital infection control committee should work together to prevent further rise in the drug resistance against this last resort of antimicrobials. A restricted and rational use of the colistin is the need of hour.

References

1. Lim LM, Ly N, Anderson D, Yang JC, Macander L, Jarkowski A. et al,. Resurgence of Colistin: A Review of Resistance, Toxicity, Pharmacodynamics, and Dosing *Pharmacotherapy*. 2010;30(12):1279–1291.
2. Talbot GH, Bradley J, Edwards J, Gilbert D, Scheld M, Bartlett JG. Bad bugs need drugs: an update on the development pipeline from the antimicrobial availability task force of the Infectious Diseases Society of America. Clin Infect Dis. 2006;42:657–668.
3. Li J, Nation RL, Turnidge JD,Milne RW,Coulthard K, Rayner CR et al. Colistin: the re-emerging antibiotic for multidrug-resistant gram-negative bacterial infections. Lancet Infect Dis. 2006;6:589–601.
4. Falagas ME, Kasiakou SK, Tsiodras S, Michalopoulos A. The use of intravenous and aerosolized polymyxins for the treatment of infections in critically ill patients: a review of the recent literature. Clin Med Res. 2006;4:138–146.
5. Li J, Nation RL, Milne RW, Turnidge JD, Coulthard K. Evaluation of colistin as an agent against multi-resistant gram-negative bacteria. Int J Antimicrob Agents.2005;25:11–25.
6. Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis. 2005;40:1333–1341.
7. Pawar SK, Shinde RV, Patil HV, Patil SR, Karande GS, Mohite ST. Multidrug resistant gram negative bacilli in clinical isolates in a tertiary care hospital. Int J Health Sci Res. 2014;4(9):69-74.
8. Ghafur A, Vidyalakshmi PR, Murali A, Priyadarshini K, Thirunarayan MA. Emergence of Pan-drug resistance

amongst gram negative bacteria! The First case series from India. J Microbiol Infect Dis 2014;4(3):86-91.
9. Falagas ME, Bliziotis IA. Pan drug-resistant Gram-negative bacteria: the dawn of the post- antibiotic era? Int J Antimicrob Agents 2007;29:630-636.
10. Collee JG, Miles RS, Watt B, Tests for the identification of bacteria, In: Collee JG, Fraser AG, Marmion BP, Simmons A, Mackie and McCartney Practical Medical Microbiology.14th Ed. Churchill Livingstone, 1996:135-144.
11. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; CLSI document M100-S25.Wayne PA: Clinical and Laboratory Standards Institute; 2015.
12. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0, valid from 2012-01-01. EUCAST: Clinical breakpoints www.eucast.org/clinical_breakpoints/.
13. Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother. 2012;67(7):1607-1615.
14. Dhariwal AK, Tullu MS. Colistin: Re-emergence of the 'forgotten' antimicrobial agent. J Postgrad Med 2013;59:208-15.
15. Dixon RA, Chopra I. Leakage of periplasmic proteins from *Escherichia coli* mediated by polymyxin B nonapeptide. Antimicrob Agents Chemother. 1986;29:781–788.
16. Capone A, Giannellaa M, Fortini D, Giordanoc A, Meledandrid M, Ballardini M et al. High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality. Clinical Microbiology and Infection 2013;19(1):23–30.
17. Taneja N, Singh G, Singh M, Sharma M. Emergence of Tigecycline & Colistin resistant Acinetobacter Baumanii in patients with complicated UTI in North India. Indian J Med Res. 2011;133:681-684.
18. Wattal C, Goel N, Oberoi JK. Surveillance of Multidrug Resistant Organisms in a Tertiary Care Hospital in Delhi, India. J Assoc Physicians India 2010;58:S32-S36.
19. Al-Sweih NA, Al-Hubail MA, Rotimi VO. Emergence of tigecycline and colistin resistance in Acinetobacter species isolated from patients in Kuwait hospitals. J Chemother 2011;23:13-16.
20. Skov R, Monnet D. Plasmid-mediated colistin resistance (mcr-1 gene): three months later, the story unfolds. Euro Surveill. 2016; 21(9):pii=30155. DOI: http://dx.doi.org/10.2807/1560-7917. ES.2016.21.9.30155.
21. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016 Feb;16(2):161-8.
22. Samonis G, Matthaiou DK, Kofteridis D, Maraki S, Falagas ME. In Vitro Susceptibility to Various Antibiotics of Colistin-Resistant Gram-Negative Bacterial Isolates in a General Tertiary Hospital in Crete, Greece. Clin Infect Dis 2010;50(12):1689–1691.
23. Garbati MA, Abdulhak AB, Baba K, Sakkijha H. Infection due to colistin-resistant Enterobacteriacae in critically-ill patients. J Infect Dev Ctries 2013;7(10):713-719.
24. Bowler PG, Duerden BI, Armstrong DG, Wound Microbiology and Associated Approaches to Wound Management. Clin Microbiol Rev. 2001 April 14(2):244-269.
25. Chen S, Hu F, Zhang X, Xu X, Liu Y, Zhu D .Independent Emergence of Colistin-Resistant *Enterobacteriaceae* Clinical Isolates without Colistin Treatment. J Clinical Microbiol 2011;49(11):4022–4023.
26. Marchaim D, Chopra T, Pogue JM, Perez F, Hujer AM et al, Outbreak of Colistin-Resistant, Carbapenem-Resistant *Klebsiella pneumoniae* in Metropolitan Detroit, Michigan. Antimicrob Agents Chemother. 2011;55(2);593–599.

How to cite this article: Pawar SK, Karande GS, Shinde RV, Pawar VS. Emergence of Colistin Resistant Gram Negative Bacilli, in a Tertiary Care Rural Hospital from Western India. Indian J Microbiol Res 2016;3(3):308-313.