e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(5); 619-625

Original Research Article

Detection of Colistin Resistance Among Multi-Drug Resistant Gram-Negative Bacterial Isolates Isolated from Clinical Specimens of ICU Patients

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Received: 10-02-2023 / Revised: 13-03-2023 / Accepted: 30-04-2023
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Conflict of interest: Nil

Abstract

Introduction: Hospital-associated infections caused by multidrug-resistant (MDR) Gram negative bacteria (GNB), especially *Klebsiella pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*, represent a growing problem worldwide Colistin is considered to be one of the last resort antibiotic of multi-drug resistant (MDR) gram-negative bacilli (GNB). The increase in colistin usage has resulted in the emergence of colistin resistance in GNB.

Materials and Methods: The study included clinical specimens received from intensive Care Units (ICUs), of Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar from June 2021 to August 2022 in the Department of Microbiology. The objective of this study was to estimate the prevalence of colistin-resistance (CLR) in MDR isolates collected from different intensive care units (ICUs). The Gram-negative isolates showing colistin resistance by Kirby-Bauer's disc diffusion method was included and further subjected to broth microdilution method for confirmation of colistin resistance.

Results: A total of 336 (8.02%) Gram negative bacilli isolated from intensive care units. K. pneumoniae 136 (40.47%) was the predominant isolate, followed by Escherichia coli (33.33%), Pseudomonas aeruginosa (23.51%), Acinetobacter baumannii (2.67%). 58(17.26%) of the 336 isolates, were found to be resistant to colistin by Kirby Bauer's disc diffusion, which were subjected to broth-microdilution method, for confirmation of colistin resistance, following which only 11 (18.96%) isolates showed colistin resistance. The predominant resistant isolate was Klebsiella pneumoniae isolate followed by P.aeruginosa, A.baumannii and E.coli. The Colistin resistant Gram-negative isolates showed high sensitivity to Tigecycline and meropenem.

Conclusion: It is recommended to reduce the colistin usage as it has been considered a last resort drug. Microbiologist, consultant and hospital infection control committee should work together to prevent further rise of resistance. In our study Tigecycline is found sensitive against colistin resistant Gram-negative bacilli.

Keywords: Colistin, Multidrug resistant, Gram Negative Bacilli, Minimum inhibitory concentration.

Introduction

Hospital-associated infections with multidrug-resistant (MDR) Gram-negative bacilli (GNB), chiefly with *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, E.coli and Acinetobacter baumannii*, represent a growing problem globally.[1] Colistin is an older class of antibiotic restricted for human usage in last four decades due to neurotoxicity and nephrotoxicity.[2] Colistin is considered a crucial treatment option for management of multidrug resistant gram negative bacilli. Because of the limitations of treatment options for multidrug-resistant (MDR), pan drug-resistant and extensively drug resistant (XDR) bacterial infection treatment, colistin was reintroduced as a last option, notably by carbapenemase producing enterobacterales, Pseudomonas aeruginosa, and Acinetobacter baumannii.[3,4]

Colistin is used particularly in critical clinical conditions such as bacteremia/sepsis and pneumonia associated with mechanical ventilation (VAP) in the intensive care unit. For other clinical conditions, colistin is seen as an alternative treatment such as urinary tract infections, osteomyelitis, joint infections, meningitis, pneumonia, infections of the gastrointestinal tract, pyoderma, soft tissue infections, eye infections, and ear infections.[4] Due to the injudicious use of this antibiotic especially in intensive care settings, Gram negative bacteria showing resistance to colistin are increasingly encountered. Colistin resistance is often associated with carbapenem resistance, and such organisms are classified as extensively-drug-resistant (XDR).[5]

The presence of colistin resistance lay pressure on researchers to develop methods to detect colistin resistance. Several methods have been developed to detect colistin resistance, such as broth microdilution method (BMD), colistin broth disk elution method, CHROM agar COL, ResaPolymyxin NP test, rapid polymyxin NP test, and the colistin agar method.[6] The disk diffusion method has been found to be inefficient and unreliable for colistin susceptibility testing. This assertion is due to the absence of an established breakpoint for disk diffusion for colistin susceptibility testing. The CLSI and EUCAST advised broth micro-dilution to assess colistin susceptibility due to established breakpoint.[7]

Materials and Methods

This cross-sectional study was conducted from August 2021 to October 2022 in the Department of Microbiology, with different clinical specimens collected from intensive care unit of Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, Telangana. Institutional human ethical clearance was obtained from the institutional ethical committee, CAIMS before the commencement of the study and the inform consent form was taken from all participants, included in this study.

Inclusion criteria: Colistin resistant clinical isolates from various clinical specimens, collected from patients admitted in intensive care units were included in the study.

Exclusion criteria: Repeat isolates from same patient, repeat specimens were excluded from study to avoid duplication of isolate. Organisms that are intrinsically resistant to colistin such as *Stenotrophomonas, Burkholderia, Proteus, Serratia, Morganella,* and *Providencia* were excluded for screening of colistin resistance.

Study tool: All clinical samples, obtained like sputum, endotracheal aspirate, broncho alveolar lavage fluid, body fluids, urine and pus were processed by means of standard microbiological methods, inoculated into blood agar and MacConkey agar. Blood and were inoculated in BacT/ALERT broth bottle and incubated at 37°C.

The Gram-negative bacteria presenting resistance to 10 µg Colistin (Methane Sulphonate) disks (HiMedia Laboratories) were put on the Muller Hinton agar (MHA) plate for screening of colistin resistance by Kirby- Bauer's disc diffusion method following standard laboratory protocols.

Colistin Broth microdilution: Colistin resistance were detected by the gold standard broth microdilution method, the Colistin powder (Sigma Aldrich), with a potency of 15,000 units/mg was used to determine minimum inhibitory concentration (MIC) by Microbroth dilution. Colistin of double strength was used as CLSI requires a potency of 30,000 units/mg. Hence. 100ml of 2X strength Muller Hinton Broth (21g/L) was prepared. The concentration of colistin tested ranged from 0.25 to 32 µg/ml. The strength of the organism in each well in microtiter plate is $5x10^5$ CFU/ml as per CLSI. After adding media, drug and organism to the wells, the microtiter plate was incubated overnight at 37°C.[7] Quality control of the test was done by standard ATCC strain *E.coli* 25922, *P. aeruginosa* 27853 and the results were noted on the next day.

Results

A total of 4186 Gram negative bacilli were isolated in the study period, of which 336 (8.02%) were isolated from intensive care units. K. pneumoniae 136 (40.47%) was the predominant isolate, followed by Escherichia coli (33.33%), P. aeruginosa (23.51%), A. baumannii (2.67%). Out of the 336 isolates, 58(17.26%) were found to be resistant to colistin by Kirby Bauer's disc diffusion. The 58 colistin resistant isolates by Kirby-Bauer disk diffusion method were subjected to broth-microdilution method, for confirmation of colistin resistance, following which only 11 (18.96%) isolates showed colistin resistance. Eleven colistin-resistant isolates were reported over a period of 15 months (June 2021–August 2022).

Out of 11 colistin resistant isolates, 9 (81.81%) were obtained from males and 2 (18.18%) from females. Maximum resistant isolates were detected in 41-50 years age group (Table:1). Respiratory samples were the most common source for colistin resistance, 5 out of the 11 resistant isolates (45.4%) were detected from respiratory samples followed by urine and blood (Table:2).The predominant resistant isolate was Klebsiella pneumoniae isolate followed by *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Escherichia coli.*8 (72.7%) of the total 11 colistin resistant strains were having MIC value of ≥ 16µg/ml and 2 isolates have MIC value of ≥ 8µg/ml and 1 isolate with MIC value ≥ 4µg/ml.(Table:3)

All the colistin resistant Klebsiella species, exhibited hundred percent resistance to Fluoroquinolones, Piperacillin tazobactam, Gentamicin, Cefoperazone sulbactam, Ceftazidime, Cefotaxime, and Cefepime. The Klebsiella isolates were highly sensitive to Tigecycline and Meropenem. All the colistin resistant Pseudomonas aeruginosa isolates showed hundred percent resistance to fluoroquinolones, Gentamicin, ceftazidime, cefepime, Imipenem and Piperacillin tazobactum. Highest sensitivity was seen with Tigecycline and Meropenem for the colistin resistant Pseudomonas isolates All the isolates of colistin resistant Acinetobacter baumannii were resistant to fluoroquinolones, Piperacillin tazobactam, imipenem, amikacin, Gentamicin, Ciprofloxacin, cefepime, Cefoperazone sulbactam and cefotaxime. Overall Tigecycline showed the highest sensitivity among the colistin resistant isolates. (Table:4)

Table 1: Demographic characteristics:

Characteristics	Percentage
Gender	
Male	9 (81.8%)
Female	2 (18.1%)
Age group	
<20 yrs	1
21-30yrs	1
31- 40yrs	2
41- 50yrs	5
51-60yrs	2

Table 2: Distribution of the isolates showing colistin resistance by Broth microdilution method.

S. No.	Sample	Total Samples (n=338)	Colistin resistant isolates by disk diffusion (n=58)	Colistin resistant isolates by Broth microdilution (n=11)
1	Respiratory samples	129	21	5(45.4%)
2	Pus	9	3	1(9.09%)
3	Urine	114	21	3(27.2%)
4	Blood	86	13	2(18.1%)
Total		**338**	**58**	**11**

Table 3: Gram-negative isolates showing colistin resistance by Broth-microdilution method.

S.No	Organism	Colistin resistant isolates by Broth microdilution. (*n=11*)
1	Klebsiella pneumoniae	5 (45.4%)
2	Pseudomonas aeruginosa	3 (27.2%)
3	Acinetobacter baumanii	2 (18.1%)
4	Escherichia coli	1 (9.09%)
Total		**11**

Table 4: Antimicrobial Resistance pattern of colistin resistant Gram negative bacilli.

Antibiotics	Pseudomonas aeruginosa(n=3)	Klebsiella pneumoniae(n=5)	Escherichia coli (n=2)	Acinetobacter baumannii(n=1)
Tigecycline	1(33.3%)	2(40%)	1(50%)	-
Meropenem	1(33.3%)	3(60%)	1(50%)	1(100%)
Amikacin	2(66.6%)	4(80%)	1(50%)	1(100%)
Imipenem	3(100%)	4(80%)	1(50%)	1(100%)
Gentamicin	3(100%)	5(100%)	2(100%)	1(100%)
Piperacillin tazobactam	3(100%)	5(100%)	2(100%)	1(100%)
Ceftazidime	3(100%)	5(100%)	2(100%)	1(100%)
Cefepime	3(100%)	5(100%)	2(100%)	1(100%)
Ciprofloxacin	3(100%)	5(100%)	2(100%)	1(100%)
Cefeperazone sulbactam	3(100%)	5(100%)	2(100%)	1(100%)
Cefotaxime	3(100%)	5(100%)	2(100%)	1(100%)

Discussion

The patients admitted in the intensive care units (ICU) are more prone for nosocomial infections which is the major cause of mortality. Anti-microbial resistance is an increasing worry in the treatment of nosocomial infections particularly in ICU patients.[8] 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.[9] Within years after the reuse of colistin, there have been reports of colistin resistant strains. Indiscriminate antibiotic use in India is leading to cases of bacteria resistant to colistin. The detection of colistin resistance in clinical samples has become more essential as use of colistin has increased for treatment of MDR-GNB and carbapenem resistant organisms.[10] Several methods have been proposed and used for detection of carbapenem resistance, but there is still need to find an appropriate low cost and low skill in developing. Due to lack of appropriate testing methods, the BMD method is considered as the gold standard to detect colistin resistance.

The present study highlights an increasing prevalence of colistin resistance in Gram-negative infections especially in the ICU settings, the prevalence of colistin resistant Gram-negative bacilli (n=11) was 18.96%, similar to a study by Taneja et al.,[11] in a study from North India who reported 16% of the carbapenem resistance, other studies by Chand Wattal et al.,[12] reported 8% and 6% by Satyajeet Krishnarao Pawar et al.[13] Male preponderance of Colistin resistance in GNB was seen in our study, a highest number were isolated from males compared to females similar findings were reported by Rama Alhamw et al.,[14] 51% of the patients were male and 49% were female and Poonam AR,[10] reported 62.5% males and 37.5% females.

Elderly patients admitted to the ICUs are at high risk from colistin resistant bacteria owing to reduced immunity and multiple co-morbidities, the most common age group 41-50yrs in concordance with Deepayan Biswas et al., *Klebsiella pneumoniae* 5(45.4%), was found to be a major Gram negative colistin resistant bacilli in the study, similar to a study by Rajalakshmi Arjun[1] reported 87.5% of all isolates and 64.7% by Poonam et al.[10] In contrary Pawar et al. and Jain S,[15] reported Pseudomonas aeruginosa as the most common isolate, whereas we isolated 3 (27.2%) colistin resistant GNB.

Maximum number of colistin resistant isolates were isolated from respiratory specimens, similar to Poonam A et al.,[10] Some other studies reported urine,[1,15] pus,[13]and blood[16,17] where they also found colistin resistance organisms maximum in tracheal secretion (33.73%). The colistin resistant isolates isolated in our study exhibited high level resistance to ß-lactams including Cefepime, Ceftazidime, piperacillin-tazobactum, Gentamicin and Cefotaxime. The isolates showed maximum sensitivity against tigecycline (63.6%), similar to a study by Rajalakshmi Arjun et al.,[1] reported 75% sensitivity. We observed 45.4% sensitivity to meropenem, similar resistance pattern was also reported in a study by Deepayan Biswas et al.,[18] with respect to meropenem (57.69%).

Conclusion

As antimicrobial resistance is on the rise, regular surveillance of colistin resistant organisms is extremely important in the current scenario. Clinicians should be attentive due to the development of colistin resistance, it is recommended to reduce the colistin usage as it has been considered a last resort drug. Microbiologist, consultant and hospital infection control committee should work together to prevent further rise of resistance against such last resort of antimicrobials, as this will enable formulation of appropriate antibiotic policies. In our study Tigecycline is found

sensitive against colistin resistant Gram-negative bacilli. A combination of colistin and tigecycline may be useful to prevent further rise of pan drug resistant bacteria.

References

1. Arjun R, Gopalakrishnan R, Nambi PS, Kumar DS, Madhumitha R, Ramasubramanian V. A Study of 24 Patients with Colistin-Resistant Gram-negative Isolates in a Tertiary Care Hospital in South India. Indian J Crit Care Med. 2017 May;21(5):317-321.
2. Surojit Das, Subhanita Roy, Samadrita Roy, Gaurav Goelv, Subir Sinha, Purva Mathur, Kamini Walia, Sanjay Bhattacharya. Colistin Susceptibility Testing of Gram-Negative Bacilli: Better Performance of Vitek2 System than E-Test Compared to Broth Microdilution Method as the Gold Standard Test. Indian Journal of Medical Microbiology; January-March 2020; (1).
3. Piyatip Khuntayaporn, Krit Thirapanmethee and Mullika Traidej Chomnawang. An Update of Mobile Colistin Resistance in Non-Fermentative Gram-Negative Bacilli. Frontiers in Cellular and Infection Microbiology. June 2022; 12.
4. Juhi Sharma, Divakar Sharma, Amit Singh,and Kumari Sunita· Colistin Resistance and Management of Drug Resistant Infections. Canadian Journal of Infectious Diseases and Medical Microbiology Volume 2022, Article ID 4315030, 10 pages.
5. Syed B, Ishaque S, Imran A, Muslim O, Khalid S, Siddiqui AB. Emergence of colistin-resistant gram-negative rods in intensive care units: A cross-sectional study from a developing country. SAGE Open Medicine. 2022;10.
6. Shubham Chauhan, Narinder Kaur, Adesh K. Saini, Jyoti Chauhan, Harit Kumar. Assessment of colistin resistance in Gram negative bacteria from clinical samples in resource-limited settings. Asian Pacific Journal of Tropical Medicine 2022; 15(8): 367-373.
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Thirty-one informational supplement. M100-S31. CLSI, Wayne, PA, USA; 2021.
8. Panigrahi K, Pathi BK, Poddar N, Sabat S, Pradhan S, Pattnaik D, Patro S, Praharaj AK. Colistin Resistance Among Multi-Drug Resistant Gram-Negative Bacterial Isolates from Different Clinical Samples of ICU Patients: Prevalence and Clinical Outcomes. Cureus. 2022 Aug 23; 14(8):e28317.
9. 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.
10. Poonam AR, Bilolikar AK, Sahu S. A comparative study among different methods of detection of colistin resistant gram-negative bacilli in a tertiary care hospital.
11. N. Taneja, G. Singh, M. Singh, M. Sha rma. Emergence of tigecycline and colistin resistant *Acinetobacter baumanii* in patients with complicated urinary tract infections in north India. Indian J Med Res, 2011; 133: 681-684
12. Wattal C, Goel N, Oberoi JK, Raveendran R, Datta S, Prasad KJ. Surveillance of multidrug resistant organisms in tertiary care hospital in Delhi, India. J Assoc Physicians India. 2010 Dec;5 8 Suppl:32-6.
13. S.K. Pawar, G.S. Karande, R.V. Shind e, V.S. Pawar. Emergence of colistin resistant gram-negative Bacilli in a tertiary care rural hospital from Western India. Indian J Microbiol Res, 2016;3: 308-313.
14. Rama Alhamwi, Lütfiye Öksüz. Detection of colistin resistance via four methods in multidrug-resistant Gram-negative rods isolated from blood

cultures. J Infect Dev Ctries. 2022; 16(11):1748-1756.
15. S. Jain. Emergence of Colistin Resistance among Gram-Negative Bacteria in Urinary Tract Infections from Super Specialty Hospital of North India. International Journal of Infectious Diseases. 2018;73S: 3–398.
16. Pragasam AK, Shankar C, Veeraraghavan B, Biswas I, Nabarro LEB, et al. Molecular mechanisms of colistin resistance in Klebsiella pneumoniae causing bacteremia from India — A first report. Front Microbiol. 2017; 7:2135.
17. Gupta A, Kumar S, Rastogi N, Sagar S, Aggarwal R, et al. Colistin-resistant Klebsiella pneumoniae in surgical polytrauma intensive care unit of level-1 trauma center: First case series from trauma patients in India. Indian J Crit Care Med. 2018; 22(2):103–106.
18. Deepayan Biswas, Wihiwot Valarie Lyngdoh, Sheryl Lanong, Clarissa Jane Lyngdoh, Prithwis Bhattacharyya, Nari Mary Lyngdoh. Detection of Colistin Resistance in Gram-Negative Pathogens: A One Year Cross-sectional Study in a Tertiary Care Centre in Northeast India. International Journal of Medicine and Public Health, Vol 12, Issue 4, Oct-Dec 2022.