Prevalence and Characterization of Multi Drug Resistant Gram Negative Bacilli Isolates from a Tertiary Care Centre of Western U.P., India

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Abstract

Infections with MDR GNB are associated with mortality rates 21% higher than those of non resistant GNB and results in longer in patient stays and higher treatment costs. Several Indian studies have reported prevalence of carbapenemase producing Enterobacteriaceae, Pseudomonas and Acinetobacter species in a range of 11% to 81%, because of ample variation reported in prevalence and incidence of carbapenemases reported from different geographical region from time to time, we aimed to determine prevalence of carbapenemase producing organism and carbapenemase encoding genes among clinical MDR-GNB isolates from our area and also to assess the performance of the phenotypic tests. This was a cross sectional study. A total of 510 multi drug resistant isolates included were subjected to MHT and MBL E strip Test to detect carbapenamase production. In addition these isolates were subjected to PCR assay to confirm presence of carbapenamase genes encoding for these enzymes. The study found carbapenemase prevalence of 58.6% by phenotypic tests. blaNDM was the most common gene (24.7%) found by PCR assay followed by blaKPC (14.9%), blaVIM (9.6%) and blaOXA-48 (8.6%). Awareness of the prevalence and incidence of the carbapenem resistance and carbapenemase enzymes is crucial in the prevention of their spread and selection of appropriate treatment options. Study shows high prevalence rate of carbapenam resistant gram negative bacilli in this area, which indicates danger of limited treatment options and requirement of continuous detection of these cases to limit spread of resistant cases.

Keywords: Carbapenemase producing GNB, carbapenem resistant GNB, carbapenemase encoding genes.
INTRODUCTION

Bacterial resistance to anti-microbial treatment is emerging as one of the major public health problem. Carbapenamases may be defined as beta lactamases that significantly hydrolyze at least imipenem or meropenem. Resistant to carbapenams is mostly due to the production of carbapenemases, which are β- lactamase enzymes with a capacity to hydrolyze not only the carbapenams but also all the other beta lactam agents. The most common carbapenemases include verona integron metallo-beta–lactamases types (VIM), imipenemase (IMP) types, Klebsiella pneumoniae carbapenemase (KPC), oxacillinase-48 (OXA-48), and New Delhi metallo-beta-lactamase-1 (NDM-1), encoded by carbapenem resistance determining gene bla\text{VIM}, bla\text{IMP}, bla\text{KPC}, bla\text{OXA-48} and bla\text{NDM} respectively. Phenotypic assay are used to identify carbapenemase activity while molecular assay have been developed to identify carbapenemase encoding genes. Recently, increasing resistance to carbapenams in health care associated infections has been reported worldwide. Resistance to carbapenams becomes a real threat to the survival of patients with infections caused by MDR-GNB as mortality in such infections has been reported up to 50% who acquire blood stream infections and overall mortality rates are 21% higher than those of non resistant GNB and results in longer in patient stays and higher treatment costs. Several Indian studies have reported prevalence of carbapenemase producing Enterobacteriaceae, Pseudomonas and Acinetobacter species in a range of 11% to 81%. This study set out to determine the burden of carbapenam resistance, prevalence of carbapenemase producing organism and carbapenemase encoding gene among clinical MDR-GNB isolates obtained from patients. We aimed to determine performance of Modified Hodge test (MHT) and Metallo β-lactamase (MBL) E Test by comparing them with results of Polymerase chain reaction (PCR) assay.

MATERIALS AND METHODS

Study design and setting

This was a cross sectional laboratory based prospective study which was carried out in Microbiology department of Teerthankar Mahaveer Medical College and Research Centre, Moradabad, Uttar Pradesh, India, during the period of April 2016 to December 2018.

Sample collection

A total of 2562 non-duplicate samples from patients suspected of infection caused by Gram-negative bacteria like Urine, Pus, Blood, Body fluids, Tracheal secretion, Sputum, HVS, Foley’s Tip etc were collected as per standard sample collection technique reported earlier.

Collected samples were subjected to conventional methods i.e. Gram staining, Culture, Biochemical tests. Out of 2562 samples 1507 were gram negative and out of 1507 gram negative bacterial isolates 510 were Multi Drug Resistant. Drug susceptibility test was done by Kirby Bauer disk diffusion method with following antibiotic disks Imipenem (IPM) 10µg, Meropenem (MRP) 10µg, Ertapenem (ETP) 30µg, Cefixime (CFM) 5mg, Cefepime (CPM) 30mg, Ceftazidime(CAZ) 30µg, Ceftazidime/Clavulanic Acid (CAC) 30µg, Ceftriaxone (CTX) 30µg, Cefoperzone/Subactum (CFS) 30µg, Ciprofloxacin (CIP) 5µg, Piperacillin/ Tazobactam (PIT) 10µg, Amikacin (AK) 30µg, Tigecycline (TGC) 15µg and interpreted according to CLSI guidelines. This was in order to find out MDR Strain and also to find relation between resistance to these drugs and carriage of carbapenemase gene. Multi Drug Resistant (MDR) strains were differentiated according to criteria given by Mattner et al. In brief, isolates that were resistant to three different classes of antibacterials but sensitive to carbapenems were included and isolates that were resistant to any one carbapenem but sensitive to other anti-bacterials were also included.

Detection of carbapenemase production

Phenotypic detection of carbapenemase production was done by MHT and MBL E Test. MHT was performed and interpreted according to guideline provided by CDC. MBL E test strip were obtained by HiMedia Pvt. Ltd and test was performed and interpreted according to kit insert provided with kit.

PCR amplification for carbapenemase genes

The entire molecular / PCR test (DNA extraction, amplification and gel electro-phoresis) were done in molecular laboratory of Subharti Medical College, Meerut.

DNA extraction was done using Qiagen DNeasy blood and tissue kit following
manufacturer’s instructions, for reaction mixture preparation, commercially available Genei® Master Mix kit was used. Manufacturer’s instruction manual was followed for using the kit. Primers of PrimeX targeting bla\textsubscript{VIM}, bla\textsubscript{KPC}, bla\textsubscript{OXA-48} and bla\textsubscript{NDM} were obtained from Valine Life Sciences, India, as described in study by Asthana S et al. For reaction mix preparations following contents were added Molecular grade water 15µl, Master Mix in kit 25µl, Primers 0.2µl and Template DNA 10µl. The amplification was done using Applied Biosystem Veriti 96 well thermal cycler. For bla\textsubscript{KPC}, bla\textsubscript{OXA-48} and bla\textsubscript{NDM} the programme was initial denaturation at 94°C for 5 minutes followed by 30 cycles of 30 seconds denaturation at 94°C, annealing at 55°C for 30 seconds and extension at 72°C for 1 minute. An additional final extension step was performed at 72°C for 7 minute. For bla\textsubscript{VIM} the same programme was used except that annealing temperature was adjusted to 45°C for 60 seconds and had a final extension step of 72°C for 10 minutes.

5µl of PCR product were analyzed by electrophoresis in 1.0% agarose stained with ethidium bromide to detect the specific amplified product by comparing with 100 base-pair standard DNA ladder and visualized under gel doc. system. For quality control, of MHT well characterized strains were used. E. coli ATCC 25922 was used as a susceptible strain, K. pneumoniae ATCC BAA-1705 as a positive control while K. pneumoniae ATCC BAA-1706 was used as a negative control and for PCR tests, the following control strains were used; K. pneumoniae ATCC strain BAA-1705 for bla\textsubscript{KPC}, K. pneumoniae ATCC strain BAA-2146 for bla\textsubscript{NDM} and E. coli ATCC BAA-2523 for bla\textsubscript{OXA-48}.

RESULTS

Distribution and Characteristics of Isolates Included in study

A total of 2562 samples were processed during study period of April 2016 to December 2018 in which 1507 were gram negative bacilli and out of 1507 gram negative bacterial isolates 510 were Multi Drug Resistant. MDR strains were differentiated according to criteria given by Mattner et al. most of which were resistant to three different classes of anti-bacterial. Overall 25.1% isolates were resistant to one or more carbapenem tested. Individually, 11%, 8.1%, 6% resistant rate was observed by Imipenem, Ertapenem and Meropenem respectively. Out of imipenem, ertapenem and meropenem resistant isolates genes were present in 59% 64%, and 68.8% isolates respectively. 132 isolates were resistant to three different classes of anti-bacterial but sensitive to carbapenems tested. Distribution of antibiotic resistant isolates is shown in Table 1. Out of 510 MDR strains 267 were from male and 243 from female patients. The age of patients ranged from 1day to 82 years with a median of 35 years. Most common species isolated among MDR-GNB was E. coli (34.7%, 177/510) followed by K. pneumoniae (18.2%, 93/510) and P. aeruginosa (9.4%, 48/510). It was also observed that highest frequency of E. coli was from Gyne ward and that of K. pneumoniae was from Medicine ICU. Distribution of isolates from various sources is shown in Table 2. Highest numbers of MDR organisms were from MICU followed by SICU. Most common sample was Urine (140/510) followed

Table 1. Cross Tabulation showing total drug resistant isolates and number of isolates with gene detected

| Drug Resistant | Gene Detected | No gene Detected | Total Isolates |
|---------------|---------------|------------------|---------------|
| Imipenem      | 92            | 74               | 166           |
| Ertapenem     | 75            | 47               | 122           |
| Meropenem     | 57            | 33               | 90            |
| Carbapenem resistant MDR-GNB | 224 | 154 | 378 |
| Carbapenem sensitive MDR-GNB | 17 | 115 | 132 |

Total | 241 | 269 | 510 |
by Pus (113/510) and Blood (85/510). Majority of Urine samples was received from FMW, Gyne and MMW. Majority of Pus samples were from MMW and Blood samples were from Medicine ICU and Surgical ICU as shown in Table 3.

**Prevalence of Carbapenemase producing organism based on MHT and MBL E-test**

Carbapenemase activity was detected in 15.6% (80/510) isolates by MHT method, 23.5% (120/510) by MBL E test method. 19.4% (99/510) isolates were positive for both tests. Therefore, total number of isolates positive by MHT was 179/510 (35.0%) and by E test was 219/510 (42.9%) correlation of phenotypic results with

Table 2. Shows Ward wise Distribution of Species isolated

| Wards   | E. coli | K. pneumoniae | P. aeruginosa | A. lwoffi | C. freundi | E. aerogenes | Other Organism* | Total |
|---------|---------|---------------|---------------|-----------|------------|--------------|----------------|-------|
| ICCU    | 02      | 01            | —             | 01        | —          | —            | —              | 04    |
| MICU    | 14      | 14            | 04            | 09        | 09         | —            | 13             | 63    |
| NEU. ICU| 03      | 02            | 03            | 06        | 02         | 01           | 09             | 26    |
| NICU    | 05      | 05            | —             | 01        | —          | 02           | 05             | 18    |
| PICU    | 01      | —             | 01            | —         | 02         | 01           | 02             | 06    |
| SICU    | 14      | 07            | 07            | 06        | 05         | 03           | 07             | 49    |
| ENT     | —       | —             | 01            | —         | —          | —            | 02             | 03    |
| FMW     | 18      | 08            | 01            | 01        | 01         | 01           | 05             | 34    |
| FOW     | 01      | —             | —             | —         | —          | —            | 01             | 02    |
| FSW     | 06      | 04            | —             | —         | 01         | 01           | 05             | 17    |
| GYNE    | 22      | 02            | —             | 01        | 01         | 01           | 04             | 31    |
| L/R     | 06      | 03            | —             | 01        | —          | 01           | 01             | 12    |
| OBS     | 14      | 03            | 01            | 03        | 05         | 03           | 05             | 34    |
| MMW     | 14      | 07            | 03            | —         | —          | 01           | 02             | 27    |
| MOW     | 07      | 08            | 09            | 05        | 02         | 03           | 04             | 38    |
| MSW     | 13      | 09            | 06            | —         | 03         | 03           | 06             | 40    |
| URO     | 01      | —             | —             | —         | —          | —            | —              | 01    |
| OPD     | 09      | 05            | 01            | 01        | —          | 04           | 09             | 29    |
| PEDIA   | 04      | 02            | —             | 01        | —          | —            | 01             | 08    |
| PVT.    | 05      | 03            | 01            | —         | —          | 01           | 01             | 11    |
| TBC     | 05      | 03            | 05            | 04        | 01         | 01           | 08             | 27    |
| E/W     | 15      | 05            | 05            | 02        | —          | 01           | 01             | 29    |
| TOTAL   | 177     | 93            | 48            | 41        | 32         | 28           | 91             | 510   |

Others Organism Include K. oxytoca (n=21), E. cloacae (n=17), C. koseri (n=13), P. mirabilis (n=4), P. vulgaris (n=3), Pseudomonas Species (n=29), A. baumannii Complex (n=4)

ICCU: Intensive Critical care Unit, MICU: Medicine Intensive care Unit, Neu ICU: Neuro Intensive care Unit, NICU: Neonatal Intensive care Unit, PICU: Paediatric Intensive care Unit, SICU: Surgical Intensive care Unit, FMW: Female Medicine Ward, FOW: Female Orthopaedic Ward, FSW: Female Surgery Ward, L/R Labour Room, MMW: Male Medicine Ward, MSW: Male Surgery ward, URO: Urology Ward, PVT.: Private ward, OPD: Out Patient Department, E/W: Emergency ward

that of number of gene detected is shown in Table 4. Among 510 MDR isolates total 299 were carbapenemase producers by phenotypic methods and 211 were non carbapenemase producers. Table 5.

**Prevalence of genes encoding for Carbapenemase enzymes**

Based on PCR assays 47.2% (241/510) of the isolates were positive for one or more gene. E.coli was species with highest number of gene detected, whereas percentage wise highest rate of gene among MDR isolates were detected in E. cloacae (64.7%) followed by C. freundi (62.5%). 82 out of 177 MDR E. coli strains were positive for one or more gene followed by K. pneumoniae (40/93, 43%), A. lwoffi (25/41, 60.9%), P. aeruginosa (20/48, 37.9%) and C. freundi (20/32, 62.5%). 82 (16.07%) isolates had presence of one or
more genes. Distribution of gene in various MDR isolates is shown in Table 6. All target genes were unevenly distributed among the isolated species with overall highest prevalence of \( \text{bla}\,_{\text{NDM}} \) (24.7%) followed by \( \text{bla}\,_{\text{KPC}} \) (14.9%), \( \text{bla}\,_{\text{VIM}} \) (9.6%) and \( \text{bla}\,_{\text{OXA}} \) (8.6%). Species wise distribution of genes detected is shown in Table 7.

### Correlation of phenotypic and genotypic tests
Out of 80 isolates positive by MHT 51 isolates showed presence of one or more gene and in 29 isolates no gene was detected. Out of 120 isolates positive by MBL E test genes were detected in 104 samples and in rest of 16 isolates which were phenotypically positive but no gene

### Table 3. Distribution of type of samples obtained from different wards

| Wards  | Urine | Pus | Blood | Sputum | ET. Secr. | Other Samples | Total |
|--------|-------|-----|-------|--------|-----------|----------------|-------|
| ICCU   | 02    | —   | —     | 01     | —         | 01             | 04    |
| MICU   | 08    | 01  | 20    | 07     | 20        | 07             | 63    |
| NEU. ICU | 02 | —     | 02    | 01     | 18        | 03             | 26    |
| NICU   | —     | —    | 10    | —      | —         | 08             | 18    |
| PICU   | —     | —    | 04    | —      | —         | 03             | 07    |
| SICU   | 07    | 09  | 16    | 02     | 12        | 03             | 49    |
| ENT    | —     | 03  | —     | —      | —         | —              | 03    |
| FMW    | 21    | 02  | 05    | 02     | —         | 04             | 34    |
| FOW    | —     | 01  | —     | 01     | —         | —              | 02    |
| FSW    | 04    | 07  | 02    | —      | 02        | 02             | 17    |
| GYNE   | 22    | 05  | —     | —      | —         | 04             | 31    |
| L/R    | 03    | —   | —     | —      | —         | 09             | 12    |
| OBS    | 16    | 05  | —     | —      | —         | 13             | 34    |
| MMW    | 21    | —   | 02    | 02     | —         | 02             | 27    |
| MOW    | —     | 35  | 02    | 01     | —         | —              | 38    |
| MSW    | 05    | 18  | 07    | 03     | 03        | 04             | 40    |
| URO    | 01    | —   | —     | —      | —         | —              | 01    |
| OPD    | 13    | 15  | 01    | —      | —         | —              | 29    |
| PEDIA  | 02    | 03  | 03    | —      | —         | —              | 08    |
| PVT.   | 03    | 03  | 02    | 01     | 01        | 01             | 11    |
| TBC    | 01    | 03  | —     | 13     | —         | 10             | 27    |
| E/W    | 09    | 03  | 09    | 08     | —         | —              | 29    |
| TOTAL  | 140   | 113 | 85    | 42     | 56        | 74             | 510   |

*Other Samples Include: - BAL (n=12), Bronchial Biopsy specimen (n=2), Catheter Tip (n=1), CSF (n=2), D&E Specimen(n=1), Drain Fluid (n=3), Peritoneal Fluid (n=7), Rectal Swab (n=7), Vaginal Swab (n=3), Pleural Fluid (n=1), Foley’s Tip (n=18), HVS (n=17)

### Table 4. Cross tabulation of results of phenotypic tests with gene detection

| MHT Result | MBL E-test Result | Total no. of Samples | Number of samples in which Gene found | Number of samples in which gene not found |
|------------|-------------------|----------------------|---------------------------------------|------------------------------------------|
| Positive   | positive          | 99                   | 86                                    | 13                                       |
| Positive   | negative          | 80                   | 51                                    | 29                                       |
| Negative   | positive          | 120                  | 104                                   | 16                                       |
| Negative   | negative          | 211                  | nil                                   | 211                                      |
| Total number of sample Processed | | 510 | 241 | 269 |


was detected in them. 99 isolates which were positive for both test MHT and MBL E test 86 were positive for gene detection and in 13 isolates no gene was detected. Table 4

Sensitivity and specificity of Modified Hodge test and MBL E test was calculated considering PCR as gold standard. MHT gave better performance for detection of Class A and Class D genes, sensitivity and specificity for \( \text{bla}_{KPC} \) was 93.4% and 75.1% and sensitivity and specificity for \( \text{bla}_{OXA-48} \) calculated was 84.7% and 69.8% whereas MBL E test is better for MBL detection, sensitivity and specificity for \( \text{bla}_{NDM} \) was 99.2% and 78.3%. Overall sensitivity and specificity of MHT found was 56.8% and 78.8% and sensitivity and specificity of E-test was 82.4% and 86.7% respectively. Statistically it was also evident that MBL E Test had strong association with detection of \( \text{bla}_{NDM} \) and \( \text{bla}_{VIM} \) (p<0.05) and MHT showed a good association with detection of \( \text{bla}_{KPC} \) and \( \text{bla}_{OXA-48} \) genes. (p<0.05).

**DISCUSSION**

Antibiotic resistance to reserve antibiotic class is on a continuous rise among gram negative bacteria especially in the family Enterobacteriaceae and among species of Acinetobacter and Pseudomonas (EPA Species)

Recently, a newspaper article reported 13% of mortality rate in India is due to antibiotic resistant cases, which is more than double when compared to developed nations where mortality rate due to drug resistant cases is 2-7%\(^1\). Worldwide several studies had reported increased prevalence of carbapenemase producing organisms\(^5,19,20\). Our findings show out of 510 MDR strains 52.3% were from males and 47.6% were from females. Ratio of male to female patient in this study was 1:1.09 this shows almost equal distribution of Antibiotic resistant strains among both sexes. We found most common MDR organism was \( E. coli \) followed by \( K. pneumoniae \) and \( P. aeruginosa \) and the hotspot zone of these organism were medical and surgical ICU’s. Similar findings were reported by Mathias \textit{et al}. from Ludhiana, Diwakar J. \textit{et al}. from Etawah and Manohar \textit{et al}. from Tamil Nadu region\(^8,9,21\). These findings may have vital role in making of hospital infection control policy. This study shows a prevalence rate of carbapenemase enzyme of 58.6% by phenotypic tests among EPA species.

| Table 5. Distribution of Number of Carbapenemase Producing and Non Carbapenemase producing isolates from different wards |
| --- |
| ICU’S | CPP | CPN | Total Isolates |
| MICU | 44 | 19 | 63 |
| SICU | 34 | 15 | 49 |
| PICU | 3 | 4 | 7 |
| NICU | 6 | 12 | 18 |
| NEURO ICU | 20 | 6 | 26 |
| ICCU+ CCU | 3 | 1 | 4 |
| Total | 110 | 57 | 167 |

| WARDS | CPP | CPN | Total |
| --- |
| MSW | 26 | 14 | 40 |
| MOW | 19 | 19 | 38 |
| MMW | 12 | 15 | 27 |
| FMW | 20 | 14 | 34 |
| FSW | 8 | 9 | 17 |
| FOW | 1 | 1 | 2 |
| GYNE | 15 | 16 | 31 |
| L/R | 8 | 4 | 12 |
| OBS | 18 | 16 | 34 |
| E/W | 19 | 10 | 29 |
| PEDIA | 4 | 4 | 8 |
| ENT | 3 | 0 | 3 |
| URO | 1 | 0 | 1 |
| TBC | 14 | 13 | 27 |
| PVT. | 7 | 4 | 11 |
| Total | 175 | 139 | 314 |

| Table 6. Species wise distribution of Gene detected in number of MDR isolates |
| --- |
| Organism Isolated | Total Number of MDR organisms | Genes Detected In |
| --- |
| \( E. cloacae \) | 17 | 11(64.7%) |
| \( C. freundii \) | 32 | 20(62.5%) |
| \( A. Iwoffii \) | 41 | 25(60.9%) |
| \( K. oxytoca \) | 21 | 12(57.1%) |
| \( P. mirabilis \) | 4 | 2(50%) |
| \( E. coli \) | 177 | 82(46.3%) |
| \( C. koseri \) | 13 | 6(46.1%) |
| \( K. pneumoniae \) | 93 | 40(43.0%) |
| \( P. aeruginosa \) | 48 | 20(41.6%) |
| \( E. aerogenes \) | 28 | 11(39.2%) |
| \( Pseudo. Spp \) | 29 | 11(37.9%) |
| \( Acb complex \) | 4 | 1(25%) |
| \( P. vulgaris \) | 3 | 0(0%) |
| Total | 510 | 241 |
### Table 7. Distribution of different Carbapenemase genes detected in various Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* species.

| Group          | Organism     | NDM | KPC | VIM | OXA | NDM, KPC | NDM, VIM | NDM, OXA | KPC, VIM | KPC, OXA | VIM, OXA | NDM KPC | NDM VIM | NDM OXA | KPC OXA | NO. isol. |
|----------------|--------------|-----|-----|-----|-----|----------|----------|----------|----------|----------|----------|----------|---------|---------|---------|----------|
| Enterobacteria  | *E. coli*    | 28  | 14  | 10  | 11  | 06       | 04       | 02       | 05       | 01       | 01       | 02       | 01      | 01      | 02      | 82       |
|                | *K. pneumoniae* | 15  | 07  | 08  | 01  | 04       | 01       | 01       | 02       | —        | —        | —        | 01      | 01      | —       | 40       |
|                | *K. oxytoca*  | 06  | 01  | —   | 01  | 02       | 01       | 01       | —        | —        | —        | —        | —       | —       | —       | 12       |
|                | *E. aerogenes* | 06  | 01  | —   | 01  | —        | 02       | —        | —        | —        | —        | —        | 01      | —       | —       | 11       |
|                | *E. cloacae*  | 04  | 00  | —   | 01  | 02       | 01       | 02       | 01       | —        | —        | —        | —       | —       | —       | 11       |
|                | *C. freundii* | 10  | 01  | 01  | 01  | 02       | —        | 02       | 01       | —        | —        | —        | 01      | 01      | —       | 20       |
|                | *C. koseri*   | 02  | 01  | —   | —   | —        | 01       | —        | —        | —        | —        | —        | —       | —       | 01      | 06       |
|                | *P. mirabilis* | 01  | 01  | —   | —   | —        | —        | —        | —        | —        | —        | —        | —       | —       | —       | 02       |
|                | *P. vulgaris* | —   | —   | —   | —   | —        | —        | —        | —        | —        | —        | —        | —       | —       | —       | —         |
|                | Total         | 72  | 26  | 19  | 16  | 16       | 06       | 06       | 05       | —        | 03       | 01      | 02      | 02      | —       | 184      |
| Pseudomonas     | *P. aeruginosa* | 08  | 05  | 04  | 01  | 01       | —        | —        | —        | —        | 01       | —       | —       | —       | —       | 20       |
|                | *Pseudo. spp* | 05  | 02  | 03  | —   | —        | —        | —        | —        | —        | 01       | —       | —       | —       | —       | 11       |
|                | Total         | 13  | 07  | 07  | 01  | 01       | —        | —        | —        | —        | —        | —       | —       | —       | —       | 31       |
| Acinetobacter   | *A. lwoffi*   | 11  | 04  | 02  | 02  | 01       | —        | 01       | —        | 02       | —        | —        | 02      | —       | —       | 25       |
|                | *Acb Complex* | 01  | —   | —   | —   | —        | —        | —        | —        | —        | —        | —        | —       | —       | 01      | 26       |
|                | Total         | 12  | 04  | 02  | 02  | 01       | —        | 01       | —        | 02       | —        | —        | 02      | —       | —       | 26       |
| Grand Total     |              | 97  | 37  | 28  | 19  | 18       | 06       | 11       | 06       | 07       | 02       | 03      | 03      | 02      | 02      | 241      |
resistant to three different classes of antibacterials or resistant to any one of the carbapenem tested. Our phenotypic prevalence is lower than that reported by Diwakar et al. from Etawah, Mate et al. from Imphal and Saini et al. from Jaipur who reported phenotypic prevalence of 81.8%, 60% and 83% respectively⁹,¹⁰,¹², whereas, higher as compared to that reported by Mathias et al. from Ludhiana, Gupta et al. from Varanasi and Singh et al from Navi Mumbai⁸,²³,²⁴. They reported phenotypic prevalence of 57%, 50% and 43.7% respectively. The difference in these findings could be because of variation in geographical regions which occur from time to time and also because of different inclusion criteria and test done. Our prevalence is also much higher than that reported in studies from western countries like United States, Canada, and Latin America¹⁹,²⁵,²⁶. These differences may be due to restricted use of antibiotics in these countries compared to India where many drugs are available over the counter without prescription of a clinician. In parallel, we found prevalence of genes encoding for carbapenemase was 47.2%. Variable rate of genotypic prevalence has been reported by various Indian studies ranging from 18% to 100%⁷-¹¹,²³. The difference might be due to different target genes as in some studies only single class of gene was targeted whereas in our study common genes of all classes of carbapenemase were included. The most prevalent gene among 510 MDR GNB isolates was blaNDM (27.4%) This was in accordance to studies elsewhere in India viz., Delhi, Guwahati, Mumbai, Vellore and Puducherry reporting blaNDM as the commonest gene detected²⁷-²⁹. Although in western world most common gene encoding carbapenemase found is blaKPC (27.4%). This was in accordance to studies elsewhere in India viz., Delhi, Guwahati, Mumbai, Vellore and Puducherry reporting blaKPC as the commonest gene detected²⁷-²⁹. Although in western world most common gene encoding carbapenemase found is blaNDM. Whereas a study from Africa reported highest prevalent gene were blaIMH types while another one reported blaVIM as the most common gene encoding for carbapenemase enzyme¹-². These findings are suggestive of inter-regional spread of the specific mechanism of carbapenem resistance. In our study we found 58(11.3%) samples were phenotypically positive but no gene was detected in them by PCR. This might be due to limited number of genes targeted in our study as well as to other mechanisms of resistance such as porin loss or mutations.

When we compared the performance of phenotypic tests to results obtained by PCR, it was found that Modified Hodge test was more sensitive and specific for Class A enzyme i.e. blaKPC (93% sensitive and 75.11% specific) and Class D enzyme i.e. blaOXA whereas MBL E test performed better for detection of Class B enzymes i.e. blanDM and blavIM. Similar results were reported by Girlich et al.³¹

CONCLUSION

Carbapenemases are globally distributed and their prevalence and incidence vary considerably across each continent, nation, region and even centre to centre, so awareness of the prevalence and incidence of the carbapenem resistance and carbapenemase enzymes is crucial in the prevention of their spread and selection of appropriate treatment options. Our study shows high prevalence rate of carbapenemase producing gram negative bacilli in this area, which indicates danger of limited treatment options and requirement of continuous detection of these cases to limit spread of resistant cases. We also found that combination of two phenotypic tests MHT and E strip Test can be done together to rule out false negative results whereas E Test should be done on regular basis to detect MBL as MBL encoding genes were more prevalent in our region as it is not feasible to do PCR on regular basis on every sample.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.
AUTHOR’S CONTRIBUTION
SM performed the tests, collected data, did data analysis and wrote the manuscript. UF guided the study and reviewed the manuscript. SM and UF approved it for publication.

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DATA AVAILABILITY
All datasets generated or analysed during this study are included in the manuscript.

ETHICS STATEMENT
The study protocol was carefully reviewed and approved by the Institutional Ethics Committee of the Teerthankar Mahaveer Medical College and Research Centre, Moradabad U.P. India.

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