Indian Journal of Microbiology Research 2020;7(1):15–19

Content available at: iponlinejournal.com

Indian Journal of Microbiology Research

Journal homepage: www.innovativepublication.com

Original Research Article

# Characterization of beta-lactamases among MDR gram negative bacilli from a tertiary care hospital in central Kerala

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ARTICLE INFO

*Article history:*
Received 03-05-2019
Accepted 14-11-2019
Available online 08-04-2020

*Keywords:*
MDR GNB
ESBL
AmpC betalactamases
Carbapenemases

ABSTRACT

**Introduction:** Infections and mortality associated with multidrug- resistant Gram-negative bacilli (MDR GNB) have dramatically increased in all parts of the world. The present study was conducted to screen the burden of Gram negative multidrug resistance in our tertiary care hospital and to pave a stepping stone for the infection control team to tackle the problem effectively.

**Materials and Methods:** A total of 300 non-repetitive MDR GNB isolates from the specimens received in our diagnostic Microbiology lab oratory for a period of one year were screened phenotypically for Extended spectrum $\beta$ -lactamases (ESBL), AmpC betalactamases and Carbapenemases. Colistin and Tigecycline susceptibility E-test were also performed randomly on some carbapenemase producing organisms.

**Results:** Among the 300 MDR GNB isolates, ESBL and AmpC producers were 56% and 24% respectively. E.coli was the most common organism with ESBL (73%) and AmpC (46%) resistance mechanisms. Carbapenemase producers were 14.6% with predominant isolate being Acinetobacter spp (40%). Among the risk factors, 13% patients had immunosuppressive conditions like diabetes, 31% had insitu urinary catheters, 57.7% and 29.4% had prior antibiotic usage and hospitalisations respectively. Twenty five carbapenemase producing MDR GNB tested for Colistin MIC were in the susceptible range. 71% of Acinetobacter spp tested for Tigecycline showed resistant MIC values.

**Conclusion:** Multidrug resistance has a significant adverse impact on clinical outcomes of patients & leads to higher costs due to consumption of health-care resources. Adherence to simple measures like hand hygiene & careful attention to barrier precautions can help in preventing the spread of these infections to a greater extent.



## 1. Introduction

The discovery of antimicrobial agents to prevent and treat infections is one of the most important developments of modern medicine. Evidence exists that antibiotic-resistance genes were present in the era before antibiotic therapy was available, and they probably originated from antibiotic-producing bacteria.[1] In Gram negative bacteria, the antibiotic susceptibility patterns of pathogens from time to time and place to place has become matter of concern due to the emergence of beta-lactamase, extended spectrum beta-lactamases (ESBL), Amp-C beta-lactamases and Carbapenemases resistance worldwide.[2–5] These enzymes collectively can hydrolyze almost all $\beta$-lactam drugs, which are used most frequently for the treatment of serious infections.[6]

Globalization, rapid travel and vanishing borders may have helped the organisms find a safe place to travel via the human body. Uncontrolled infections and irrational use of antibiotics in the hospital are closely related to the emergence of resistant strains. Patients with unrecognized colonization with ESBL producers[7] and Carbapenemase Producing Enterobacteriaceae (CPE) have served as reservoirs for transmission during outbreaks.[8] As a preventive stratergy for arresting the transmission of

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https://doi.org/10.18231/j.ijmr.2020.004

CPE, the US CDC[9] recommends for all acute and longterm care facilities the following core measures: hand hygiene, contact precautions, patient isolation and dedicated staff, minimization of the use of invasive devices — particularly urinary catheter-s, promotion or reinforcement of antibiotic stewardship, and screening for CPE.

Recognizing the importance of drug resistance, WHO[10] had selected combating antimicrobial resistance as the theme for World Health Day 2011. i.e. 'Combat drug resistance: No action today, no cure tomorrow'. The present study was undertaken to find the proportion of different types of multidrug resistance mechanisms and factors associated with multidrug resistance in Gram negative bacilli, from Government Medical College, Thrissur which may have tremendous implications in the infection control and antibiotic policy of the hospital, because knowledge of the status of multidrug resistance in a geographical area is important in formulating the institutional antibiotic policy.

## 2. Materials and Methods

A prospective study was conducted in the Department of Microbiology, Government Medical College hospital, Thrissur during the period February 2012 to January 2013. The isolates included non-repetitive MDR Gram-negative bacilli recovered from different clinical specimens during the period of study. Biochemical identification of the isolates was done as per standard recommended procedures.[11]

Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per CLSI guidelines.[12] The routine antibiotic discs used were Ampicillin (25$\mu$g/30$\mu$g), Gentamicin (10$\mu$g), Amikacin (30$\mu$g), Ciprofloxacin (5$\mu$g), Cephalexin (30$\mu$g), Cefotaxime (30$\mu$g), Nitrofurantoin (300$\mu$g), Cot-rimoxazole (1.25/23.75). For non-fermenters, the antibiotic panel included Piperacillin (100$\mu$g), Ceftazidime (30$\mu$g), Ciprofloxacin (5$\mu$g), Gentamicin (10$\mu$g) and Amikacin (30$\mu$g) (Hi-Media Laboratories Private Limited, Mumbai).

Those isolates which were resistant to three or more classes of antibiotics were designated as MDR and were further evaluated. A random 300 MDR E.coli, Klebsiella spp, Pseudomonas spp and Acinetobacter spp. isolates were selected for this study. A detailed history was taken regarding the risk factors associated with multidrug resistance using a proforma for each isolate. Those isolates resistant to third generation cephalosporins were considered as potential ESBL producers and phenotypic screening of the same was done by double disk synergy test (DDST) using Ceftazidime (30$\mu$g), Cefotaxime (30$\mu$g) and Amoxicillin-clavulanic acid (20$\mu$g/10$\mu$g) combination discs.[12] To the lawn culture of the organism to be tested, ceftazidime and cefotaxime discs were placed 20mm centre to centre from amoxycillin/clavulanic acid (20$\mu$g/10$\mu$g). A clear extension of the edge of the ceftazidime or cefotaxime inhibition zone toward the disc containing clavulanate was interpreted as synergy, in dicating the presence of an ESBL.[13] Carbapene mase, Amp-C enzyme production were also screened by Kirby Bauer disc diffusion method using M eropenem (10$\mu$g), Imipenem (10$\mu$g), Cefoxitin(30$\mu$g), Cefipime(30$\mu$g) discs.[12] The MDR isolates found resistant to cefoxitin (30$\mu$g) were considered as potential AmpC $\beta$-lactamase producer. Isolates resistant to Imipenem (10$\mu$g) or Meropenem (10$\mu$g) were classified as Carbapenemase producers.[14,15] Some of the carbapenem resistant isolates were further checked for susceptibility to Colistin[16] and Tigecycline[17] by E-test.

## 3. Results

During the one year period, a total of 300 MDR Gram-negative bacilli isolates from various specimens received in the Department of Microbiology, Government Medical College hospital, Thrissur were included in the present study. The highest percentage of specimens in this study were from patients of age group 41-70 years constituting 54.66% (164/300), and mean age was 44 years. Male to female ratio was 1.34:1 (172:128), showing male preponderance in patients included in the present study. Out of 300 samples, 142 (47.3%) were urine, 44 (14.6%) were pus aspirates, 53 (17.7%) were pus swabs, 47 (15.66%) were respiratory specimens including sputum, tracheal aspirates and 14 (4.7%) were blood. The predominant MDR isolate obtained was E.coli constituting 56.7% of the total, and others included 21.7% Klebsiella spp, 11% Acinetobacter spp and 10.7% Pseudomonas spp. The details are given in the Figure 1.

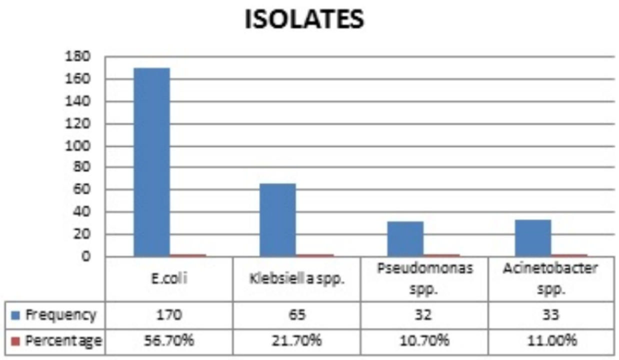


**Fig. 1:** Frequencies of MDR gram negative isolates

In this study various risk factors associated with multidrug resistance were analyzed. 57.7% (173/300) patients had prior antibiotic usage, 29.4% (88/300) patients had previous hospitalization and 20.8% (62/300) had history of admission to ICUs. Immunosuppressive and interventional risk factors associated with drug resistance were also analyzed. Diabetes and chronic disease conditions affecting kidney, lung, liver and cardiovascular system predominated with 13% (39/300) and 8% (24/300) respectively. Steroid intake (14/300, 4.7%), chronic non healing ulcers (9/300, 3%), malignant conditions (7/300, 2.3%) and HIV patients (3/300, 1%) were the other immunosuppressive conditions. Among the interventions, 77.3% (232/300) patients had peripheral venous lines, 31% (93/300) had bladder catheterization, 28% (84/300) had history of blood transfusion, 21% (63/300) had recent surgery.

Among the 300 MDR GNBs, resistance patterns to various antibiotics were also analyzed (Figure 2). All the 300 isolates were resistant to A mpicillin, Cephalexin. Ceftriaxone resistance was 98.3% (295/300). Resistance percentage to Ciprofloxacin, Cotrimoxazole and Gentamicin were 95% (285/300), 90.3% (271/300), 87% (261/300) respectively. In urinary isolates Nitrofurantoin resistance was found to be 66.7% (200/300). Antibiotics Amikacin (37.7%, 113/300), Meropenem (16.33%, 51/300), Imipenem (13.66%, 43/300) showed minimum resistance among the 300 samples.

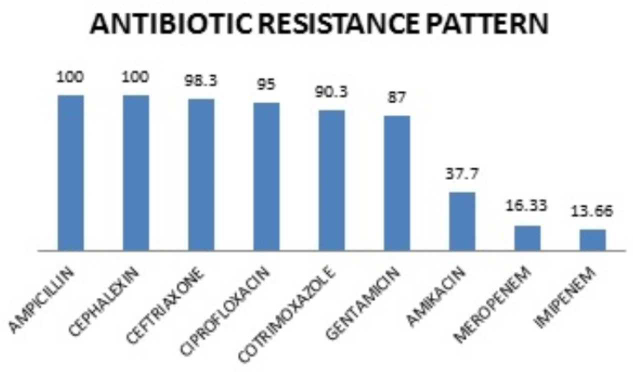


**Fig. 2:** Antibiotic resistance pattern of MDR GNB isolates

Multidrug resistance mechanisms screened in these MDR isolates were ESBL, AmpC beta lactamases and Carbapenemases. Their frequency is given in Table 1.

Distribution of these resistance mechanisms among the MDR isolates analysed. E.coli were the predominant organism with ESBL and AmpC Beta lactamases. Acinetobacter spp outnumbered Enterobacteriaceae in carbapenemase production. The details are given in Table 2.

Carbapenemase enzyme distribution varied among various specimens and departments in the present study. Isolates from 7% of total urine samples (10 out of 142), 22.7% of pus aspirates (10 out of 44), 18.86% of pus swabs (10 out of 53), 21.27% of respiratory specimens (10 out of 47) and 28.57% of blood (4 out of 14) were found to be carbapenemase producing. Specimens received from Medicine and Newborn ICU department were harbouring the highest number of carbapenemase producing isolates in the present study.

Out of 44 carbapenemase producers, 25 isolates were tested for Colistin susceptibility using E test. All the isolates were in the susceptible MIC range for Colistin according to EUCAST. Tigecycline susceptibility E test was tested on 20 carbapenemase producing isolates. The MIC of E.coli isolates was in the susceptible range constituted 30% (6/20) of the total carbapenemase producing isolates tested for tigecycline MICs whereas Acinetobacter spp. showed resistant (71%) and intermediate (29%) MIC values. The details are given in Table 3

## 4. Discussion

The emergence of multidrug resistant (MDR) nosocomial pathogens, resistant to all currently available antibiotics has been recognized as a public health threat in recent times. The spread of these resistant strains coupled with the decline in the discovery and development of newer effective antibiotics over the last two decades poses a severe impact on our health care system by the depletion of most of the available therapeutic options for MDR bacterial infections. Currently there is renewed interest in the usage of polymyxins, as they are the only treatment option for these MDR and pan-drug resistant (PDR) Gram-negative infections.[18]

To understand the resistance pattern in our hospital, 300 non- repetitive MDR GNB were screened for other mechanisms of antibiotic resistance like ESBL, AmpC beta lactamases and carbapene mase production along with their clinicoepidemiological details. According to Lortholary et al[19] and Masaeli Milad et al,[20] the risk factors associated with multidrug resistance were found to be prior exposure to antibiotics, immunosuppressive conditions like DM, ICU hospitalization. Previous antibiotics and urinary catheter were identified as independent risk factors for MDR GNB acquisition in Gudiol et al[21] study. In the present study the findings related to risk factors were in accordance with the above authors' data.

In the present study, by the initial screening technique the predominant resistant mechanism observed was due to ESBL production (56%, 168/300), followed by AmpC (24%, 72/300) and carbapenemase (14.6%, 44/300) production. Meyer & Picoli[22] in 2011 in their study with fifty-eight bacterial isolates K. pneumonia with reduced susceptibility to third generation cephalosporins and/or cefoxitin detected a high frequency of ESBL (48.3%), followed by the AmpC plasmid/ESBL (15.5%), and two carbapenemase strains and the resistance frequencies were similar to the present study.

A study by Gudiol et al[21] also stated that the most frequent mechanism of resistance was extended-spectrum $\beta$-lactamase (ESBL) production (45%), mainly by Escherichia coli, followed by Amp-C cephalosporinase hyper production (24%). In the present study, E.coli constituted 73% (122/168) of the ESBL producers, 19% (32/168) by Klebsiella spp, 5% (9/168) by Pseudomonas

**Table 1:** Results of phenotypically detected multidrug resistance mechanisms

| Betalactamase enzymes | No of isolates | Percentage |
|---|---|---|
| ESBL | 168 | 56% |
| AmpC | 72 | 24% |
| Carbapenamases | 44 | 14.6% |

**Table 2:** Frequencies of MDR mechanisms among GNB

| Resistance mechanisms | E.coli | Klebsiella spp | Pseudomonas spp | Acinetobacter spp |
|---|---|---|---|---|
| ESBL | 122(73%) | 32 (19%) | 9 (5%) | 5 (3%) |
| AmpC | 33 (46%) | 18 (25%) | 11(15%) | 10 (14%) |
| Carbapenamases | 9 (21%) | 9 (21%) | 8 (18%) | 18 (40%) |

**Table 3:** Frequencies of Tigecycline susceptibile carbapenemase producing isolates

| Tigecycline MIC | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| E.coli (6) | 6 | - | - |
| Acinetobacter spp (14) | - | 4 | 10 |

spp and 3% (5/168) Acinetobacter spp. Similarly E.coli and Klebsiella spp were the predominant AmpC producers with 46% (33/72) and 25% (18/72) respectively in the present study.

According to Gaur et al[23] and Wattal et al,[24] carbapene m resistance were found to be ranging from 6% to 57% in Acinetobacer spp. In the present study, carbapene mases constituted 14.6% (44/300) of the total MDR organisms screened and Acinetobacter spp predominated with 40% (18/44) of carbapenemase producing MDR bacteria, followed by 21% (9/44) E.coli, 21% (9/44) Klebsiella spp and 18% (8/300) Pseudomonas spp.

Among the 44 carbapene mase producing organisms, fourteen Acinetobacter spp, six E.coli, five Pseudomonas spp, as a treatment option were tested with Colistin E tests. All the tested isolates were sensitive to Colistin. Acinetobacter spp and E.coli strains were also tested for Tigecycline susceptibility using E- tests, all the E.coli were in the sensitive range for Tigecycline while none of the Acinetobacter strains came in the sensitive range of MIC values. A study by Behera et al[25] in 2009, tigecycline was found to be highly effective against Gram- positive bacteria (35/35) and Gram-negative members of Enterobacteriaceae (11/11), but a high prevalence of resistance was reported in members of Acinetobacter spp (20/26). According to Datta et al[26] in a ten year analysis of multi-drug resistant blood stream infections caused by Escherichia coli and Klebsiella pneumoniae in a tertiary care hospital, tigecycline was introduced in the hospital formulary from 2007 and in the same year a resistance of 14 percent was observed which further increased to 20 per cent in 2009 in K. pneumoniae. However, in E. coli the resistance to Tigecycline remained 1.7 percent in 2008 and increased marginally to three per cent in 2009. All the isolates remained sensitive to Colistin. This suggests that Tigecycline may not be as effective as Colistin in treatment of carbapenem resistant isolates. In the battle against rapidly emerging bacterial resistance we can no longer rely entirely on the discovery of new antibiotics; we must also pursue rational approaches to the use of older antibiotics such as colistin.

## 5. Conclusion

The present study reveals a change in antibiotic susceptibility patterns of pathogenic Gram-negative bacteria and emerging newer drug resistance mechanisms in this tertiary care centre. Carbapenems were the only active antibiotics against many multidrug resistant Gram-negative pathogens, particularly those with extended-spectrum beta-lactamases (ESBLs) and AmpC enzymes. The emergence and spread of carbapenemase-producing strains has become a major concern in health care institutions. So vigilance and timely recognition of infections with resistant bacteria and appropriate antibiotic therapy, is highly recommended. For integration of laboratory and clinical practice, auditing of antibiotic reports along with evaluating the impact of the report on treatment policy in the hospital could be a first step. Careful attention to barrier precautions and hand hygiene by all health care providers which can be monitored by Institutional infection control team can help in preventing the spread of these, multidrug resistant Gram-negative microorganisms.

## 6. Source of Funding

None.

## 7. Conflicts of Interest

There are no conflicts of interest.

## References

1. Medeiros AA. Evolution and Dissemination of $\beta$-Lactamases Accelerated by Generations of $\beta$-Lactam Antibiotics. Clin Infect Dis.

1997;24(Supplement_1):S19–S45.
2. Sharma AR, Bhatta DR, Shrestha J, Banjara MR. Antimicrobial Susceptibility Pattern of Escherichia coli Isolated from Uninary Tract Infected Patients Attending Bir Hospital. Nepal J Sci Technol. 2013;14(1):177–84.
3. Asati RK, Sadawarte K. Prevalence and antimicrobial susceptibility pattern of Escherichia coli causing urinary tract infection. Int J Pharma Bio Sci. 2013;4(4):927–36.
4. Goel V, Hogade SA, Karadesai SG. Prevalence of extended-spectrum beta-lactamases, AmpC beta-lactamase, and metallo-beta-lactamase producing Pseudomonas aeruginosa and Acinetobacter baumannii in an intensive care unit in a tertiary care hospital. J Sci Soc. 2013;40(1):28–31.
5. Manikandan C, Amsath A. Antibiotic susceptibility pattern of Escherichia coli isolated from urine samples in Pattukkottai Tamilnadu. Int J Curr Microbiol App Sci. 2014;3(10):449–57.
6. Fam N, Diab M, Helmi H, Defrawy IE. Phenotypic detection of metallo-$\beta$-Lactamases and extended spectrum $\beta$-Lactamases among Gram negative bacterial clinical isolates. Egy J Med Microbiol. 2006;15(4):719–29.
7. Paterson DL, Bonomo RA. Extended-Spectrum -Lactamases: a Clinical Update. Clin Microbiol Rev. 2005;18(4):657–86.
8. Gijon D, Curiao T, Baquero F, Coque TM, Canton R. Fecal Carriage of Carbapenemase-Producing Enterobacteriaceae: a Hidden Reservoir in Hospitalized and Nonhospitalized Patients. J Clin Microbiol. 2012;50(5):1558–63.
9. Centers for Disease Control and Prevention (CDC). Guidance for control of infections with carbapenem-resistant or carbapenemase-producing Enterobacteriaceae in acute care facilities. Morb Mortal Wkly Rep. 2009;58:256–60.
10. World Health Organisation. Antimicrobial resistance; 2011. Available from: http://www.who.int/mediacentre/factsheets/fsN‘194/en/index/html.
11. Win WC, Allen SD, Janda WM, Koneman EW, Procop GW, et al. Colour Atlas & text book of diagnostic microbiology, 6th edition. Philadelphia: Lippincott Williams & Wilkins; 2006.
12. Performance standards for antimicrobial susceptibility testing: 20th informational supplement M100-S20. Wayne, Pennsylvania, USA: Clinical Laboratory Standards Institute (CLSI); 2012.
13. Manoharan A, Sugumar M, Kumar A, Jose H, Mathai D, Group. ICMR-ESBL study group. Phenotypic & molecular characterization of AmpC $\beta$-lactamases among Escherichia coli, Klebsiella spp. & Enterobacter spp. From five Indian Medical Centres. Indian J Med Res. 2012;135:359–64.
14. Jacoby GA. Amp C -Lactamases. Clin Microbiol Revi. 2009;22(1):161–82.
15. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended Broad-Spectrum -Lactamases Conferring Transferable Resistance to Newer -Lactam Agents in Enterobacteriaceae: Hospital Prevalence and Susceptibility Patterns. Clin Infect Diseases. 1988;10(4):867–78.
16. European Committee on Antimicrobial Susceptibility Testing. Colistin: Rationale for the clinical breakpoints, version 1.0, 2010.
17. Brink AJ, Bizos D, Boffard KD, Feldman C, Grolman DC, et al. Guideline: Appropriate use of tigecycline. South Afr Med J. 2010;100(6):388–94.
18. Balaji V, Baliga PR, Jeremiah SS. Polymyxins: Antimicrobial susceptibility concerns and therapeutic options. Indian J Med Microbiol. 2011;29(3):230–42.
19. Lortholary O, Fagon JY, Hoi AB, Slama MA, Pierre J, et al. Nosocomial Acquisition of Multiresistant Acinetobacter baumannii: Risk Factors and Prognosis. Clin Infect Dis. 1995;20(4):790–6.
20. Masaeli M, Faraji T, Ramazanzadeh R. Risk Factors Associated with Resistance in Metalo beta-lactamase Producing Enterobacteriaceae Isolated from Patients in Sanandaj Hospitals. Curr Drug Ther. 2012;7(3):179–83.
21. Gudiol C, Tubau F, Calatayud L, Garcia-Vidal C, Cisnal M, Sánchez-Ortega I. Bacteraemia due to multidrug-resistant Gram-negative bacilli in cancer patients: risk factors, antibiotic therapy and outcomes. J Antimicrob Chemother. 2011;66(3):657–63.
22. Meyer G, Picoli SU. Phenotypes of beta-lactamases in Klebsiella pneumoniae from emergency hospital of Porto Alegre. J Bras Patol Med Lab. 2011;47(1):24–31.
23. Gaur A, Garg A, Prakash P, Anupurba S, Mohapatra TM. Observations on carbapenem resistance by minimum inhibitory concentration in nosocomial isolates of Acinetobacter species: an experience at a tertiary care hospital in North India. J Health Popul Nutr. 2008;26:183–8.
24. 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;58:32–8.
25. Behera B, Das A, Mathur P, Kapil A, Gadepalli R, Dhawan B. Tigecycline susceptibility report from an Indian tertiary care hospital. Indian J Med Res. 2009;129:446–50.
26. Datta S, Wattal C, Goel N, Oberoi JK, Raveendran R, Prasad KJ. A ten year analysis of multi-drug resistant blood stream infections caused by Escherichia coli &Klebsiella pneumoniae in a tertiary care hospital. Indian J Med Res. 2012;135:907–12.

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**Cite this article:** Ardra M , Swaminathan A, Nair K P. **Characterization of beta-lactamases among MDR gram negative bacilli from a tertiary care hospital in central Kerala**. *Indian J Microbiol Res* 2020;7(1):15-19.