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# Occurrence Of Metallo-B Lactamase Among Gram-Negative Bacilli In A Tertiary Care Hospital & Their Antibiogram Patterns

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***Abstract:***
*Metallo-beta-lactamases (MBLs) are enzymes that confer resistance to a broad spectrum of beta-lactam antibiotics, including carbapenems, which are often considered the last line of defense against multidrug-resistant bacterial infections. The emergence of MBL-producing bacteria has significantly compromised treatment options, particularly for Gram-negative pathogens, which are increasingly resistant worldwide. This study investigates the prevalence of MBL-producing Gram-negative bacilli in a tertiary care hospital in India, aiming to provide insights into their distribution and resistance patterns. A total of 150 Gram-negative bacterial isolates were obtained from various clinical specimens, including urine, pus, blood, sputum, and stool. Antimicrobial susceptibility was assessed using the Kirby-Bauer disc diffusion method. MBL production was detected using the imipenem-EDTA combined disk test and the double disk synergy test. Among the isolates, Escherichia coli was the most frequently identified organism (50%), followed by Klebsiella spp. (20.66%) and Pseudomonas aeruginosa (10%). While 82.22% of isolates remained sensitive to imipenem, resistance to ampicillin was the highest (85.9%). MBL production was confirmed in 30 isolates, with a positivity rate of 63.33% using the combined disk test. The notable prevalence of MBL-producing Gram-negative bacilli underscores a serious challenge in antimicrobial therapy. These findings emphasize the urgent need for ongoing surveillance, robust infection control strategies, and prudent antibiotic stewardship in healthcare settings to curb the spread of multidrug-resistant organisms.*
***Keyword:*** *Nosocomial Infection, Metallo-β Lactamase,Antibiogram Pattern, Gram negative bacilli*

**Background**
Metallo-beta-lactamase (MBLs) is an enzyme that makes bacteria resistant to beta-lactam antibiotics, including carbapenem family. The spread of MBLs severely limits therapeutic options for infection by pathogens. MBLs are produced by many Gram negative and some Gram positive bacteria, including Staphylococcus saprophyticus, Klebsiella spp., proteus spp., Enterococcus spp., and enterobacter spp. The presence and spread of MBL positive strains have been observed worldwide and in India. The most common acquired MBLs include IMP, VIM, SPM, GIM, SIM, and NDM-1. In India, the prevalence of MBLs ranges from 7.5% to 71%. Acinetobacter spp. has emerged as the main cause of nosocomial infection, while Klebsiella pneumoniae is an important nosocomial pathogen causing urinary tract and bloodstream infections. The global spread of bla NDM in GNB is predominantly related to international

travel and medical tourism, but horizontal gene transfer in endemic areas occurs outside hospitals and has been linked to household transmission and exposure from environmental sources[1-10].

Mehvash Haider et al. found that 88% of gram-negative bacteria are resistant to carbapenems, a common treatment for burn wound infections. Carbapenems are used as the last resort for treating beta-lactamase-resistant bacteria, but they face danger from emerging carbepenemases, particularly Metallo beta lactamases (MBLs). MBLs act on penicillin, cephalosporins, and carbapenems but not on monobactams. They differ from other β-lactamases by using metal ions zinc to react with the carbonyl group of the amide bond of most penicillin, cephalosporins, and carbapenems. Amp C β-lactamases confer resistance to cephalosporins and are not affected by available beta-lactamases inhibitors. Detecting MBL-producing clinical isolates is challenging due to their ability to hydrolyze all beta-lactamases, including carbapenem. Early detection of MBL-positive isolates can help prevent further spread, save antibiotics, and reduce financial burdens for patients.[11-26]

Today, hundreds of different ß-lactamase has evolved with them and hundreds of different ß-lactamase has been identified. Genes encoding ß-lactamase enzymes are found on the chromosome and on plasmids. Plasmid-mediated ß-lactamase genes are the most common ones as they can be transferred to other Gram-negative bacteria by conjugation. Many Gram-negative bacilli possess naturally occurring chromosomal mediated ß-lactamase[27].

Today, it is known that the major factor causing ß-lactamase production by pathogens is the widespread clinical use of ß-lactamse[28].

## MATERIAL AND METHOD:

**Study Design:** Cross sectional study.

**Place of study:** The present study was conducted in the Department of Microbiology, Santosh Medical College & Hospital, Ghaziabad.

**Sample size:** 150 isolates of Gram-negative bacteria were collected from randomly clinical samples of urine, pus, blood, sputum, stool and body fluid from IPD and OPD of intensive care unit(ICU) were included in this study.

**Inclusion criteria:** All Gram negative Bacilli isolate with pure growth pattern on culture plate. Gram negative Bacilli isolate showing resistance to any of the third generation cephosporins antibiotics for MBL production. Gram Negative Bacilli isolate showing resistance to Imipenem for production of MBLs.

**Exclusion Criteria:** Organisms identified other than Gram Negative Bacilli were not considered.

**Specimen**: All specimen were considered potentially infectious according to Universal safety precaution, Body fluid such as cerebrospinal fluid (CSF), pleural fluid, sputum, pus, urine and stool were collected in suitable sterile container.

**Sample processing:** This is followed by direct microscopy by gram staining.

**Inoculation to the media**: All specimens were inoculated on MacConkey agar and Blood agar. As per the recommended procedure urine sample was inoculated on Cystine Electrolyte Deficient agar (CLED). All inoculated plates were incubated aerobically at $37^0$C for 24 hours. The blood culture specimen was inoculated in blood culture bottle and incubated aerobically at $37^0$C in two automated blood culture systems. On receipt of positive signal, the particular specimen was sub cultured from the blood culture broth onto blood and MacConkey agar and incubated as aerobically at $37^0$C for 24 hours. The composition of Blood Agar is Peptone,1gm; Sodium chloride,0.5gm; Beef extract,1gm; Distilled water,100ml; Agar,1.5gm; Defibrinated sheep blood,5-10ml. When the agar base is cooled to $50^0$C, add sterile Blood agar aseptically and mix well gently. Dispense 15 ml amount to sterile petri plates aseptically. The composition of MacConkey agar used in the study is Peptone,2gm; Sodium taurocolate,0.5gm; Lactose,1gm (10% aqueous solution 10ml); Distilled water,100ml; Agar,1.5gm; Neutral red 2% in 50% ethanol,0.35ml. Mix well before pouring into sterile Petri plate.

**Identification by colony morphology, motility and biochemical properties:** Specific identification of bacterial pathogens was based on Colony characteristics, Staining characteristics, microscopic morphology, Motility and biochemical properties using standard laboratory criteria. The characteristics

of each colony type were noted. Colonies were described according to from size, shape, surface, elevation, edge, colour, density, consistency, haemolysis, pigmentation production.

**Antimicrobial sensitivity Test:** Antimicrobial Sensitivity test was done by using Kirby Bauer disc diffusion method. **Preparation of Inoculum:** Using a sterile inoculation loop, touch four or five isolated colonies are picked the organism to be tested. Suspend the organism in 5 ml of sterile peptone water. Incubate the suspension for 2 hours at 37°c. Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy. **Inoculation of the Muller-Hinton Agar (MHA) plate:** Dip a sterile swab into the inoculums tube. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet. Inoculate the dried surface of an MHA plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure even distribution of the inoculum. Rim the plate with the swab to pick up any excess liquid. Discard the swab into an appropriate container. Keep the plate for 3-5 minutes at room temperature to dry the plate surface. **Placement of the antibiotic disks:** Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using sterile forceps. Forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug beings to diffuse immediately upon contact with the agar. To add disks one at a time to the agar plate using forceps, place the MH plate on the template provided in this procedure Sterilize the forceps by cleaning them with a sterile alcohol them igniting. Using the forceps carefully remove one disk from the cartridge Partially remove the lid of the petri dish. Place the disk on the plate over one of the dark spots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air. Continue to place one disk at a time onto the agar surface until all disk should not be placed closer than 24 mm (center to center) on the MHA plate. Once all disk are place, replace the lid, invert the plates, and place them in a 37°c air incubator for 16 to 18 hours. Results can be read after 18 to 24 hours of incubation. **Measuring zone sizes:** Following incubation, measure the zone sizes to the nearest millimeter using a ruler or caliper, include the diameter of the disk in the measurement. When measuring zone diameters, always round up to the next millimeter. All measurements are made with the unaided eye while viewing the back of the petri dish. Hold the plate a few inches above a black, non-reflecting surface illuminated with reflected light. View the plate using a direct, vertical line of sight to avoid any parallax that may result in misreading. Record the zone size on the recording sheet. If the placement of the disk or the size of the zone does not allow read the diameter of the zone, measure from the center of the disk to a point on the circumference of the zone where a distinct edge is present (the radius) and multiply the measurement by 2 to determine the diameter. Growth up to the edge of the disk can be reported as a zone of 0 mm. **Interpretation and reporting of the result:** Using the published CLSI guidelines, noted the susceptibility or resistance of Klebsiella pneumoniae. For each drug, noted on the recording sheet whether the zone size is within the susceptible (S), intermediate (I), or resistant (R) range based on the interpretation chart.

**MBL Detection: imipenem (imp) edta combined disk test:** The strains which showed resistance to imipenem were further examined for the detection of MBL production using EDTA (750 µg) combined disk. The IMP-EDTA combined disk test was performed as described by Young et al. **Preparation of EDTA solution:** A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium Ethylene diamine tera acetic acid (EDTA) in 1000 ml of distilled water and adjusting it to pH 8.0 by using NaOH and the mixture was sterilized by autoclaving. **Preparation of inoculum:** Using a sterile inoculating loop or needle, touch four or five isolated colonies are picked. Suspend the organism in 5 ml of sterile peptone water. Incubate the suspension for 2 hours at 37°c. Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy. **Inoculation of the MHA plate:** Dip a sterile swab into the inoculum tube.

Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet. Inoculate the dried surface of an MHA plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculums. Rim the plate with the swab to pick up any excess liquid. Discard the swab into an appropriate container. Leaving the lid slightly ajar, allow the plate to sit at room temperate at least 3 to 5 minutes, but not more than 15 minutes, for the surface of the agar plate to dry before proceeding to next step. **Placement of the antibiotic disks:** Place the appropriate antimicrobial-impreganted disks on the surface of the agar, using forceps. Forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. This should be done before replacing the petri dish lid as static electricity may cause the disks to relocate themselves on the agar surface & adhere to the lid. Using the forceps two 10-µg impenem discs were placed on the plate and 10-µg (750µg) of EDTA solution was added to one of the disc. The inhibition zones of the imipenem and imipenem-EDTA discs were compared after 16-18 h of incubation at 35°C. **Interpretation: MBL Positive strain:** Shows increase in the zone size ≥ 7mm around the imipenem disc with EDTA solution them the imipenem disc alone. **MBL Negative strain** -Shows increase in the zone size ≤ 7 mm around the imipenem disc with EDTA solution them the imipenem disc alone.

**RESULT**

**Direct microscopy by gram staining:** Gram negative rod, cocci, coccobacilli or bacilli, Single, pair, cluster, pink in colour after gram stain was observed(Figure-3).

**Colony Identification by MacConkey Agar:** On MacConkey agar, large dome shaped mucoid sticky, pink color,Lactose fermenting colonies was observed(Figure 4); oxidase Positive(figure 5); Catalase positive(Figure 6); Indole Negative(Figure 7); triple sugar iron test; a/a reaction(a=acidic)(Figure 8); The sugar fermentation test exposed that the isolates were skilled of fermenting glucose, lactose, maltose, and mannitol (Figure 9). Biochemical characterization revealed that highest percentage of MBL was in *Pseudomonas aeruginosa* (26.66%) followed by klebsiella spp.(25.80%) , Acinetobacter spp.( 9.09%) and E.coli (8.10%.) for refence use table 1&2.

**RESULTS**

Graph-1 shows of the 150 isolate, 74(49%) isolate were urine, 33(22%) isolate were pus,11(7.33%) were sputum,09(06%) were blood, 07(4.66%) Cathter tip, 06(4%) were EET,06(4%) were Ear swab and 04 (2.66%) isolate were stool. Graph -2 shows of the 150 isolates collected, 82( 55%) isolates were from female patients and 68(45%) from male patients indicating a higher admission rate of female to the hospital. Graph 3 shows about Maximum number of isolates were obtained from patient between the age group of 61-70 years (26) 17.33%, followed by the age group of 51-60 (23) 15.33% and 31-40 years (23) 15.33%, and 21-30 years (22) 14.66% and 11-20 years (18) 12%. Graph 4 tells about thehe highest number of isolates were from samples obtained from Surgery (52), followed by ICU (28), Medicine(28), Gynaecology (17), Pediatrics (17) and ENT (8). Graph -5 shows 75 (50%) E.coli predominated the population of isolated organisms followed by 31 (20%) Klebseilla species, 15 (10%) Pseudomonas aeruginosa,12 (08%) Citrobacter species,11(07%) Acinetobacter species and 06 (04%)Proteus species. Graph-6 shows E.coli were found predominantly in Surgery (28) ,followed by Medicine (15),Klebieilla species was found predominantly in ICU (11), followed by Surgery (11),Pseudomonas aeruginosa was found predominantly in Surgery (07), followed by ICU (05), Acinetobacter species was found predominantly in Gynaecology (03), followed by pediatrics (03), Citrobacter spcies was found predominantly in surgery (05,) followed by gynecology (4), proteus species was found predominantly in ICU (02), followed by Medicine (02). Graph-7 shows E.coli was the commonest isolate in Urine( 46), followed by Pus (16), blood( 04), Stool( 04), Ear swab( 02), Sputum( 02), ETT( 0), Catheter Tip(0). Klebsiella species was commonly isolates in Urine( 10), followed by Pus(

09), Sputum( 04), Catheter Tip (04), Ear Swab( 02), ETT( 01), blood( 01), Stool( 0).Pseudomonas aeruginosa was commonly isolate Pus( 05), followed by Urine( 03), sputum( 03), ETT( 02), CatheterTip( 01), blood (01) Ear Swab(0), Stool( 0). Acinetobacter species was commonly isolate Urine( 03), followed by E.T.T(03), Sputum( 02), Pus (01), blood (01), Catheter Tip( 01), Stool( 0), Ear Swab(0).Citrobacter species was isolate Urine (09), followed by blood( 02), Pus( 01), Ear Swab (01). Sputum( 0), Catheter Tip( 0), ETT( 0),Stool(0).Proteus species was commonly isolate Urine (02), Pus (02), blood (0), Sputum (0), Cateter Tip (01), Ear Swab (01), ETT (0), Stool (0). Graph-8 shows the Maximum number of Enterobacteriaceae isolates were found to be sensitive to Imipenem (82.22%), followed by Gentamicin ( 55.55% ) and Cefepime (42.22%) .Maximum no. of isolates were found to be resistant to Ampicillin (85.9%),followed by Ceftriaxone (82.96.%),and Levofloxacin(62.96%). Graph-9 shows the maximum number of Pseudomonas aeruginosa isolates were found to be sensitive to Levofloxacin and Tobramycin (66.66%), followed by Imipenem (60%). Maximum number of Pseudomonas aeruginosa isolate were found to be resistant to Ceftrazidime (55.55 %) followed by Aztreonam (46.66%).

**Screening for MBL production**

All isolate were screened for MBL production by disc diffusion method, out of which, thirty (30) isolates were found to be positive for MBL production. Those thirty isolate were subjected to MBL confirmatory test i,e. IMP-EDTA Combined disc test and IMP-EDTA double disc synergy test. 19 Isolate were positive for MBL production by IMP-EDTA combined disc test and out of which 09 Isolate were also positive for MBL production by IMP-EDTA double disk synergy test.

The tables obtainable in the investigation provided data on Metallo-β-lactamase (MBL) producing Imipenem-resistant Gram-negative bacteria (GBN) and their exposure using different screening methods. Table No-3 tells us that out of 30 isolates (representing 150 total cases), 19 (63.33%) were identified as MBL-producing, while 11 (36.66%) were non-MBL producers. This specifies a high prevalence of MBL-mediated resistance amid the tested isolates. Table No-4 grants the results of the IMP-EDTA Combined Disc Test, a technique used to detect MBL production. The test recognized 19 (63.33%) isolates as positive and 11 (36.66%) as negative, aligning with the MBL prevalence observed in Table No-3. Meanwhile, Table No-5 reports findings from the IMP-EDTA Double Disc Synergy Test (DDST), another screening technique for MBL detection. This test acknowledged only 9 (30%) isolates as MBL-positive, while 21 (70%) tested negative, signifying that DDST may have lower sensitivity compared to the Combined Disc Test.The assessment of these two screening methods highpoints that the IMP-EDTA Combined Disc Test detected more MBL-producing insulates than the Double Disc Synergy Test, demonstrating it may be a more consistent technique for identifying MBL-mediated carbapenem resistance. These conclusions are crucial for diagnosing antibiotic-resistant infections and picking appropriate treatment approaches, as MBL-producing bacteria pose noteworthy therapeutic challenges, in figure 17. Double –disc synergy test (DDST) using imipenem and EDTA (upper half) Produce large synergistic zone of inhibition towards the imipenem+EDTA disk and imipenem- EDTA combind disc test (Lower half) Produce more than 7mm larger zone of Inhibition than the imipenem disk as noted in figure 17 and is a evident for MBL positive strain; Shows increase in the zone size ≤ 7 mm around the imipenem disc i.e, MBL Negative Strain with EDTA solution them the imipenem disc alone as you can verify in figure 18. The Table 6 explains about the effectiveness of the IMP-EDTA Combined Disc Test and the IMP-EDTA Double Disc Synergy Test (DDST) for metallo-beta-lactamase (MBL) production in bacterial isolates is strikingly shown by their comparison. The Combined Disc Test is considerably more sensitive than the Double Disc Synergy Test; out of thirty tested samples, it found 19 positive cases while the Double Disc Synergy Test found just 9 positives. Using Yates's correction and the Chi-square test produced a p-value of 0.01, which is statistically significant ($p < 0.05$). This implies that a real variation in the diagnostic accuracy of the two tests explains the observed variations between them rather than chance.

**Interpretation and reporting of the report:** Using the published CLSI guidelines, noted the susceptibility or resistance of Klebsiella pneumoniae. For each drug, noted on the recording sheet whether the zone size is within the susceptible (S), intermediate (I), or resistant (R) range based on the interpretation chart.

## DISCUSSION

In any nosocomial setting, Carbapenems (Imepenem, Meropenem) are used as the 'last resort' for treatment of MDR gram negative bacterial infection[29]. However, since last 15 years, acquired resistance to this life saving antimicrobial have been increasingly reported noy only in Pseudomonas and Acinetobacter species, [30-31] but also among another member of Enterobacteriaceae. This resistance is mainly mediated by MBLs. Of the 150 samples, 82(55%) were from female patients while 68 (45%) were from male patients indicating higher admission rate of female than male to the hospital. In our study gram negative bacilli isolated from the various samples were 17.33% in the age group of 61-70 years, followed by 15.33% in the age group 51-60 years and 31-40 years. Of the 150 bacterial isolates, E.coli 75 (50%) was isolated maximum followed by Klebsiella species 31 (20.6%), Pseudomonas aeruginosa 15 (10%), Citrobacter species 12 (8%), Acinetobacter species 11 (7.3%) and Proteus species 6 (4%).However, a study done by EL Kholy Amani A et al [32] which indicated Klebsiella species (21.08%) as the most common isolate followed by E. coli( 18.98%), Pseudomonas aeruginosa (6.5%) and Acinetobacter species (4.8%) respectively. In our study antibiotic sensitivity of Enterobacteriace family showed maximum sensitivity to Imipenem (82.22%) followed by Gentamicin (55.55%), Cefepime (42.22%) and Levofloxacin (37.03%). A Study by Kholy Amani et al. [33] showed somewhat similar pattern with maximum sensitivity to Imipenem (86%) followed byAmikacin (66.6%), Gentamicin (42%), Cefepime (40%). Levofloxacin (30%). Our study showed maximum resistance to Ampicillin (85.9%) followed by Ceftriaxone (8296%), Levofloxacin (62.96%) and Cefepime (57.77%). However, Vemula Sarojamma et al reported highly resistant to Imipenem (84%), Amikacin (56%), and Gentanicin (40%) in their study. In our study Pseudomonos aeruginosa showed maximum sensitivity to Piperacillin/Tazobactum (80%) followed by Tobramycin 66.66% and Levofloxacin (66.66%), and Imipenem (60%) respectively. Resistance pattern of Pseudomonas however was maximum by Ceftazidime (55.35%) followed by Aztreonam (46.66%) and Imipenem (33.33%), Levofloxacin (33.33%),Tobramycine (33.33%), and Piperacilli/ Tazobactam (33.33%) respectively. Varaiya et a 2016[34-36] found that (40%) were susceptible to Piperacillin-Tazobactam (66.67%) were sensitive to ampicillin. In our study all 30 Imipenem resistant Gram-negative bacterial isolate were tested by two different screening method namely MP-EDTA combind disc test and IMP-EDTA double disk synergic test. 19 (66.33%) were positive combind disc method and 09 (30%) were positive double disk synergic test. Other similar studies showed 100% positive for MBL combind disc method, whereas 93.68% positivity by double disk synergic method. This study is comparable to study of Stood et al. (100%), Irfan et al. [37-38] (100%), Attal et al. (88.89%), and Fam et al (87.5%).[39-40]Franklin et al. have reported 100% sensitivity of CDST method and 79% sensitivity of DDST method.[140] The combined disc method using Imipenem + EDTA was found to be superior to Imipenem EDTA double disk synergy test( DDST) ($p=0.01$) which is in concordance with the studies conducted by yan at al [127-128] and Behera at al)[41-42]. In India most studies have used the imipenem-EDTA combind disc test and dobule disc synergy test using imipenem-EDTA according to which MBL production ranged from (7-65%). [135-136] 86.4% of MBL production was reported by Vinod et al. (Wattal et al 2010). MBL production has been reported as 70.8% from North India.[43-44] MBLs in the bacterial community (Lee et al. 2003).observed a high rate of prevalence of Carbapenem resistance( 47%), similar a high rate was observed in a study from Tanzania (35%) Mushi et al. 2014. A slightly lowered rate of MBL production 24.1% was noted in another study (Mushi et al. 2013[39-44] Although both tests are simple and easy to perform in the laboratory and helpful in MBL detection in any set up but combined disc test is better test as compared to double disk synergic test as it is devoid of visual misinterpretation. The study showed that Imipenem resistant isolate can be routinely screened for MBL production using simple method such as combined disc test, Imipenem and Imipenem-EDTA combined disc test which will be crucial step toward large scale monitoring of these emerging resistant determinants. MBL has become a serious problem worldwide and several aspects of them are worrying the community. These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination [45]. They compromise the activity of wide – spectrum antibiotics creating major therapeutic difficulties with significant impact on the outcome of

patient appropriate antimicrobial selection, surveillance system and effective infection control procedure are the key factor in their control.

## CONCLUSION

Among all gram negative bacilli isolates,( 50%) 0f E.coli were more prevalent followed by( 20.66%) Klebsiella species ( 10%) of Pseudomonas aeruginosa, Citrobacter spp.(8%), Acinetobacter spp.(7.33%) and proteus spp.(4%) in different clinical sample .Maximum number of isolate were obtained from the patient between the age group of 61-70 years (26) 17.33%, followed by the age group of 51-60 years (23) 15.33% and 31-40 years (23) 15.33%, and 21-30 years (22) 14.66% years. E coli was the first most common pathogen in urine and second most common in pus whereas Pseudomonas aeruginosa was most common pathogen in pus and ear swab. In the present study maximum number of isolates were found to be sensitive to Imipenem (82.22%), followed by Gentamicin (55.55%) and Cefepime (42.22%). Maximum no. of isolates was found to be resistant Ampicillin (85.9%), followed by Ceftriaxone (82.96.%), and Levofloxacin (62.96%). Of the 30 imipenem resistant strains, 63.33% were positive for MBL- production by Combined Disk Test while 30% were positive for MBL production by Double Disk Synergy Test. Highest percentage of MBL was in Pseudomonas aeruginosa( 26.66%) followed by klebsiella spp.(25.80%) , Acinetobacter spp.( 9.09%) and E.coli (8.10%.). The present study suggests that both tests are simple and easy to perform in the laboratory and helpful in MBL detection in any setup but CDST is better test as compared to DDST as it is devoid of visual misinterpretation. The spread of multidrug-resistant GNB in the hospital setting is now seen as a globalized threat. In the majority of hospitalized patients in ICUs are especially exposed to the risk. In the absence of molecular detection technique, the combind disc test provides a sensible choice for phenotypic detection of MBL production and can be implemented in clinical laboratory on a daily basia. In addition, routine surveillance Of MBL producing bacteria is crucial for establishing appropriate empirical antimicrobial therapy and restraining their spread in hospital environment.

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| Table 1. Key Identification Characteristics for the Most Common Enterobacteriaceae(29) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | KIA | GAS | H2S | MR | VP | IND | CIT | PAD | URE | MOT | LYS | ARG | ORN | ONPG |
| Tribe I: Escherichieae | | | | | | | | | | | | | | |
| Genus: Escherichia | | | | | | | | | | | | | | |
| E.coli | A/A | + | - | + | - | + | - | - | - | + | + | -/+ | +/- | + |
| Genus: Shigella | | | | | | | | | | | | | | |
| Group A.B.C | ALK/A | - | - | + | - | -/+ | - | - | - | - | - | - | - | - |
| S. sonnei | ALK/A | - | - | + | - | - | - | - | - | - | - | - | + | + |
| Tribe II: Edwardsielleae | | | | | | | | | | | | | | |
| Genus: Edwardsiella | | | | | | | | | | | | | | |
| E. Tarda | ALK/A | + | + | + | - | + | - | - | - | + | + | - | + | - |
| Tribe III: Salmonelleae | | | | | | | | | | | | | | |
| Genus: Salmonella | ALK/A | + | + | + | - | - | + | - | - | + | + | +/- | + | - |
| Tribe IV: Citrobactereae | | | | | | | | | | | | | | |
| Genus: Citrobacter | | | | | | | | | | | | | | |
| C. freundii | A/A: | + | + | + | - | - | + | - | +/- | + | - | +/- | -/+ | + |
| | ALK/A | | | | | | | | | | | +/- | + | + |
| C. koseri | ALK/A | + | - | + | - | + | + | - | +/- | + | - | | | |
| Tribe V: Klebsielleae | | | | | | | | | | | | | | |
| Genus: Klebsiella | | | | | | | | | | | | | | |
| K. pneumonia | A/A | ++ | - | - | + | - | + | - | + | - | + | - | - | + |
| K. oxytoca | A/A | ++ | - | - | + | + | + | - | + | - | + | - | - | + |
| Genus: Enterobacte | | | | | | | | | | | | | | |
| E.aerogenes | A/A | ++ | - | - | + | - | + | - | - | + | + | - | + | + |
| E. cloacae | A/A | ++ | - | - | + | - | + | - | +/- | + | - | + | + | + |
| Genus: Hafnia | | | | | | | | | | | | | | |
| H. Alvei | ALK/A | + | - | -/+ | + | - | - | - | - | + | + | - | + | + |
| Genus: Pantoea | | | | | | | | | | | | | | |
| P. agglomerans | A/A: | -/+ | - | -/+ | +/- | -/+ | +/- | -/+ | -/+ | + | - | - | - | + |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ALK /A | | | | | | | | | | | | | |
| Genus: Serratia | | | | | | | | | | | | | | |
| S.marcescens | ALK /A | + | - | - /+ | + | - | + | - | - | + | + | - | + | + |
| Tribe VI: Proteeae | | | | | | | | | | | | | | |
| Genus: Proteus | | | | | | | | | | | | | | |
| P.vulgaris | ALK /A | +/- | + | + | - | - | +/- | + | ++ | +* | - | - | - | - |
| P.mirabilis | ALK /A | + | + | + | + /- | + | -/+ | + | ++ | +* | - | - | + | - |
| Genus: Morganella | | | | | | | | | | | | | | |
| M.morganii | ALK /A | + | - | + | - | + | - | + | ++ | + | - | - | + | - |
| Genus: Providencia | | | | | | | | | | | | | | |
| P.rettgeri | ALK /A | - | - | + | - | + | + | + | ++ | + | - | - | - | - |
| P.stuartii | ALK /A | - | - | + | - | + | + | + | -/+ | +/- | - | - | - | - |
| P.alcalifaciens | ALK /A | +/- | - | + | - | | + | + | - | + | - | - | - | - |
| Tribe VII: Yersineae | | | | | | | | | | | | | | |
| Genus: Yersinia | | | | | | | | | | | | | | |
| Y.enterocolitica | ALK /A | - | - | + | - | +/- | - | - | +/- | - | - | - | + | + |
| KIA, Kligler' iron agar; H2S, hydrogen sulfide; MR, methyl red; VP, Voges-Proskauer; IND, indole; CIT, PAS, phenylalanine deaminase; URE, urease; MOT, motility; LYS, lysine; AGR, arginine; ORN, ornithine; ONPG, o-nitrophenyl-β-D-galactopyranside;++, strong positive reaction; +, 90% or more strains negative; +/-, 50-90% of strains positive; -/+, 50-90% of strains negative; shaded areas indicate key reactions. | | | | | | | | | | | | | | |
| * Swarming motility demonstrated on oninhibitory media. | | | | | | | | | | | | | | |
| Î Nonmotile at 36°C, motile at 22°C | | | | | | | | | | | | | | |

Table no. 2- BIOCHEMICAL TEST: Pseudomonas Aeruginosa

| | |
|---|---|
| Catalase test | Positive |
| Oxidase test | Positive |
| Indole test | Negative |
| TSI | A/A no gas,no $H_2S$ production, metallic sheen. |
| Sugar fermentation test | |
| Glucose | Negative.Oxidatively broken down to form acid only. |
| Lactose | Negative |
| Mnnitol | Negative |
| Methyle red test | Negative |

| | |
|---|---|
| Voges – ProsKauer test | Negative |
| Citrate utilization test | Positive |
| Urease test | Positive |

Table No-3 MBL PRODUCING IMIPENEM RESISTANCE ORGANISMS

| Total GBN (%) | MBL Producing GBN n (%) | Non-MBL Producing GBN n (%) |
|---|---|---|
| 30 (150) | 19 (63.33) | 11 (36.66) |

Table No -4 MBL SCRENING BY IMP-EDTA COMBINED DISC TEST

| Number (%) | IMP-EDTA Combined Disc Test |
|---|---|
| 19 (63.33) | Positive |
| 11 (36.66) | Negative |
| 30 (150) | Total |

Table No- 5 MBL SCRENING BY IMP-EDTA DOUBULE DISC SYNERGY TEST

| Number (%) | IMP-EDTA Double Disc Synergy Test |
|---|---|
| 09 (30) | Positive |
| 21 (70) | Negative |
| 30 (150) | Total |

Imipenem-EDTA Combined Disk Test With IMP-EDTA Double Disk Synergy Test.

TABLE NO. 6-COMPARISON OF IMP-EDTA COMBINED DISCS TEST WITH IMP-EDTA DOUBLE DISC SYNERGY TEST

| | Total | Positive | Negative |
|---|---|---|---|
| IMP-EDTA COMBINED DISC TEST | 30 | 19 | 11 |
| IMP-EDTA DOUBLE DISC SYNERGY TEST | 30 | 09 | 21 |

p value = 0.01 hence will be consider as significant.
(p value was determined using chi-square test will yet correction)

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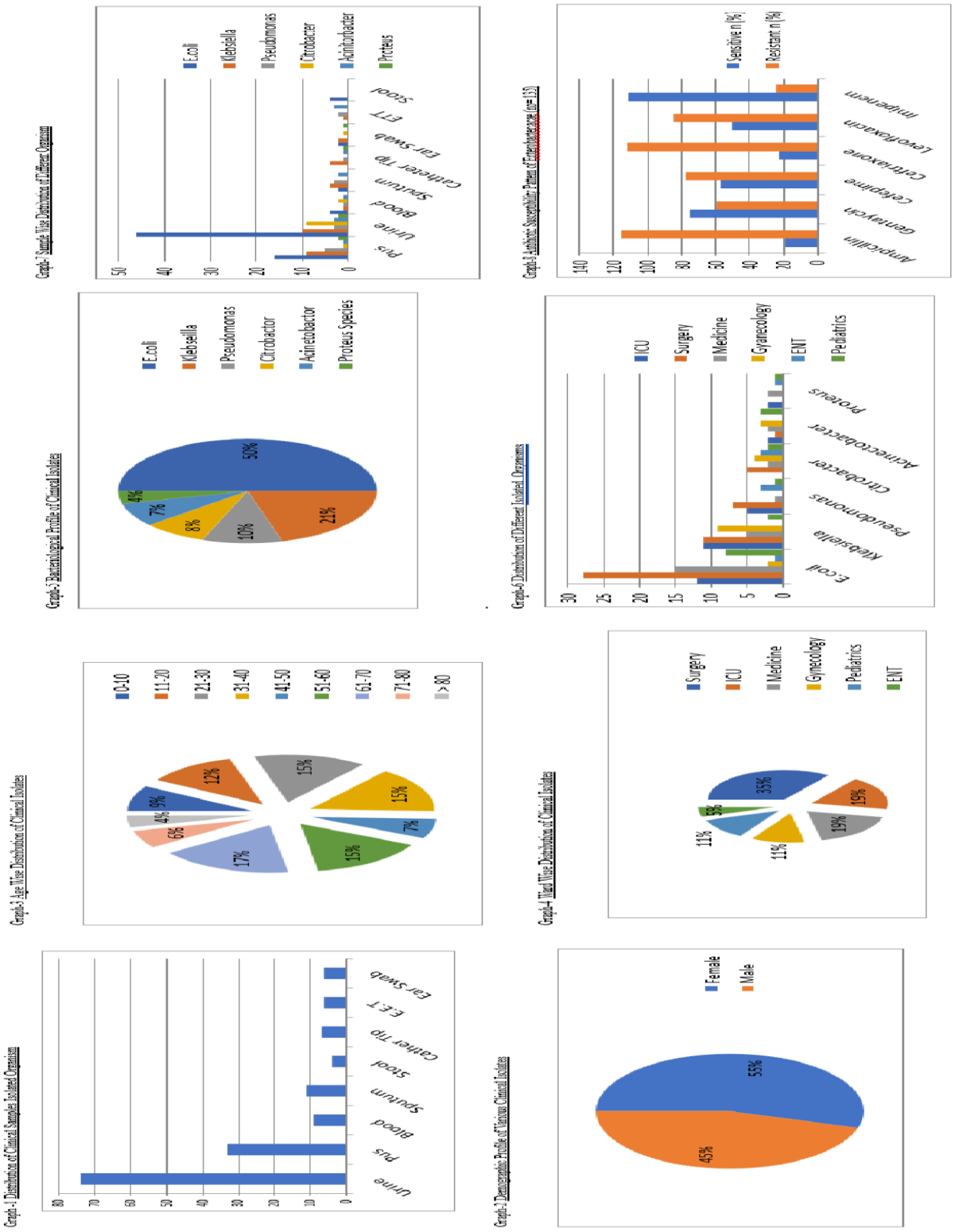

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