

Original Article



Emergence of Colistin Resistant Gram-Negative Bacteria in a Tertiary Care Rural Hospital in 2019

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Abstract

Background: Colistin-resistant Gram-negative bacteria is a rapidly emerging global threat generated a sense of public alarm. **Objective:** To combat this challenge a study was designed to evaluate the fast spreading infections by colistin-resistant pathogens in the tertiary care rural hospital of Bangladesh. **Materials and Methods:** To study isolation of pathogenic gram-negative bacilli, clinical sample (n-640) of hospitalized patients of Khwaja Yunus Ali Medical College Hospital in Enayetpur, Bangladesh during the 1st quarter of the year 2019 were used. The bacterial isolates were screened for meropenem and colistin-resistance. **Results:** A total of 156 bacterial isolates were studied which included *Escherichia coli* (n-112), *Klebsiella pneumoniae* (n-14), *Pseudomonas aeruginosa* (n-27), and *Salmonella typhi* (n-3). Antibiotic sensitivity testing showed that 32/156 (20%) and 119/156 (76%) isolates were resistant to meropenem and colistin, respectively, whereas 50/156 (32%) isolates were resistant to both antibiotics. *Escherichia coli*, *K. pneumoniae*, *pseudomonas aeruginosa*, and *Salmonella typhi* isolates respectively were 112/156 (72%), 14/156 (9%), 27/156 (17%), and 3/156 (2%). **Conclusion:** Colistin is typically used as salvage therapy, or last-line treatment, for MDR gram-negative infections. But there is worrisome therapeutic scenario in our study finding of colistin resistance is 76% in Gram-negative bacteria of the clinical isolates. The restricted and rational use of colistin drug is the need of hour.

Key words: Colistin (Polymyxin E), Meropenem, Multidrug-Resistant.

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Introduction

Antibiotic resistance, which started in the 1970s among Gram-negative bacteria, is a crucial global problem.¹ The rapid increase in the prevalence of Gram-negative pathogens that are resistant to fluoroquinolones and aminoglycosides as well as all beta lactams, including carbapenems, monobactam, cephalosporins and broad-spectrum penicillin, has prompted to solve this challenge by one of the oldest antibiotics Colistin in the polymyxin group using as a last therapeutic option for critically ill patients suffering from infections caused by multidrug resistant gram negative bacteria. Colistin (polymyxin E) is a polypeptide bactericidal agent available in two forms for clinical use as (polymyxin B and polymyxin E). Colistin sulfate and colistimethate sodium. Colistimethate sodium is to hydrolyze to colistin sulfate on parenteral

administration and acts like bactericidal drug by disrupting lipopolysaccharide (LPS) molecules of gram negative bacterial outer membrane.² It is also established that neither new development of antimicrobials nor an alternative drug working activity against multidrug-resistant (MDR) gram-negative bacteria, all are bound to widespread use of colistin. As a result, there are reports of emergence of older antibiotics like colistin resistance to gram negative bacteria of Enterobacteriaceae.^{3,4,5}

The colistin resistant aerobic Gram-negative bacteria 70.75% cases was found in an intensive care unit patient in Netherlands.⁶ A study in Italy found 50% colistin resistant *E.coli*.⁷ Considering the widespread occurrence of colistin resistance and the impending danger associated with it,

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we screened gram-negative bacteria belonging to the family Enterobacteriaceae isolated from the patients of Khwaja Yunus Ali medical College Hospital has prompted more accurate and careful monitoring of resistance to this polypeptide.¹

Materials and Methods

It was Laboratory based prospective study carried out at the Department of Microbiology of Laboratory Services, Khwaja Yunus Ali Medical College Hospital, Enayetpur, Sirajganj, Bangladesh from January 2019 to March 2019 (3 months). The clinical specimens from which Colistin resistant pathogenic isolates were included in the study. The colistin resistant pathogens isolated repeatedly from the same patient's repeat specimen were excluded from study to avoid duplication of isolate. In the Department of Microbiology 640 clinical specimens were received from different wards like ICU, CCU during this period. The specimens included were urine, stool, blood, pus, sputum, throat swab and aspirated fluid. Processing of the specimens was done on blood agar, chocolate agar, Mac Conkey's agar and eosin methylene blue agar (EMB Agar) media (Merck, Germany) for double checking and taking pure culture.⁸ After incubation of plates at 37°C for 18-24 hours, the isolated colonies were identified by Gram staining and standard biochemical tests such as triple-sugar iron (TSI) and Simmons citrate.⁹ Antimicrobial susceptibility testing (AST) was performed using disk diffusion method (Kirby-Bauer) on Mueller-Hinton agar (Merck) plates according to the Clinical and Laboratory Standards Institute (CLSI) Guidelines.¹⁰ The tested antibiotic panel were meropenem, ampicillin, amoxicillin, amoxiclav, amikacin, azithromycin, azitreonam, ceftriaxone, cefixim, cefotaxime, cefuroxin, cephradine, ceftazidime, ciprofloxacin, Clindamycin, gentamicin, trimethoprim/sulfamethoxazole, nitrofurantoin, levofloxacin, teicoplanin, vancomycin and colistin. The phenotype of Enterobacteriaceae was defined as MDR according to the International Expert proposal for Interim Standards Guidelines.¹¹

This study was approved by the Institutional Review Board (IRB) and Ethics Committee of Khwaja Yunus Ali Medical College Hospital.

Results

Among the admitted patients during 1st quarter of 2019,640 different clinical samples were microbiologically investigated. A total of 156 (79%) bacterial isolates were identified as member of Enterobacteriaceae containing E. coli 112 (72%), Klebsiella pneumoniae14 (9%), Pseudomonas aeruginosa 27 (17%) and Salmonella typhi 3 (2%). (Figure 1)

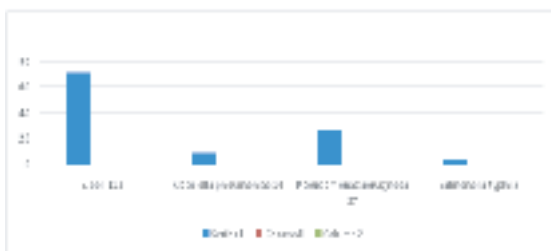


Figure 1: Pattern of Colistin Resistant Gram-Negative Bacteria

In the microbiology laboratory all the isolates were found to be multiple-drug-resistant by the disk diffusion method. Throughout this study, results were interpreted using the CLSI guidelines. Total 156 gram negative bacilli were isolated in the study period. While 37 (23.7%) were sensitive to colistin while 119 (76.3%) were resistant. (Figure 2)

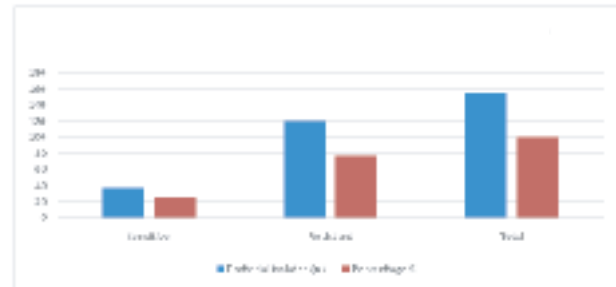


Figure 2: Sensitivity Pattern of Colistin Resistance of Bacterial Isolates

Table I: Distribution pattern of colistin resistant gram negative bacilli in different clinical specimens

| Specimen | <i>E. coli</i> (n=112) | <i>Klebsiella pneumoniae</i> (n=14) | <i>Pseudomonas aeruginosa</i> (n=27) | <i>Salmonella typhi</i> (n=03) | Total |
|-----------------|------------------------|-------------------------------------|--------------------------------------|--------------------------------|-----------|
| Urine | 44 | 05 | 02 | 00 | 51 (42) |
| Stool | 07 | 00 | 00 | 00 | 07 |
| Blood | 00 | 00 | 02 | 03 | 05 |
| Pus | 20 | 00 | 12 | 00 | 32 (26) |
| Sputum | 01 | 02 | 02 | 00 | 05 |
| Aspirated fluid | 08 | 03 | 03 | 00 | 14(11) |
| Total | 86 (71) | 10 (8) | 23 (19) | 03 (2) | 122 (100) |

*Figure in parenthesis indicate percent

Table I shows frequency of distribution of colistin resistant isolates. Maximum 86(71%) were Escherichia coli, while Pseudomonas spp.23 (19%), Klebsiella spp.10 (8%) were next the greatest number of isolates. Among various clinical specimens urine 51(42%) showed maximum colistin resistant isolates, while 32(26%) were isolated from pus and 14 (11%) were isolated from sputum. Urine was the major contributory source for isolation of Esch. coli, Pseudomonas spp and Klebsiella spp.

Table II: Antimicrobial sensitivity pattern of multidrug resistant gram negative bacilli

| Antibiotics | Sensitive | Resistant | Sensitive % | Resistant % |
|----------------|-----------|-----------|-------------|-------------|
| Ampicillin | 61 | 95 | 39.1 | 60.9 |
| Amoxicillin | 47 | 109 | 30.12 | 69.88 |
| Amoxiclav | 101 | 55 | 64.74 | 45.26 |
| Amikacin | 131 | 85 | 83.97 | 16.03 |
| Azythromycin | 77 | 79 | 49.35 | 50.65 |
| Azitronem | 107 | 49 | 68.58 | 31.42 |
| Ceftriaxone | 100 | 56 | 64.10 | 35.90 |
| Cefixim | 87 | 69 | 55.76 | 44.24 |
| Cefotaxime | 91 | 65 | 58.33 | 41.67 |
| Cefuroxin | 93 | 63 | 59.61 | 40.39 |
| Cephadrine | 81 | 85 | 45.6 | 54.4 |
| Cephazidime | 128 | 28 | 82.05 | 17.95 |
| Ciprofloxacin | 72 | 84 | 46.1 | 54.9 |
| Clindamycin | 103 | 53 | 66.02 | 34.08 |
| Cloxacillin | 102 | 54 | 65.38 | 34.62 |
| Gentamicin | 123 | 33 | 78.85 | 21.15 |
| Livofloxacin | 97 | 59 | 62.17 | 37.83 |
| Meropenem | 114 | 42 | 73.07 | 26.93 |
| Nitrofurantoin | 101 | 55 | 64.74 | 35.26 |
| Teicoplanin | 145 | 11 | 92.94 | 7.06 |
| Vancomycin | 123 | 33 | 78.85 | 21.15 |
| Colistin | 37 | 119 | 23.71 | 76.29 |

Table II shows antimicrobial sensitivity pattern of multidrug resistant gram negative bacilli. Maximum sensitivity was for Teicoplanin 92.94% (145) followed by Amikacin 83.97% (131), Cephazidime 82.05% (128) and Gentamicin/Vancomycin 78.85% (123).

Discussion

The spread of antibiotics resistance to a wide variety of antibiotics such as beta-lactams, aminoglycosides and carbapenems is a global challenge to the health systems. To cope up this vital problem colistin is increasingly used as one of the last available treatment options for patients with severe infections caused by MDR Enterobacteriaceae, Acinetobacter baumannii, and Pseudomonas aeruginosa.¹² However, its nephrotoxicity and neurotoxicity impacts have reduced its application as a routine prescribed drug.¹³

Colistin resistance follows the increasing trend in consumption of colistin in human medicine, especially in countries with high rates of carbapenem-resistant gram-negative bacilli, including Italy.¹⁴ Now, acquired resistance to colistin is extremely worrying considering that colistin is used as a last resort antibiotic against carbapenem-resistant Gram-negative bacteria, especially Enterobacteriaceae.¹⁵

During the 1st quarter of 2019, in this study 20% and 76% Gram negative bacilli found resistant to meropenem and colistin, respectively. Whereas the two years earlier study carried out in Tamil Nadu of our neighboring country India a

quite different scenario of resistance was found as 65% and 33% Gram-negative bacteria resistant to meropenem and colistin, respectively.¹⁵ Indiscriminate antibiotic use is the cause of rapid spread of carbapenem and colistin resistance among Gram-negative bacteria has become a major threat for the treatment of infectious diseases.

In a study from Singapore during 2006, 30% of *P. aeruginosa* isolates were found resistant to colistin.¹⁶ But in our study far more colistin resistance 81% found in *P. aeruginosa*. This highly significant difference in colistin resistance from Singapore's one-year study on only 102 strains of Enterobacteriaceae may be explained as it was carried out 13 years ago. Now it must be increased as its resistance spread rapidly. Our study carried during three months on 156 isolated gram-negative bacilli in comparison to one year. This proves emergence of colistin resistance is far greater than other countries of the world.

Colistin resistance in gram negative bacilli develops by mutational mechanism was thought but by research the transmission through plasmid mediated colistin resistance (*mcr-1* gene) was reported 1st in China on 18 November 2015 in food animals, foods and humans.^{17,18}

Conclusion

This document is unique, as it is the first in vitro Colistin resistance report of short period study from a tertiary health care delivery center of Bangladesh. Colistin resistance is an alarming concern because it is used as last resort of treatment in healthcare facilities. From the results of this study, we recommend, strict infection control guidelines and antimicrobial stewardship should be implemented to overcome the new resistance spread and without susceptibility testing no antibiotics should be prescribed.

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References

1. Edward JE, Gilbert D, Rie LB, Bad bugs, no drugs: no ESCAPE! An update from the infectious diseases society of America. Clin Infect Dis 2009;48(1):1-12.
2. Bergen PJ, Li J, Rayner CR, Nation RL. Colistin methanesulfonate is an inactive prodrug of colistin against *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2006;50:1953-1958.

3. 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.
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. Halaby T, Naiemi NA, Kluytmans J, Palen JVD, Christina MJE, Grauls V, et al. Emergence of Colistin Resistance in Enterobacteriaceae after the Introduction of Selective Digestive Tract Decontamination in an Intensive Care Unit. *Antimicrobial Agents and Chemotherapy* 2013;57(7):3224-3229
7. Principe L, Piazza A, Mauri C, Anesi A, Multicenter prospective study on the prevalence of colistin resistance in *Escherichia coli*: relevance of *mcr-1*-positive clinical isolates in Lombardy, Northern Italy. *Infect Drug Resist* 2018; 11: 377-385.
8. 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.
9. Till PM. *Bailey & Scott's Diagnostic Microbiology* 14th ed. Missouri: Elsevier; 2016.
10. Clinical and Laboratory Standards Institute; Performance Standards for Antimicrobial Susceptibility Testing. CLSI document M100-S25. Wayne PA: Clinical and Laboratory Standards Institute 2015.
11. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18(3):268-281.
12. Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microbiol Rev* 2017;30(2):557-596.
13. Poirel L, Kieffer N, Liassine N, Thanh D, Nordmann P. Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of *Escherichia coli*. *Lancet Infect Dis* 2016;16: 281.
14. European Centre for Disease Prevention and Control (ECDC). Plasmid-mediated colistin resistance in Enterobacteriaceae. Stockholm: ECDC, 2016 June.
15. 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. *Journal of Medical Microbiology* 2017; 66:874-883.
16. Tan T, Ng SY. The in-vitro activity of colistin in gram-negative bacteria. *Singapore Med J* 2006;47:621-624.
17. 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.
18. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. 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;16(2):161-168.