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A STUDY ON DETECTION OF COLISTIN RESISTANCE BY BROTH MICRO DILUTION METHOD FOR AEROBIC GRAM NEGATIVE BACILLI

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ABSTRACT **Background:** Colistin (Polymyxin E), plays a crucial role as the last line of defense against Gram-negative bacteria which are multi- drug resistant in nature. Colistin resistance is on a rise worldwide. Colistin resistance among resistant Gram-negative bacteria is vital due to lack of antibiotics for further treatment. **Aim & Objectives:** To determine the invitro susceptibility to Colistin among aerobic Gram-negative bacteria and also to determine Colistin MIC among MDR in Gram-negative bacteria. **Materials and Methods:** It is a prospective study conducted in the Department of Microbiology, at Guntur medical college. 200 aerobic Gram-negative bacterial isolates were processed for Colistin MIC. Institutional Ethical Approval was taken before the commencement of the study. Descriptive analysis was done and data was presented as frequency (n) and percentages (%). **Results:** Out of 200 samples, 6% were found to be resistant.94% were found to be intermediate susceptible. Among 12, Klebsiella has maximum colistin resistant (42 %), followed by Acinetobacter (n=25%) and Pseudomonas (n=2; 16%) and least were E. coli (16 %). Among 12 Colistin resistance, MIC ranges >16µg/ml were 34%, 8µg/ml were 12%, 4 µg/ml were 16%. Among 12 Colistin resistance, Carbapenem resistance was in 83% and remaining 17% which were carbapenem sensitive, were resistant to both Ceftazidime and Amikacin. Ceftazidime resistance was seen in 100% isolates.**Conclusions:** The implementation of surveillance programs for early identification and early infection control is necessary. Clinicians should be accurate in the treatment strategy.

KEYWORDS : Colistin resistance, Gram-negative bacteria, Broth Micro Dilution, MIC, MDR

INTRODUCTION

Colistin, also called polymyxin E, was first introduced in 1950 and was used until the early 1980s and was abandoned because of high incidence of Nephrotoxicity.1 With the emergence of multi drug resistant Gram-negative bacteria, there has been a resurgence in the use of colistin to strengthen the ammunition to fight against infections globally.

Recent studies Indian has also showed nephrotoxicity is reversible and less frequent manner than expected.3 In vitro, Broth Micro Dilution method gives Minimum Inhibitory Concentration (MIC) break point of Colistin which is useful for therapeutic purpose.

Our study aims to determine the invitro susceptibility to Colistin among aerobic Gram-negative bacteria and also objectifies to determine Colistin Minimum Inhibitory Concentration among multi drug resistant Gram-negative bacteria.

MATERIALS AND METHODS

Study design

It is a prospective study conducted in the Department of Microbiology for a period of 7 months (January 2022-July 2022), at Guntur Medical College. Ethical committee approval was taken before the commencement of the study. A total of 200 aerobic Gram-negative bacterial isolates were processed for Colistin MIC.

Study procedure

Non duplicate aerobic Gram negative bacilli isolates (n=200) from different clinical samples i.e from blood, pus, aspirates, sterile fluids, urine were included in the study and were processed for colistin MIC using the Micro Broth Dilution method

1. Cation-adjusted Mueller-Hinton broth (CAMHB) was used.

2. Primary stock solution was prepared so that final concentration of active colistin sulphate (as calculated against referenced pure salt) was1mg/ml as per CLSI MO7-A11.

3. The above prepared primary stock solution (1000µg/ml) was used to make desired concentration of working stock solution.

4.Working stock solution was prepared 4 X final drug concentration i.e to achieve final concentration of 16µg/ml, working stock solution of 64µg/ml was prepared.

5. Dilution of colistin was prepared in 10 micro centrifuge tubes by adding 936µl of autoclaved MHB in 1st well and 500 µl in 2-10 MCT. From Primary stock solution 64µl was added to 936µl of autoclaved MHB.500 µl from the 1st MCT was transferred to 2nd MCT and perform twofold serial dilutions upto 10 MCT to get concentrations as 64 µg/ml ,32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml and so on.

Preparation of 96 well round bottom microtiter plate

Step1: 50µl of MHB broth was added to all wells of column 1 to 10, 75µl was added in column 11 and 100 µl in column 12 of the microtiter plate.

Step2: 25µl of Colistin dilution 64µg/ml was added to column 1, 32µg/ml to column 2 and so on till 0.125 µg/ml in column 10 of the microtiter plate so that the final conc. in microtiter plate wells were be16 µg/ml, 8 µg/ml, 4 µg/ml and so on.

Column 11 was growth control containing only media and bacterial inoculum while column 12 was media control containing only media 100µl. Each well of the microtiter plate should finally contain total volume of 100µl.

Inoculum preparation:

- A standardized inoculum of 0.5 McFarland was prepared using the direct colony suspension method compared with Densitometer.
- 0.5 McFarland suspension was diluted 1:75 times by adding 10µl to 740µl of autoclaved MHB medium. From this diluted suspension, 25µl was added to each of the wells in column 1 to 11 to yield bacterial concentration of approximately 5 x104 CFU/well.

- The microtiter plates were incubated at 35± 2oC for 16 to 20 h for Enterobacteriaceae and Pseudomonas, and 20-24hrs for Acinetobacter.

- MIC of Colistin was read as the lowest concentration of colistin that completely inhibits growth of the organism in the microdilution wells as detected by the unaided eye.

Results were interpreted as Intermediate Susceptible if MIC ranges ≤2 µg/ml; Resistance ≥4 µg/ml as per CLSI guidelines:M07-A11 and M100-30th Edition 2020.

Quality control of the test was done by standard ATCC strain Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853.

RESULTS

- Out of 200 GNB isolates from different samples of Pus, blood, urine, respiratory specimens , most common isolated gram negative bacilli was Klebsiella sp.(n=88), followed by Pseudomonas sp. (n=52), E. coli (n=32), Acinetobacter sp.(n=28).
- Out of 200 GNB 12(6%) were found to be resistant for colistin MIC.188(94%) were found to be intermediate susceptible for Colistin MIC.

Out of 12 Colistin resistant:

- 67% were males(n=8) 33%were females(n=4).
- The mean age for resistant isolates was 64.3%.
- Resistant isolates are from ICU in patients with CKD, COPD, Cancer, Chronic lung disease.
- Among 12 ,Klebsiella sp. was found to have maximum colistin resistant (n=5;42 %), Acinetobacter (n=3; 25%) followed by Pseudomonas (n=2; 16%), E.coli (n=2; 16 %). (Fig-1).
- Klebsiella(n=5) isolated were from ICU, in urine 40%, BAL 20%, blood 20%. Pus 20%, Acinetobacter (n=3) from ICU BAL 67%, from blood 33%, Pseudomonas (n=2)both were from BAL(100%) E.coli(n=2) from pus 50%, blood50%. (Fig-2.)
- Among 12 colistin resistance ranges >16µg/ml were 34%(n=4),8µg/ml were 12% (n=6), 4 µg/ml were 16%(n=2).
- Among 12 colistin resistance, Carbapenem resistance was seen in 83% (n=10) whereas other 17% (n=2) which were carbapenem sensitive were found resistant to both ceftazidime and amikacin. Ceftazidime resistance was seen in 100%(n=12) isolates. (Fig-3).

Out of 188 Colistin Intermediate susceptible:

- Among 188, Klebsiella sp. found to be Colistin susceptible (n=83; 44 %), Acinetobacter (n=25; 13%) Pseudomonas (n=50; 26%), E. coli (n=30; 15 %).
- Among 188 Colistin intermediate susceptible MIC ranges 2 µg/ml wer17% (n=32), 1 µg/ml were 42% (n=80),0.5 µg/ml were5.3% (n=10),0.25 µg/ml were 12% (n=24), 0.125 µg/ml were 4.2% (n=8),0.006 µg/ml were 13% (n=26),0.003 µg/ml were 4.2% (n=8).

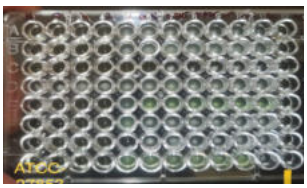

Figure-1 Colistin resistant Organisms (n=12)

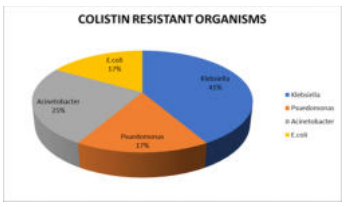

Figure-2 Colistin resistant organisms

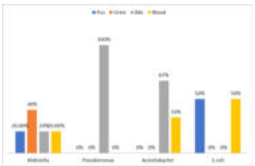

Figure-3 Colistin resistant organisms isolated from different samples

DISCUSSION

The emergence of drug-resistant GNB is increasing and posing a great challenge to clinicians and microbiologists.[4] The drug available for these infections is colistin but increasing use of colistin is leading to increasing resistance to this drug. Colistin resistance is mainly mediated by modification of lipopolysaccharide moiety of Gram-negative bacilli.[5,6] Like any other antibiotic use of colistin, can be transmitted horizontally from one patient to another[8]. Inappropriate dosing might lead to continued spread of colistin resistance among carbapenem-resistant strains and other pathogens.[7,9]

In this study the mean age for resistant isolates was 64.3% which is comparable to Rajya Laxmi Arjun et al[10] reported 58.3% also with Ghafur et al[11] (Chennai 2014)

Out of 200 GNB in this study 6% were found to be resistant for colistin MIC almost in correlation with Wichai Santimaleeworagun et al [12] study, Thailand (2020) which was 6.4% whereas Prashanth Manohar et al [13] study, Chennai (2017) it was 30%. Satyajeet Krishna Rao Pawar et al [14] study (Karad 2016) which was 9.98% resistant Whereas 94% were found to be intermediate susceptible for Colistin MIC which was almost correlating with Satyajeet Krishnarao pawar [14]12 et al study (Karad 2016) which was 90.02%.

In this study 42% were Klebsiella species, followed by, 25% Acinetobacter, 16.5%Pseudomonas 16.5% E.coli. Which was comparable with Prashanth Manohar et al study, Chennai (2017) observed 33% of colistin resistance in Klebsiella, 30% in Pseudomonas, 25% in Acinetobacter. Kanwal Preet Sodhi et al,[15] Ludhiana study (2020) who observed 8.7 % of Colistin MIC resistance in Klebsiella sp,1% in Pseudomonas,1% in Acinetobacter sp.

Among 12 colistin resistance, MIC ranges >16µg/ml were 34%,8µg/ml were 12%, 4 µg/ml were 16% which was compared with study (Chennai 2017) Colistin MIC was >16 µg/ml in 58.33%, 8 µg/ml 12.5%, and 4 µg/ml 29.17%) isolates.

Among 12 colistin resistance, Carbapenem resistance was seen in 83% (n=10) whereas other 17% (n=2) which were carbapenem sensitive were found resistant to both ceftazidime, amikacin, Thailand (2020) which was 73% resistant to carbapenem and remaining 27% were seen resistant to ceftazidime, amikacin.

Among Colistin susceptible MIC breakpoint with 2 µg/ml was 17% and 1 µg/ml were 42% which showed the shifting ranges to resistance breakpoint which was alarming towards rising trend of Colistin resistance.

CONCLUSION

The recent reports on resistance to the antimicrobials are alarming, as the gene has the potential to spread to other bacteria.

Organism already resistant to other antibiotics could become resistant to Colistin in the healthcare settings.

It is of crucial importance that microbiology laboratories perform susceptibility testing for these new antimicrobials by Broth micro dilution method in order to conduct surveillance programs, to permit the early identification, to focus on infection control procedures, to formulate local antibiogram and to guide the clinicians to choose the antibiotic treatment options at the national level to characterize the AMR scenario across the country and provide guidance for policy and practice.

REFERENCES

1. Falagas ME, Kasiakou SK. Colistin: The revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clinical Infectious Diseases. 2005;40(9):1333–41.
2. CDC's 2019 Antibiotic Resistance (AR) Threats Report.
3. Lauren M Lim et al. pharmacotherapy, dec2010.resurgence of colistin.
4. National Programme on Antimicrobial Resiatance Containment,NCDC, India February 2021.
5. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for bacteria that Grow Aerobically; —Eleventh Edition. CLSI document M07-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
6. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Thirtieth edition. CLSI document M100-A30 edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
7. Colistin breakpoints for Pseudomonas aeruginosa and Acinetobacter species, CLSI

rationale document MR01, Romney M Humphries, Accelerate diagnostic Inc, USA. September 2018.
8. Giamarellou H, Poulakou G. Multidrug-resistant gram-negative infections: What are the treatment options? Vol. 69, Drugs. 2009. p. 1879–901.
9. Dijkmans AC, Wilms EB, Kamerling IM, et al.. Colistin: revival of an old polymyxin antibiotic. Ther Drug Monit. 2015;37(4):419–427.
10. Arjun R, Gopalakrishnan R, Senthur Nambi P, Suresh Kumar D, Madhumitha R, Ramasubramanian V. A study of 24 patients with colistin-resistant Gram-negative isolates in a tertiary care hospital in South India. Indian Journal of Critical Care Medicine. 2017;21(5):317–21.
11. Ghafur A. Emergence of Pan-drug resistance amongst gram negative bacteria! The First case series from India. Journal of Microbiology and Infectious Diseases. 2014;4(3):86–91.
12. Santimaleeworagun W, Thunyaharn S, Juntanawiwat P, Thongnoy N, Harindhanavudhi S, Nakeesathit S, et al. The prevalence of colistin-resistant gram-negative bacteria isolated from hospitalized patients with bacteremia. J Appl Pharm Sci. 2020;10(2):56–59.
13. Manohar P, Shanthini T, Ayyanar R, Bozdogan B, Wilson A, Tamhankar AJ, et al. The distribution of carbapenem- And colistin-resistance in Gram-negative bacteria from the Tamil Nadu region in India. J Med Microbiol. 2017;66(7):874–883.
14. Satyajeet Krishnarao Pawar, Geeta Satish Karande, Ravindra Vasantrao Shinde, Vaishali Satyajeet PawarIndian J Microbiol Res 2016;3(3):308-313.
15. Sodhi K, Mittal V, Arya M, Kumar M, Phillips A, Kajla B. Pattern of colistin resistance in Klebsiella isolates in an Intensive Care Unit of a tertiary care hospital in India. J Infect Public Health. 2020;13(7):1018–1021.