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# Comparison of antimicrobial resistance pattern among clinical isolates of gram negative bacilli from intensive care units and general wards in a tertiary care hospital

**Gomathi Maniyan[1]*, Vijayalakshmi Arumugam[2], Nithya Gomatheswari[3], Malathi Murugesan[4]**
[1,4]Postgraduate student, Department of Microbiology, Chengalpattu Medical College, Chengalpattu, Kanchipuram, Tamil Nadu, India.
[2]Professor & HOD, Department of Microbiology, Chengalpattu Medical College, Chengalpattu, Kanchipuram, Tamil Nadu, India.
[3]Assistant Professor, Department of Microbiology, Thoothukudi Medical College, Thoothukudi.

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**Abstract:** Increasing antimicrobial resistance is a worldwide concern. The prevalence of resistance among hospitalized patients varies in different location. The right choice of antibiotic is utmost importance to initiate empirical therapy especially in critical care areas. To compare and assess the differences in the pattern of antimicrobial resistance shown by Gram Negative Bacilli (GNB) isolates from general wards and Intensive Care Unit (ICU) patients. This is a retrospective study conducted in a tertiary care hospital on 100 (50 + 50) isolates of GNB from clinical samples collected from General ward and ICU patients respectively. GNB isolates were identified by standard biochemical tests and their antimicrobial susceptibility pattern was determined as per CLSI guidelines and analyzed for both the groups. The resistant strains were identified for Extended Spectrum Beta Lactamases (ESBL) and Metallo Beta Lactamase (MBL) production. In general ward isolates, Enterobacteriaceae was the commonest (78%) and nonfermenters accounts to 22%. In ICU, Enterobacteriaceae 64% and nonfermenters 36%. In ICU maximum resistance to third generation cephalosporins and fluoroquinolones was noted among nonfermenters. In contrary, in general ward, Escherichia coli showed highest resistance to almost all the drugs except aminoglycosides. Majority of the isolates in both the groups were sensitive to aminoglycosides (80%). ESBL producer in ICU was 80% and in general ward 72%. MBL production among nonfermenters in ICU was 25%. This study provides information on antibiotic resistance in different areas of the hospital. Need of the day is that, each hospital should have a comprehensively drafted and strictly implemented antibiotic policy.

**Key words:** Antimicrobial susceptibility; Extended spectrum beta-lactamase (ESBL); General ward; Intensive care unit (ICU); Metallo Beta Lactamase (MBL).

## Introduction

Increasing antimicrobial resistance is a worldwide concern. The prevalence of resistance among hospitalized patients is an alarming rise and also varies in general ward and intensive care units in accordance with the geographical and regional location. In almost all the cases antimicrobial therapy is initiated empirically keeping in mind the high morbidity, longer hospital stay and higher mortality.[1,2] The right choice of antibiotic is utmost importance to initiate empirical therapy especially in critical care areas. Antimicrobial resistance is a major health problem in current era. Exposures to broad-spectrum antimicrobials and cross infection are important contributing factors to antimicrobial resistance.[3] Limited antimicrobial usage, appropriate treatment, avoidance of cross infection, and adherence to good hand hygiene are the remedial measures shown to reduce multidrug-resistant organisms, leading to reductions in nosocomial infections with these pathogens and in subsequent attributable mortality.[4]

To compare and assess the differences in the pattern of antimicrobial resistance shown by GNB isolates from general ward and Intensive care unit patients so that the study can provide guidelines for choosing an effective antibiotic and helps for the implementation of the antibiotic policy.

## Materials and Methods

This is a retrospective study conducted in tertiary care hospital on 100 (50 + 50) isolates of GNB from clinical samples collected from General ward and ICU patients over a period of six months. Different clinical samples such as blood, urine, pus, sputum, swabs were collected from different specialties. The samples were processed and GNB isolates were identified by standard biochemical tests and categorized separately. Their antimicrobial susceptibility pattern was determined by Kirby Bauer disc diffusion method as per CLSI (Clinical Laboratory Standards Institute) guidelines and analyzed for both the groups. Isolates showing resistant zone of inhibition to third generation

***Corresponding Author:**
**Dr. Gomathi Maniyan,**
Postgraduate student,
Department of Microbiology,
Chengalpattu Medical College,
Chengalpattu, Kanchipuram, Tamil Nadu, India.
**E-mail:** gomathym2013@gmail.com

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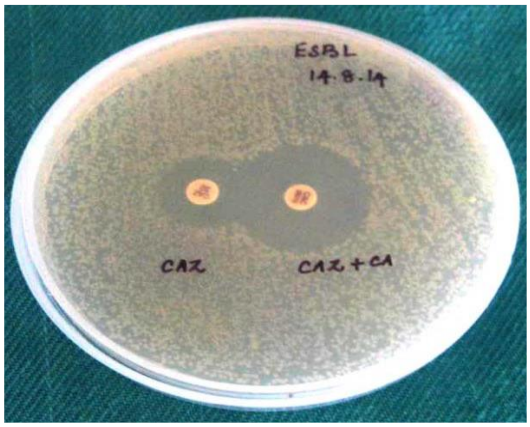
cephalosporins i.e. ceftazidime (30µg), cefotaxime (30µg) and to fourth generation cephalosporins, cefepime (30µg) were screened for ESBL production. The resistant strains were identified for ESBL and MBL production by using phenotypic methods.

**ESBL detection:** ESBL producing isolates were characterized phenotypically for ESBL production using double disc synergy test (DDST) as recommended by the Clinical Laboratory Standards Institute (CLSI)[8]. The test was done by using both cefotaxime (30µg) and ceftazidime (30µg) alone and in combination with clavulanic acid. > 5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was taken as positive result for ESBL production.

**MBL detection:** Among the nonfermenters, *Acinetobacter* isolates which were found to be resistant to Imipenem, Meropenem by Kirby - Bauer disc diffusion method were selected. The resistant isolates were determined and subjected to various phenotypic detection methods such as Combined disc diffusion Test and Double disc synergy test.

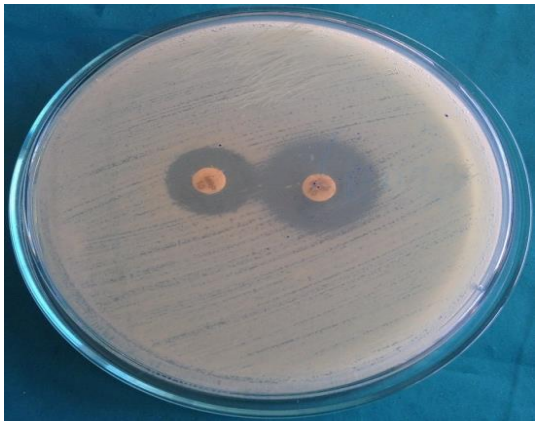
**Combined Disc Diffusion Test (CDDT):** The strain to be tested was inoculated onto MHA plate as suggested by the CLSI. Two (10µg) Imipenem or Meropenem discs were placed on the plate at the distance of 20mm and 10 µl of 0.5 M EDTA solution was added to one of them to obtain the desired concentration (750 µg). After18 hours of incubation, the zone diameter of Imipenem, Meropenem and Imipenem EDTA, Meropenem EDTA discs were compared. The increase in inhibition zone with Imipenem EDTA, Meropenem with EDTA disc ≥5mm than the Imipenem, Meropenem disc alone was considered as MBL positive.

**Figure 1:** Phenotypic confirmatory method for ESBL



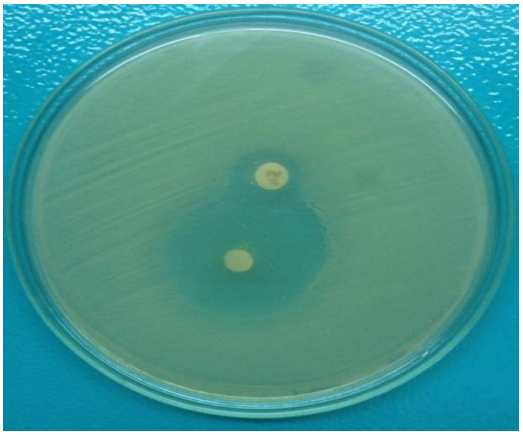

**Double Disc Synergy Test (DDST):** Lawn culture of the test organism was prepared over Mueller-Hinton agar plate as per CLSI guideline. A plain sterile disc was kept 20 mm apart from either Imipenem or Meropenem (10µg) dis. 5µl of EDTA was added to plain disc and incubation was done at 37˚C overnight. Presence of an extended zone from Imipenem or Meropenem disc towards EDTA was interpreted as positive.

**Figure 2:** Imipenem-EDTA combined disc test for MBL detection



**I – Imipenem IE – Imipenem EDTA**

**Figure 3:** Double disk synergy test for MBL detection



## Results

In general ward isolates, Enterobacteriaceae were the commonest (78%) and non-fermenters accounts to 22%. In ICU nonfermenters accounted for 36% and Enterobacteriaceae were 64%. In ICU, maximum resistance to third generation cephalosporins and fluoroquinolones was noted among nonfermenters. In contrary, *Escherichia coli* isolated from general wards showed highest resistance to almost all the drugs except aminoglycosides. Majority of the isolates in both the groups were sensitive to aminoglycosides (80%). ESBL producers in ICU was 80% and in general ward 72%. MBL production among nonfermenters in ICU was 25%. None of the isolates showed MBL production in general wards.

In the present study, high prevalence of ESBL producing Enterobacteriaceae among hospitalised patients was observed and were found to be multidrug resistant and is in agreement with other studies.[5,6]

**Table 1:** Prevalence of Gram negative bacterial isolates from clinical samples of General ward

| Organism | Urine | Blood | Pus | Sputum | Throat swab | Vaginal swab | Total |
|---|---|---|---|---|---|---|---|
| *E.coli* | 10 | - | 5 | 2 | - | 2 | 19 |
| *Klebsiella sp.,* | 6 | - | 5 | 4 | 1 | - | 16 |
| *Pseudomonas sp.,* | 2 | - | 5 | 1 | - | - | 8 |
| *Acinetobacter sp.,* | - | - | 2 | 1 | - | - | 3 |
| *Citrobacter sp.,* | 1 | 1 | 2 | - | - | - | 4 |

**Table 2:** Prevalence of Gram negative bacterial isolates from clinical samples from ICU

| Organism | Urine | Blood | Pus | Sputum | ET tube | Total |
|---|---|---|---|---|---|---|
| E.Coli | 16 | - | - | - | - | 16 |
| Klebsiella sp., | 2 | 8 | - | 2 | 2 | 14 |
| Pseudomonas sp., | 2 | 4 | 4 | - | - | 10 |
| Acinetobacter sp., | - | 6 | 2 | - | - | 8 |
| Citrobacter sp., | - | 2 | - | - | - | 2 |

**Table 3:** Prevalence of ESBL production among bacterial isolates

| Organism | Ward | | | ICU | | |
|---|---|---|---|---|---|---|
| | Total | NO. | % | Total | NO. | % |
| E.Coli | (n=19) | 18 | 94.73 | (n=16) | 12 | 75 |
| Klebsiella | (n=16) | 8 | 50 | (n=14) | 12 | 85.7 |
| Pseudomonas | (n=8) | 4 | 50 | (n=10) | 8 | 80 |
| Acinetobacter | (n=6) | 2 | 66.7 | n=8) | 6 | 75 |
| Citrobacter | (n=4) | 4 | 100 | (n=2) | 2 | 100 |

**Table 4:** Prevalence of ESBL production among various clinical specimens

| Specimen | Ward | | ICU | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Sputum | 3 | 8.3 | 4 | 10 |
| Pus | 14 | 38.9 | 6 | 15 |
| Urine | 16 | 44.4 | 16 | 40 |
| Blood | 1 | 2.8 | 12 | 30 |
| Vaginal swab | 1 | 2.8 | - | - |
| Throat swab | 1 | 2.8 | - | - |
| ET tube | - | - | 2 | 5 |

**Table 5:** Comparison of antimicrobial resistance pattern between General ward and ICU

| Organism | *E. coli* % | | *Klebsiella* % | | *Pseudomonas* % | | *Acinetobacter* % | | *Citrobacter* % | |
|---|---|---|---|---|---|---|---|---|---|---|
| Drugs | Ward | ICU | Ward | ICU | Ward | ICU | Ward | ICU | Ward | ICU |
| AK | 11 | 25 | 31 | 14.3 | 12.5 | 20 | 33.3 | 25 | 75 | 100 |
| CO | 73.7 | 87.5 | 69 | 85.7 | 87.5 | 100 | 66.7 | 75 | 100 | 100 |
| CF | 89 | 100 | 68.8 | 85.7 | 100 | 100 | 100 | 75 | 100 | 100 |
| NX | 70 | 87.5 | 66.7 | - | - | 100 | - | - | 100 | 100 |
| CE | 89 | 75 | 50 | 85.7 | 75 | 80 | 66.7 | 75 | 100 | 100 |
| CI | 95 | 87.5 | 62.5 | 85.7 | 75 | 100 | 66.7 | 75 | 100 | 100 |
| PT | 27.3 | 25 | 15.38 | - | - | - | - | 50 | - | - |

AK-Amikacin, CO-Cotrimixazole, CF-Ciprofloxacin, NX-Norfloxacin, CE-Cefotaxime, CI-Ceftriaxone, PT-Piperacillin Tazobactum

## Discussion

ESBL have become a widespread serious problem. The enzyme production is increasing by many strains of pathogenic bacteria with a potential for dissemination. Presence of ESBL compromises the activity of wide spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients. The continued emergence of ESBL presents diagnostic challenges to the clinical laboratories. In the present study, ESBL producers in ICU was 80% and in general ward 72%. On the contrary in various other studies ESBL production rate varies from 17% to 70% [9-13]. In present study Urine samples (84.4%) were the major source of ESBL producing strains followed by pus, blood and sputum. However, in other studies urine was the major source of ESBL producers [10,14,15]. One of the investigator however, reported blood as major source of ESBL producers[6]. Among various GNB isolated highest ESBL production was observed in *E. coli,* which is in accordance with other studies[10,,15]. In present study high prevalence of ESBL producing *Enterobacteriaceae* among hospitalized patients was observed and is in agreement with findings of other investigators [6,17]. In the present study, high prevalence of ESBL producing enterobacteriaceae among hospitalized patients was observed. MBL production among

nonfermenters in ICU was 25% and were found to be multidrug resistant and is in agreement with other studies[5,6]

In the present study, *Pseudomonas spp*. showed higher resistance to ciprofloxacin. Higher resistance was observed with cephalosporins in all isolates. In the present study all the ESBL isolates were found to be Multi Drug Resistant (MDR). These findings are in agreement with other studies [10,16,19]. Phenotypic tests for ESBL detection need to be confirmed whether an ESBL is produced. Some ESBL may fail to reach a level to be detected by disk diffusion tests but result in treatment failure in the infected patient. In present study high frequency of ESBL positive strains were observed in strains of *E. coli* and were shown to produce ESBLs as investigated by disc diffusion test. Hence it is advisory

## Conclusion

The present study highlights the presence of ESBL and MBL producing bacteria which were multidrug resistant. In view of this emerging drug resistance, practice of routine ESBL and MBL testing along with conventional antibiogram will be useful for all cases which will help in the proper management of the patient and also prevent further development of bacterial drug resistance. This study provides information on antibiotic resistance in different areas of the hospital. It emphasis on the need for each hospital to have a comprehensively drafted and strictly implemented antibiotic policy.

## References


1. Tam VH, Rogers CA, Chang KT, Weston JS, Caeiro JP, Garey KW, Impact of multidrug-resistant Pseudomonas aeruginosa bactremia on patient outcomes, Antimicrob Agents Chemother. 2010 Sep; 54(9):3717-22. doi: 10.1128/AAC.00207-10. [PubMed]

2. Shorr AF. Review of studies of the impact on gram-negative bacterial resistance on outcomes in the intensive care unit. Crit Care Med2009;37;1463-9.

3. Depuydt PO, Vandijick DM,Bekaert MA, Decruyenaere JM, Blot SI, Vogelaers DP, *et al.,* Determinants and impact of multidrug antibiotic resistance in pathogens causing ventilator associated pneumonia. Crit Care 2008; 12: R142.

4. Blot S.Limiting the attributable mortality of nosocomial infection and multidrug resistance in intensive care units. Clin Microbiol infect 2008; 14:5-13.

5. Shanthi M, Sekar U. Extended Spectrum Beta Lactamase Producing *Escherichia coli* and *Klebsiella Pneumoniae*: Risk Factors for Infection and Impact of Resistance on Outcomes. J Assoc Physicians India.2010 December;(58 Suppl):41–4. [PubMed]

6. Vemula S, Vadde R. Prevalence of ESBL-Producing *Klebsiella pneumoniae* Isolates in Tertiary Care Hospital. ISRN Microbiology. 2011; 2011:318–48. [PMC free article] [PubMed]

7. Abhilash KP, Veeraraghavan B, Abraham OC. Epidemiology and Outcome of Bacteremia Caused by Extended Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella Spp*. in a Tertiary Care Teaching Hospital in South India. J Assoc Physicians India. 2010 December; (58 Suppl):13–7. [PubMed]

8. Clinical Laboratory Standards Institute. Performance standards for antimicrobial disc susceptibility tests, IXX international Supplement (M100- S19). Wayne Pa, USA: National Committee for Clinical Laboratory Standards. 2014.

9. Gopalakrishnan R, Sureshkumar D. Changing Trends in Antimicrobial Susceptibility and Hospital Acquired Infections Over an 8 Year Period in a Tertiary Care Hospital in Relation to Introduction of an Infection Control Programme. J Assoc Physicians India. 2010 December;(58 Suppl):25–31. [PubMed]

10. Shanthi M, Sekar U. Extended Spectrum Beta Lactamase Producing *Escherichia coli* and *Klebsiella Pneumoniae*: Risk Factors for Infection and Impact of Resistance on Outcomes. J Assoc Physicians India.2010 December;(58 Suppl):41–4. [PubMed]

11. Mubarak SA, Abida AE, Hajar MA, Adeel IA. Molecular Characterization and Epidemiology of Extended Spectrum Beta Lactamase Producing Escherichia coli and Klebsiella pneumoniae Isolates in the United Arab Emirates. Med Princ Pract. 2011; 20:177–80.

12. Ali AM, Rafi S, Qureshi AH. Frequency of Extended spectrum beta Lactamase producing gram negative bacilli among clinical isolates at clinical laboratories of Army medical college, Rawalpindi. J Ayub Med Coll Abbottabad. 2004 Jan-Mar;16(1):35–7. [PubMed]

13. Patel BV, Patel PG, Raval PN, Patel MH, Patel PH, Vegad MM. Bacteriological profile and antibiogram of gram negative organisms isolated from medical and neurology intensive care unit with special reference to multi-drug resistant organisms. National journal of Medical Research. 2012 July-Sept; 2(3):335–38.

14. Iraj A, Nilufar YN. Antibiogram of Extended Spectrum Beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from Hospital Samples. Bangladesh J Med Microbiol. 2010; 04(01):32–6.

15. Saba R, Muhammad F, Shahida H. Prevalence and comparison of Beta-lactamase producing *Escherichia coli* and *Klebsiella spp*. from clinical and environmental sources in Lahore, Pakistan. African Journal of Microbiology Research. 2012 January;6(2):465–70.

16. Abhilash KP, Veeraraghavan B, Abraham OC. Epidemiology and Outcome of Bacteremia Caused by Extended Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella Spp*. in a Tertiary Care Teaching Hospital in South India. J Assoc Physicians India. 2010 December;(58 Suppl):13–7. [PubMed]

17. Wattal C, Goel N, Oberoi JK, Raveendran RS, Datta S, Prasad K J. Surveillance of Multidrug Resistant Organisms in a Tertiary Care Hospital in Delhi, *India*. J Assoc Physicians India. 2010 Dec;(58 Suppl):32–6. [PubMed]

18. Umadevi S, Kandhakumari G, Joseph NM, Kumar S, Easow JM, Stephen S, *et al.,* Prevalence and antimicrobial Susceptibility pattern of ESBL producing Gram Negative Bacilli. J Clin Diagn Res. 2011 Apr;5(2):236–39.

19. Ava B, Mohammad R, Jalil V, Yousefi Frequency of extended spectrum beta-lactamase (ESBLs) producing *Escherichia coli* and *Klebseilla pneumonia* isolated from urine in an Iranian 1000-bed tertiary care hospital. African Journal of Microbiology Research. 2010 May;4(9):881–84.

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