# A STUDY OF METALLO BETA LACTAMASES IN GRAM NEGATIVE ISOLATES IN A TERTIARY CARE HOSPITAL

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**Abstract: Background- In recent years over the counter use of Antibiotics for human therapy has resulted in selection of pathogenic bacteria resistant to multiple drugs and in some cases resistant to last resort of drugs. The common form of resistance is either through lack of drug penetration or Hyper production of AmpC type β-lactamase, and Carbapenem-hydrolyzing β-lactamases. Methods- Total 2004 gram negative bacilli were tested for MBL production. Phenotypic confirmation of MBL is done by combined disk test and E strip test. Then MIC of Colistin was done by E strip test for MBL positive strains. Results- Out of 2004 samples screened 196 isolates were Imipenem resistant. Out of 196 isolates 160 were MBL producers by Combined disk test and 162 by E strip test. Out of this 162 isolates 11 isolated were Colistin resistant by E strip test. Conclusion- highest prevalence of MBL production was seen in samples from ICU. This indicated unemperical use of higher antibiotics. This suggests there is need for rational use of Antimicrobials and Culture sensitivity for every patient should be done before starting Antibiotic therapy.**

**Index Terms: MBL, Colistin, E strip. (key words)**

## I. INTRODUCTION

Multi drug resistant bacteria have come into limelight in last few decades because mankind is affected due to these microbes which have become smarter than us and are posing threat to our destiny. Not only morbidity but mortality is also going on rise when these microscopic creatures are invading us[1]. We need to save ourselves from these devils or else mankind will be an endangered species. β-lactam antibiotics are among the most widely prescribed antibiotics worldwide[2]. The emergence of resistance to these agents in the past two decades has resulted in major clinical crisis[3]. Antibiotic resistance among Gram negative bacilli(GNB) is rapidly expanding problem due to the organisms ability to mutate, and to acquire and transmit plasmids and other mobile genetic elements encoding resistance genes[4] The major defence of GNB against β-lactam antibiotics is the production of β-lactamases[5,6]. In the early 1960s, Ampicillin, Carbenicillin, and the narrow-spectrum Cephalosporins were frequently used to treat GNB infection. However, plasmid-mediated β-lactamase mediated resistance has caused these antibiotics to lose their efficacy.Gram-negative bacteria have at their disposal of plethora of resistance mechanisms that they can sequester and, eluding the action of Carbapenems and other β-lactams. The common form of resistance is either through Lack of drug penetration (i.e., outer membrane protein [OMP] mutations and efflux pumps) or by Hyper production of AmpC type β-lactamase, and Carbapenem-hydrolyzing β-lactamases. MBL are unique group of β-lactamases that have a zinc ion at their active site. These Metallo enzymes hydrolyze Carbapenems but have poor ability to hydrolyze monobactams like Aztreonam. They are not inhibited by Tazobactam or Clavulanic acid[10] but are readily inhibited by metal chelators like EDTA and thiol based compounds like 1,10- o phenanthrolin and 2-mercaptopropionic acid[7,8]. Five different types of MBLs whose prevalence are increasing rapidly are IMP, VIM, SPM, GIM and SIM[9,10,11] among them VIM and IMP are most predominant[12]. The first strain producing MBL i.e., *Pseudomonas aeruginosa* was isolated in Japan in 1988.The problem of MBL producing strains was originally confined to *Pseudomonas* and *Acinetobacter*. However, Carbapenem resistance has been observed in members of *Enterobacteriaceae* family due to spread of MBL genes. The present study was aimed at determining the prevalence of Metallo-β-lactamases (MBL) production in gram negative bacterial isolates obtained from various clinical isolateS[13].. Prevalence of MBL production in gram negative bacilli in ICU infections in Maharashtra is 19.67%[14]. MBL gene has been transferred into *Enterobacteriaceae* species, including *E. cloacae, Proteus spp. Citrobacter freundii, K. oxytoca, M. morganii* and *Providencia spp*[15]. Thus, in recent years MBL genes have spread from *P. aeruginosa* to *Enterobacteriaceae,* and a clinical scenario to be developing that could simulate the global spread of Metallo- β-lactamases.[22]Focusing on all the above problems we have undertaken this study to detect prevalence of Metallo-β-lactamase production in Gram negative isolates, to study the prevalence of Carbapenem resistance in Gram negative bacilli,to compare different phenotypic methods to detect Metallo-β-lactamase production, to study antibiotic resistance pattern of Metallo-β-lactamase producing Gram negative bacilli..

## II. MATERIAL AND METHODS-

The present cross sectional study is conducted in our department, from October 2016 to September 2018. A total of 2004 samples were screened during this period. Pus , Wound swab, Urine, Blood, Body fluids, Respiratory samples were collected as per standard protocol[16].Gram negative bacteria belonging to family *Enterobacteriaceae, Pseudomonas aeruginosa*, *Acinetobacter* spp. were processed and the isolates were identified with standard biochemical tests

### A. ANTIBIOTIC SUSCEPTIBILITY TESTING

The antibiotic susceptibility testing of the isolated organisms were carried on Mueller-Hinton Agar (MHA), by Kirby-Bauer disc-diffusion method, using 0.5 McFarland as the turbidity standard as per CLSI guidelines 2016[17]. All antibiotic discs were

obtained from Himedia Laboratories P.LTD. ATCC *E.coli* 25922 and ATCC *Pseudomonas aeruginosa* 27853 strains were used for quality control

**B. INTERPRETATION OF AST**

After overnight incubation, the zone diameters (including the 6mm disc) were measured with a ruler on the under surface of the petri dish and interpreted as sensitive, intermediate and resistant according to CLSI guidelines 2016[17].

Quality control strains used for AST were-

- ATCC *E.coli* 25922
- ATCC *Pseudomonas aeruginosa* 27853.

**C.SCREENING OF MBL**

In the present study, 2004 Gram negative bacilli belonging to family *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. were tested for MBL production

The isolates showing Imipenem resistance were considered as screening test positive for MBL detection[17,18,19].

- Enterobacteriaceae- ≤ 19 mm
- Pseudomonas- ≤ 15 mm
- Acinetobacter- ≤ 18 mm

D. **PHENOTYPIC CONFIRMATION OF MBL-**

**1. COMBINED DISC TEST-**

The test was performed as described by Yong et al[20]. Test organisms were inoculated on Muller-Hinton agar as recommended by CLSI 2016[17]. A disc of Imipenem (10 µg) and Imipenem-EDTA (10/750 µg) were place at 25 mm apart. The inhibition zones of the Imipenem and Imipenem-EDTA discs were compared after 16-18 hours of incubation at $35^0$C[20].

**Interpretation-**

In the Combined Disc test, if the increase in Inhibition zone of Imipenem-EDTA was ≥ 7mm than the Imipenem disc alone, it was considered as MBL positive[20]

**2. E-STRIP TEST-**

Bacterial suspension of 0.5 McFarland was spread on Muller-Hinton agar by sterile swab stick. Then E-strip which contains double sided seven dilution range of Imipenem with fixed concentration of EDTA was placed on surface of medium and the plates were incubated at $35^0$C for 16-24 hours. The intersection of the ellipses at the strip is read from two halves i.e., at the section with Imipenem alone and Imipenem with EDTA[21].

**Interpretation-**

If the ratio of the value obtained for Imipenem to the value of Imipenem + EDTA is more than to 8 then it is taken as MBL positive

OR

If zone is observed on the side coated with Imipenem + EDTA and no zone is observed on the opposite the side coated with Imipenem, it is taken as MBL positive[21].

**E.MIC OF COLISTIN FOR MBL POSITIVE STRAINS-**

**E-strip test-**

Bacterial suspension of 0.5 McFarland was spread on Muller-Hinton agar by sterile swab stick. Then E-strip with dilution range of Colistin was placed on the surface of the medium and the plates were incubated at $35^0$C in air for 16-24 hours. After that MIC was read where the ellipse intersects the MIC scale on the strip[22].

**Interpretation-**

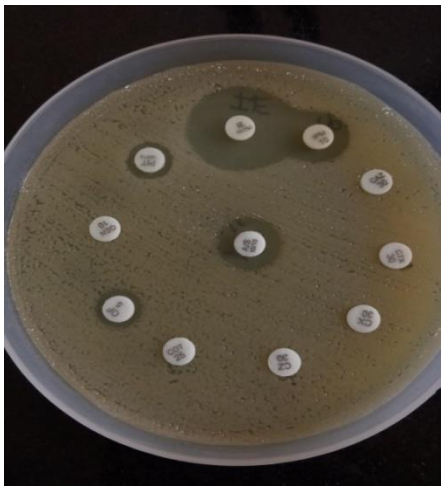
If the strain showed MIC ≤ 2µg/ml, then it was taken as sensitive and

If the strain showed MIC ≥ 8µg/ml, then it was taken as resistant[22].

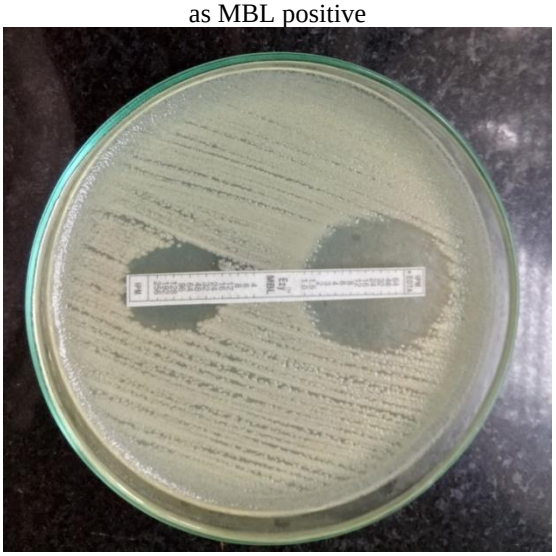
**Statistical analysis-**

Chi square test ($X^2$) was applied wherever applicable, p value <0.05 was considered as statistically significant.

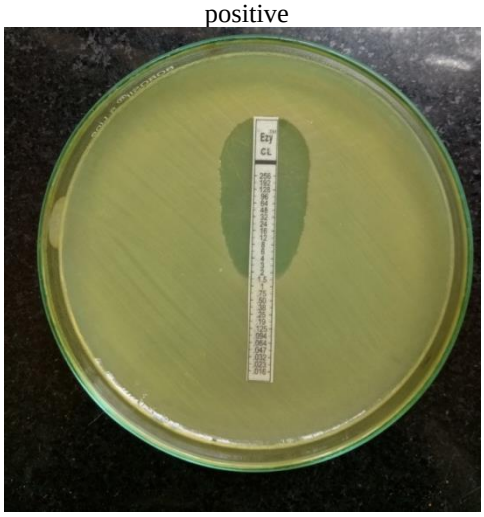
Color Plate:1 MHA plate showing Organism resistant to all antibiotics (Pan resistant)



Color Plate-2: MHA plate showing zone difference of >7mm between discs of Imipenem and Imipenem + EDTA and considered as MBL positive



Color Plate-3: showing E-test for detection of MBL. Ratio of MIC of Imipenem/Imipenem+EDTA ≥8 was considered as MBL positive

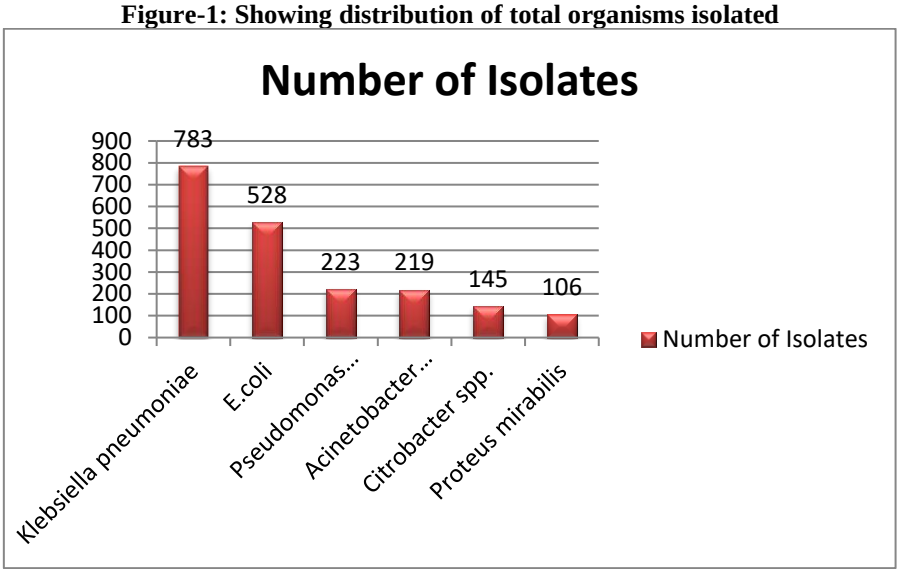


Color Plate-4: Showing MIC for Colistin by E-test.MIC of Colistin ≤2µg/ml was taken as Sensitive

**III. OBSERVATION AND RESULTS**

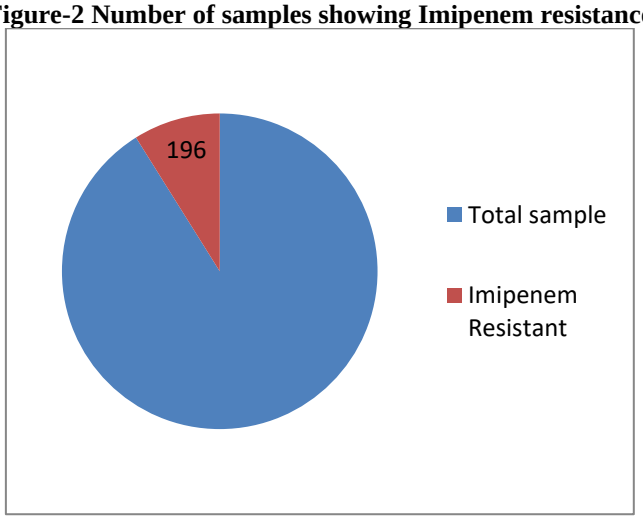
In the present study, Gram negative isolates from various clinical specimens received in the diagnostic bacteriology laboratory were included. A total of 2004 Gram negative isolates were included in the study.

**Figure-1: Showing distribution of total organisms isolated**




Out of total 2004 gram negative bacilli screened, 783(39.07%) were found to be *Klebsiella pneumoniae*, 528(26.34%) *E. coli*, 223(11.12%) *Pseudomonas aeruginosa*, 219(10.98%) *Acinetobacter baumanii*, 145(7.23%) *Citrobacter* spp. and 106(5.28%) *Proteus mirabilis.*

**Figure-2 Number of samples showing Imipenem resistance**




Out of total 2004 samples screened, 196 were found to be Imipenem resistant.

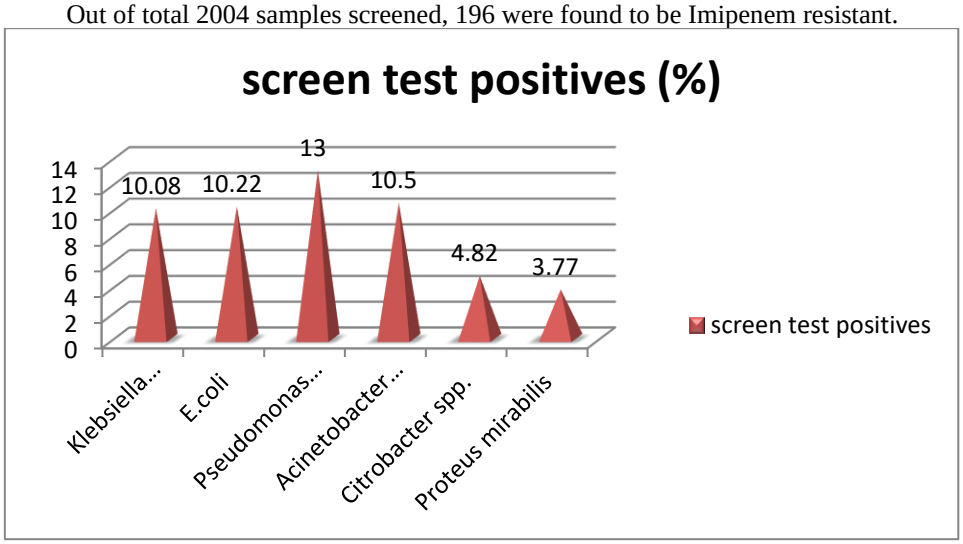



**Figure-3: Showing screening of clinical isolates for Carbapenem resistance by CLSI susceptibility criteria**

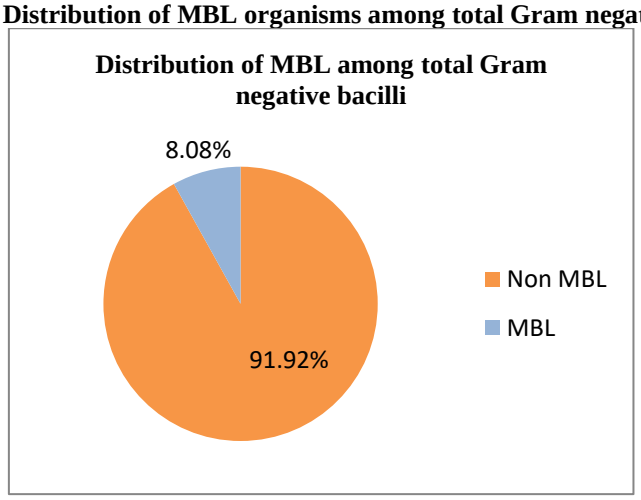
10.08% of total *Klebsiella pneumoniae* , 10.22% of total *E. coli* , 13% of total *Pseudomonas aeruginosa* , 10.5% of total *Acinetobacter baumanii* , 4.82% of total *Citrobacter* spp. and 3.77% of total *Proteus mirabilis* were screen test positive

**Table-1: Confirmation of MBL in screen test positive isolates by Phenotypic confirmatory methods**

| Sr.No. | Bacterial isolates | Screen test positive | Combined disc test | E test |
|---|---|---|---|---|
| 1 | *Klebsiella pneumoniae* | 79 | 70 | 71 |
| 2 | *E. coli* | 54 | 46 | 46 |
| 3 | *Pseudomonas aeruginosa* | 29 | 23 | 24 |
| 4 | *Acinetobacter baumanii* | 23 | 11 | 11 |
| 5 | *Citrobacter* spp. | 7 | 6 | 6 |
| 6 | *Proteus mirabilis* | 4 | 4 | 4 |
| | Total | 196 | 160 | 162 |

Out of total 196 screen test positive isolates, 160 were found to MBL producer by Combined disc test and 162 by E-test. E-test was taken as gold standard.

**Figure-4: Distribution of MBL organisms among total Gram negative bacilli**



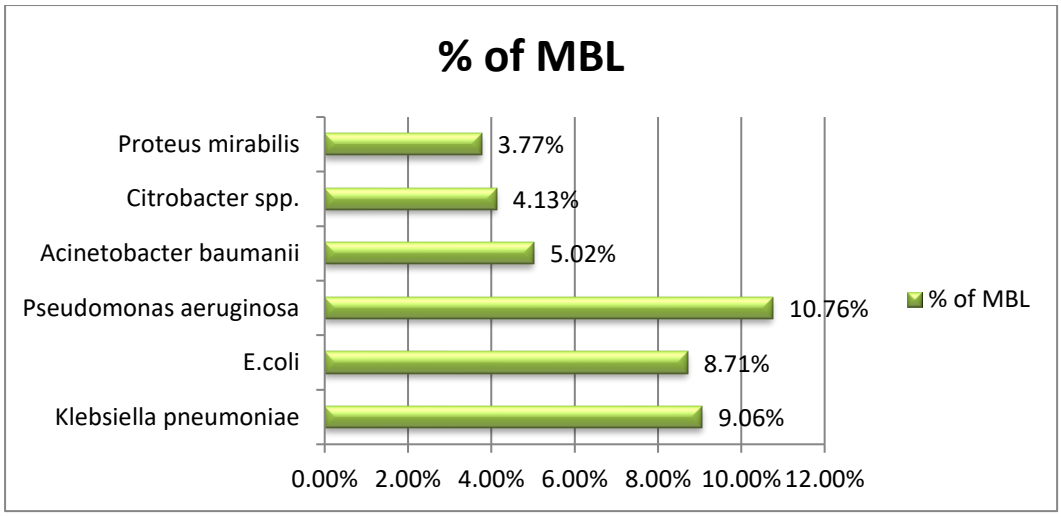

Out of total gram negative bacilli 8.08% were MBL producers

**Table-2: Showing distribution of MBL among individual wards**

| Sr.No. | Ward | Total Isolates (n=2004) | MBL (n=162) |
|---|---|---|---|
| 1. | ICU's | 263 | 52 (19.77%) |
| 2. | Burn | 275 | 29 (10.54%) |
| 3. | Surgery | 408 | 42 (10.29%) |
| 4. | Orthopaedics | 298 | 15 (5.03%) |
| 5. | OBGY | 221 | 10 (4.52%) |
| 6. | Medicine | 296 | 9 (3.04%) |
| 7. | Paediatrics | 126 | 3 (2.38%) |
| 8. | ENT | 45 | 1 (2.22%) |
| 9. | OPD | 72 | 1 (1.38%) |
| 10. | Total | 2004 | 162 (8.08%) |

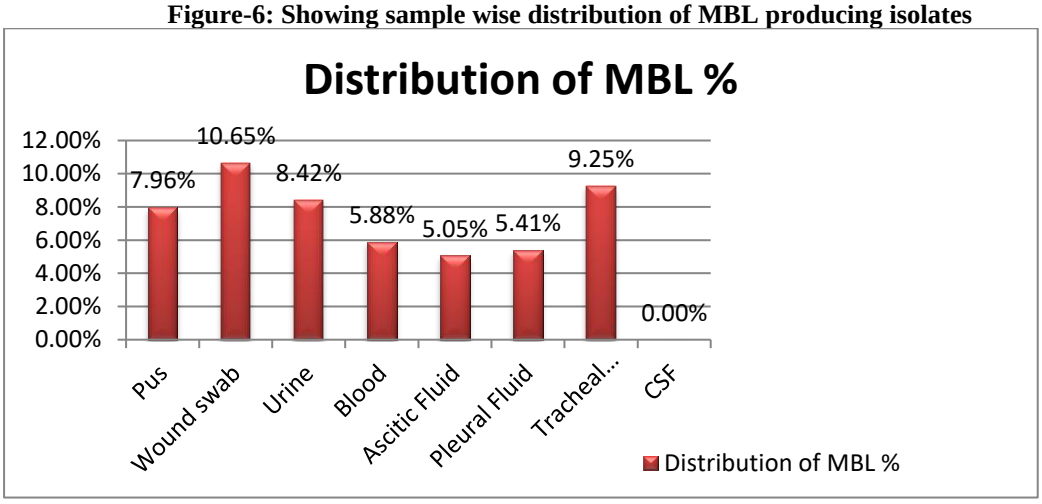
Applying Chi Square test, $p<0.05$ shows statistical significance. Highest prevalence of MBL was seen amongst isolates from ICU,s(19.77%) followed by Burns ward(10.54%), surgery wards (10.29%), orthopaedic wards(5.03%)

**Figure-5: Showing distribution of MBL among different genera**




Amongst all the organisms isolated from various clinical samples Pseudomonas aeruginosa was the predominant organism showing MBL production 10.76%, followed by Klebsiella pneumoniae 9.06%, E.coli 8.71%, acinetobacter baumanii 5.02%

**Figure-6: Showing sample wise distribution of MBL producing isolates**




MBL producing gram negative bacilli were predominantly isolated from wound swab 10.65% followed by Tracheal secretions 9.25%, urine 8.64%, pus 7.96% etc

**Table-3: Showing age wise distribution of MBL isolates**

| Age in years | No. of MBL isolates (n=162) |
|---|---|
| <1 | 1 (0.61%) |
| 1-10 | 2 (1.23%) |
| 11-20 | 39 (24.07%) |
| 21-30 | 27 (16.66%) |
| 31-40 | 20 (12.34%) |
| 41-50 | 17 (10.49%) |
| 51-60 | 39 (24.07%) |
| >60 | 17 (10.49%) |
| Total | 162 |

Maximum no. of MBL producers 24.07% are present in age group 11-20 years and 51-60 years followed by 16.66% in 21-30 years age group. Minimum no. of MBL producers 0.61% is present in <1 year age group.

**Table-4: Showing MICs of Imipenem and Imipenem+EDTA in MBL producing organisms**

| MIC(µg/ml) Imipenem | MIC(µg/ml) Imipenem+EDTA | Ratio of I/IE | No. of isolates (n=162) | Interpretation |
|---|---|---|---|---|
| 16 | 1 | 16/1=16 | 64 | MBL positive |
| 24 | 1.5 | 24/1.5=16 | 70 | MBL positive |
| 24 | 2 | 24/2=12 | 4 | MBL positive |
| 12 | 1 | 12/1=12 | 19 | MBL positive |
| 16 | 1.5 | 16/1.5=10.66 | 5 | MBL positive |

It is detected by calculating the ratio of MIC's of Imipenem and Imipenem +EDTA

If the ratio of I/IE ≥8 then it is MBL positive

**Table-5 Showing MIC of Colistin in MBL producing organisms**

| MIC (µg/ml) | No. of isolates (n=162) | Interpretation |
|---|---|---|
| 1 | 7 | Sensitive |
| 1.5 | 128 | Sensitive |
| 2 | 16 | Sensitive |
| 8 | 9 | Resistant |
| 12 | 2 | Resistant |

If MIC of Colistin is ≤2 then it is sensitive and If MIC of Colistin is ≥8 then it is resistant

**Table-6: Showing Antibiotic resistance pattern of MBL in *Enterobacteriaceae***

| **Organisms** | ***K.pneumoniae*** | ***E.coli*** | ***Citrobacter* spp** | ***P.mirabilis*** |
|---|---|---|---|---|
| **Antibiotics** | **MBL (n=71)** | **MBL (n=46)** | **MBL (n=6)** | **MBL (n=4)** |
| Ampicillin | 71 (100) | 46 (100) | 6 (100) | 4 (100) |
| Amoxicillin-Clavulanic acid | 71 (100) | 46 (100) | 6 (100) | 4 (100) |
| Cephazoline | 71 (100) | 46 (100) | 6 (100) | 4 (100) |
| Cefoxitin | 71 (100) | 46 (100) | 6 (100) | 4 (100) |
| Cefotaxime | 71 (100) | 46 (100) | 6 (100) | 4 (100) |
| Ceftazidime | 71 (100) | 46 (100) | 6 (100) | 4 (100) |
| Cefepime | 71 (100) | 46 (100) | 6 (100) | 4 (100) |
| Piperacillin-Tazobactam | 71 (100) | 46 (100) | 6 (100) | 4 (100) |
| Imipenem | 71 (100) | 46 (100) | 6 (100) | 4 (100) |
| Gentamycin | 63 (88.73) | 39 (84.78) | 5 (83.33) | 2 (50) |
| Amikacin | 54 (76.05) | 30 (65.21) | 3 (50) | 1 (25) |
| Tobramycin | 48 (67.60) | 30 (65.21) | 4 (66.66) | 1 (25) |
| Ciprofloxacin | 51 (71.83) | 31(67.39) | 3 (50) | 2 (50) |
| Cotrimoxazole | 61 (85.91) | 31 (67.39) | 4 (66.66) | 2 (50) |
| Colistin | 00 | 4 (8.69) | 00 | 00 |

Amongst the members of *Enterobacteriaceae, Klebsiella pneumoniae* showed highest resistance of 88.73% to Gentamycin, 76.05% to Amikacin, 67.60% to Tobramycin etc. *E. coli* showed resistance of 8.69% to Colistin.

**Table7: Showing Antibiotic resistance pattern of MBL in Non-fermenters**

| **Organisms** | ***P.aeruginosa*** | ***A.baumanii*** |
|---|---|---|
| **Antibiotics** | **MBL (n=24)** | **MBL (n=11)** |
| Ampi-sulbactam | ND | 11 (100) |
| Ceftazidime | 24 (100) | 11 (100) |
| Cefepime | 24 (100) | 11 (100) |
| Piperacillin-Tazobactam | 24 (100) | 11 (100) |
| Imipenem | 24 (100) | 11 (100) |
| Aztreonam | 17 (70.83) | ND |
| Gentamycin | 21 (87.50) | 8 (72.72) |
| Amikacin | 15 (62.50) | 6 (54.54) |
| Tobramycin | 19 (79.16) | 7 (63.63) |
| Ciprofloxacin | 12 (50) | 4 (36.36) |
| Cotrimoxazole | ND | 4 (36.36) |
| Colistin | 4 (16.66) | 3 (27.27) |

Amongst non fermenters, *Pseudomonas aeruginosa* showed highest resistance of 87.50% to Gentamycin, 62.50% to Amikacin, 79.16% to Tobramycin . *Acinetobacter baumanii* showed resistance of 27.27% to Colistin while *Pseudomonas aeruginosa* showed resistance of 16.66%

Table 8: **Showing Antibiotic resistance pattern in Urinary Isolates**

| **Organisms** / **Antibiotics** | ***E.coli* (n=14)** | ***K.pneumoniae* (n=11)** | ***P.aeuroginosa* (n=6)** | ***A. baumanii* (n=5)** | ***Citrobacter* spp. (n=1)** | ***P.mirabilis* (n=1)** |
|---|---|---|---|---|---|---|
| Nitrofurantoin | 9 (64.28) | 5 (45.44) | 3 (50) | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| Norfloxacin | 14 (100) | 7 (63.63) | 5 (83.33) | 5 (100) | 1 (100) | - |

*Acinetobacter baumanii*, *Citrobacter* spp. and *E. coli* showed 100% resistance to Norfloxacin 64.28% 0f *E. coli*, 50% of *Pseudomonas aeruginosa* and 45.44% of *K. pneumoniae* showed resistance to Nitrofurantoin.

## IV. DISCUSSION

One of the major problems of human medicine today is the rapid emergence, spread and increase in the resistance of multidrug resistant pathogenic bacteria to readily available antibiotics. This growing resistance of pathogens to antibiotics is a challenge to medical health practitioners when it comes to treating and managing most infections caused by these multidrug resistant organisms. Clinically important bacteria including *E. coli, K. pneumoniae and P. aeruginosa* amongst others become resistant to drug classes such as β-lactams, Fluroquinolones, Aminoglycosides and even the Co-trimoxazoles following the production of antibiotic degrading enzymes including Extended-spectrum beta lactamases (ESBLs) and Metallo-β-lactamases (MBLs) which they acquire as a result of genetic mutation and selective pressure posed by prior antibiotic usage. ESBL and MBL producing bacteria have serious clinical implications as they are usually associated with high rate of morbidity, mortality, increased length of hospital stay and high treatment cost. Several studies both nationally and internationally[23,24,25,26,27] have assessed and given proven evidences of the presence of these enzymes in both the community and hospital environments[25,28,29]. There is need for sensitive diagnostic methods for ESBL and MBL detection in order to guide therapy, monitor the development of resistance in both the community and hospital settings and to implement any interventional measures as a way of curtailing the economic and clinical loss they present. In view of the underlying circumstances, the present study detected phenotypically MBL producing Gram negative bacteria from various clinical isolates of patient that attended a Tertiary hospital in central India. As shown in Figure-1, in our study distribution of Gram negative bacilli was as follows- *Klebsiella pneumoniae* (39.07%) was the most common organism isolated. This was similar to other studies conducted by Nevine et al (41.17)[30], Shobha et al (45.62%)[31] and Datta et al (35.7%)[32]. In our study 10.08% of total *Klebsiella pneumoniae*, 10.22% of total *E. coli*, 13% of total *Pseudomonas aeruginosa*, 10.5% of total *Acinetobacter baumanii*, 4.82% of total *Citrobacter* spp., 3.77% of total *Proteus mirabilis* were screen test positive i.e, showing Imipenem resistance. Whereas Kanchanadevi et al[33] noted 16.23% of *E.coli*, 27.27% of *P.aeruginosa*, 1.24% of *K.pneumoniae*, 0.64% of *P.mirabilis* and 0.64% of *Citrobacter* spp. to be Imipenem resistant.

**Table-9: Showing MBL detection in various studies by Combined disc test.**

| Sr .No. | Study | Combined disc test (%) |
|---|---|---|
| 1. | Franklin et al[34] | 100 |
| 2. | Pandya et al[19] | 96.30 |
| 3. | Galani et al[35]. | 94.7 |
| 4. | Present study | 98.76 |

Using E-test as the gold standard all 162 isolates showed MBL production. The E-test MBL results as proved in study by Walsh et al in 2002 were in 100% agreement with the results from the genotypic and biochemical methods[36]. The E-test MBL strip has the ability to detect Metallo-β-lactamases, both chromosomally and plasmid mediated, in aerobic and anaerobic bacteria[37].This method can be used by clinical laboratories to monitor the emergence of Metallo-β-lactamases in a range of clinically significant bacteria[37].

**Table-10: Showing prevalence of MBL in different studies**

| Sr. No. | Study | Prevalence of MBL (%) |
|---|---|---|
| 1. | Pandya et al[19] | 6% |
| 2. | Yong et al[20] | 6.5% |
| 3. | Deshpande et al[38] | 7.33% |
| 4. | Chaudhary et al[39] | 8.4% |
| 4. | Present study | 8.08% |

highest prevalence of MBL was seen among isolates from ICUs (19.77%); this was followed by Burns ward (10.54%), Surgery ward (10.29%), Orthopaedic wards (5.03%), OBGY wards (4.52%), Medicine wards (3.04%) etc. Vikas kumar et al[40] showed the highest number of MBLs in ICUs (25%) Wankhede et al[14] revealed the higher number of MBL producers from ICUs (57.63%) overall maximum MBL production was shown by *P. aeruginosa* similar to study done by Kamble et al[41] amongst members of *Enterobacteriaceae* maximum MBL production was shown by *K. pneumoniae* similar to study done by Wadekar et al[42]. among all the samples, 10.65% of the total wound swabs were positive for MBL, 9.25% of tracheal secretions, 8.64% of urine, 7.96% of pus, 5.05% of ascitic fluid and 3.41% of pleural fluid,5.88% of blood were positive for MBL production. In study by Hisaaki et al[43] 4.1% of Pus and 2.1% of Blood were positive for MBL production which is comparable to our study. In study by Wadekar et al[42] 11.5% of Urine samples were positive for MBL production which is comparable to our study. High number of positivity in wound swab, tracheal secretions and urine reveals that such organisms might have been acquired by the patients from the hospital environment. So from this we can clearly state that infections possessing MBL producing organisms are predominantly hospital acquired. Total 151 isolates (93.20%) showed sensitivity to Colistin. Out of this 7 isolates showed MIC of 1µg/ml, 128 isolates showed MIC of 1.5µg/ml and 16 isolates showed MIC of 2µg/ml. Sensitivity to Colistin is 67% In study by Prasanth Manohar et al[44], 100% in study by Deshmukh et al[18] and Naveenkumar et al[45] which is comparable to our study. Amongst the members of Enterobacteriaceae family *E.coli* showed a resistance of 8.69% to Colistin *Acinetobacter baumanii* showed resistance of 27.27% to Colistin while *Pseudomonas aeruginosa* showed 16.66% whereas S.Mishra et al[46] noted resistance of 58.95% to Colistin

Overall antibiotic resistance pattern of our study is comparable with studies done by Chakraborthy et al[47], Deshmukh et al[18], S. Mishra et al[46] and Chaudhary et al[39].

## V. CONCLUSION-

Highest prevalence of MBL producing organisms was from ICUs due to the unempirical use of higher antibiotics and invasive infections. Hence it is must to perform culture and sensitivity of every patient before the augment of Antibiotic therapy. Multidrug resistance was found to be significantly higher in MBL producing organisms. Such a broad spectrum resistance is a matter of concern and necessitates the restricted use of last resort drugs like Carbapenems and other suitable alternatives.There is a need for rational use of antimicrobials and strict adherence to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. Otherwise there is coming a time when our 'magic bullets' (Carbapenems and other antibiotics) are no longer 'magic' or 'bullets'.Large number of MBL producers were sensitive to Colistin thus giving a ray of hope for patients with MBL infections, but excessive reliability on this option can cause increase in resistance and thus end of antibiotic era, because we will be left with no other options in such MDR infections.To prevent the spread of MBL producing organisms, infection control precautions like barrier nursing, cohorting of patients and nurses, attention to hand washing are essential.Development of infection control policy and hospital antibiotic prescribing guide should be followed. Education of medical and nursing staffs, patients, visitors and medical students through posters and meetings could play an important role. If clinical microbiology laboratories in particular are incompetent of providing a standard of quality in terms of promptly and correctly detecting multidrug resistant organisms in their routine work, resistant strains of pathogens will flourish, patients condition will worsen and the spread of infection in the form of uncontrolled antimicrobial resistance is foreseeable. It is becoming trendier in India for people to obtain and abuse broad spectrum antibiotics even without a doctor's prescription in over-the counter (OTC) medications. In the present study we found that Metallo-β-lactamase was the predominant cause of Carbapenem resistance. Combined disc test and E-test are simple, cost effective and highly accurate tests for MBL detection. Hence these tests can be used in Laboratories where molecular diagnostic techniques like PCR are not available..

## VI. REFRENCES-


1. Birgy A, Bidet B, Genel N, Doit C, Decre D, Arlet G and Bingen E. Phenotypic screening of Carbapenemases and Associated β-Lactamases in Carbapenem- resistant Enterobacteriaceae. Journal of Clinical Microbiology. 2012;50(4):1295-1302.
2. Arif Maqsood Ali, Brig Shahid Ahmed Abbasi, Mubasher Ahmed. Frequency of Extended spectrum Beta-Lactamases (ESBl) producing Nosocomial Isolates in a Tertiary care Hospital in Rawalpindi. Pakistan Armed Forces Medical Journal. 2009;59(2).
3. Fatima H M, Chanawong A, Kevin G K, Birkenhead D, and Hawkey P M. Detection of Extended spectrum Beta Lactamases in members of the family Enterobacteriaceae: Comparison of MAST DD test, the double disc and E test ESBL. Journal of Antimicrobial Chemotherapy. 2000; 45:881-885.
4. Nurul M A, Loo H K C, Subramaniam G, Wong E H, Selvi P, Ho S, Kamaraluzaman A, Parasakthi N. Faecal prevalence of Extended spectrum Beta Lactamase producing coliforms in a geriatric population and among hematology patients. Malaysian J Pathol. 2005;27(2):75-81.
5. V K Tukaram, S L Nilekar, N Kantikar Shivraj. Dept. of Microbiology, A. C. P. M. Medical college Dhule. ESBLs- A challenge to the Clinician. Indian Medical Gazette. 2012;85-88.
6. Gupta V. An update on newer Beta Lactamases. Indian Journal of Medical Research. 2007;126:417-427.
7. Koneman E W, Allen S D, Janda W M, Schreckenberger P C, Win W C, editors. Antimicrobial Susceptibility Testing. In: Color atlas & textbook of Diagnostic Microbiology, 7th ed. Philadelphia: JB Lipincott Co; 2017:1074-1171
8. Daoud Z, E. Hobeika, A. Choucair. Isolation of the first Metallo-Beta-Lactamase producing *Klebsiella pneumoniae* in Lebanon. Rev Esp Quimioter. 2008;21:123-126
9. Luzzaroa F, A. Endimiania, J.D. Docquierb, C. Mugnaioli and G. Amicosante et al. Prevalence and characterization of Metallo-Beta-Lactamases in clinical isolates of *Pseudomonas aeruginosa*. Diagn. Microbiol. Infect Dis. 2004;48:131-135.
10. Walsh, T R, M A Toleman, L Poirel, P Nordmann. Metallo-Beta-Lactamse: The quiet before the storm? Clinical Microbiology Reviews. 2005;18:306-325.
11. Sader H S, A O Reis, S Silbert, A C Gales. IMPs, VIMs and SPMs: The diversity of Metallo-Beta-Lactamase produced by Carbapenem resistant *Pseudomonas aeruginosa* in a Brazilian hospital. Clinical Microbiology and Infectious Diseases. 2005;11:73-76.
12. Senda K, Y Arakawa, K Nakashima, H Ito, S Ichiyama et al. Multifocal outbreaks of Metallo-Beta-Lactamase producing *Pseudomonas aeruginosa* resistant to broad-spectrum beta lactams, including Carbapenems. Antimicrobial Agents and Chemotherapy. 1996;40:349-353.
13. Watanabe S, Iyobe S, Inoue M, Mitsuhashi S. Transferable Imipenem resistance in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 1991;35:147-51.
14. Wankhede S V, Iyer V S, Bharadwaj R S. The study of MBL producers in Gram negative isolates from ICU and Wards in BJMC Pune. Indian Journal of Basic and Applied Medical Research. 2011;1(1):38-46.
15. Rolain J M, Parola P and Cornaglia G. New Delhi metallo beta lactamase (NDM-1): towards a new pandemia? Clinical Microbiology and Infection. 2010;16:1699-1701
16. Forbes B A, Daniel F Sahm, Alice S Weissfield. General issues and role of Laboratorians. In: K Fabiano, Sarahly L, Ellen Wurm, editors. Bailey and Scotts Diagnostic Microbiology, 14th edition. Mosby Elsevier;2017:56-71.
17. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Sixth Informational Supplement. CLSI document M100-S26.Wayne, PA: Clinical and Laboratory Standards Institute. 2016;33 (1).
18. Durgesh Gopalrao Deshmukh, Ajit S Damle, Jyoti K Bajaj, Jayshree B Bhakre, Neeta S Patwardhan. Metallo-β-lactamase-producing Clinical Isolates from Patients of a Tertiary Care Hospital. Journal of Laboratory Physicians. 2011;3(2):93-97.

19. Nirav P. Pandya, Sweta B. Prajapati, Sanjay J. Mehta, Kunjan M. Kikani, Pratima J. Joshi. Evaluation of various methods for detection of metallo-β-lactamase (MBL) production in gram negative bacilli. Int J Biol Med Res. 2011;2(3):775-777.
20. Dongeun Yong, Kyungwon Lee, Jong Hwa Yum, Hee Bong Shin, Gian Maria Rossolini, Yunsop Chong. Imipenem-EDTA Disk Method for Differentiation of Metallo-Beta-Lactamase-Producing Clinical Isolates of *Pseudomonas* spp. and *Acinetobacter* spp. Journal of Clinical Microbiology. 2002;40(10):3798–3801.
21. Performance standards of Antimicrobial Disc Susceptibility Tests, M100 S21 CLSI. 2011;31(1).
22. Performance standards of Antimicrobial Susceptibility Testing. Twenty Seventh Informational Supplement. M100-S27. 2017;37(1).
23. Iroha I R, Amadi S E, Adikwu M U, Esimone C O. Detection of plasmid borne ESBL enzymes in clinical isolates of *Escherichia coli* from a Community General Hospital. International J of Molecular Medicine and Advanced Sciences. 2008;4(2):46-49.
24. Spanu T, Luzzaro F, Perilli M, Amicosante G, Toniolo A, Fadda G and The Italian Study Group. Occurrence of ESBL in members of the family Enterobacteriaceae in Italy: Implication for Resistance to Beta Lactam and other Antimicrobial Drugs. Antimicrobial Agents Chemotherapy. 2002;46(1):196-202.
25. Bashir D, Thokar M A, Fomda B A, Bashir G, Zahoor D, Ahmed S, Taboli A S. Detection of MBL producing *Pseudomonas aeruginosa* at Tertiary care Hospital in Kashmir. African J of Microbial Research. 2011;5(2):164-172.
26. Sturenburg E, Sobottka I, Noor D, Laufs R, Mack D. Phenotypic detection of MBL producing *Pseudomonas aeruginosa* strains isolated from Burned patients. Iranian J of Pathology. 2008;3(1):20-24.
27. Kohler J, Karen L, Dorso, Young K, Gail G, Hammond, Rosen H, Kroop H, Silver L. In vitro activity of the potent, broad spectrum carbapenem MK-0826 (L749, 345) against broad spectrum Beta Lactamase and Extended spectrum Beta Lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* clinical Isolates. Antimicrobial Agents and Chemotherapy. 1999;43(5):1170-1176.
28. Bonnet R. Growing group of Extended spectrum Beta Lactamases: The CTX-M enzymes. Antimicrobial Agents Chemotherapy. 2004;48(1):1-14.
29. Bush K, Jacoby G A. Updated Functional Classification of Beta Lactamases. Antimicrobial Agents Chemotherapy. 2010;54(3):969-976.
30. Nevine S. Fam, Mervat M. El-Damarawy. CTX-M-15 Extended-Spectrum Beta-Lactamases Detected from Intensive Care Unit of an Egyptian Medical Research Institute. Research Journal of Medicine and Medical Sciences. 2008;3(1):84-91.
31. Shobha K.L, Ramachandra L, Rao G, Majumder S, Rao S P. Extended Spectrum Beta-Lactamases (ESBL) In Gram Negative Bacilli At A Tertiary Care Hospital. Journal of Clinical and Diagnostic Research. 2009;3:1307-1312.
32. Priya Datta, Archana Thakur, Bibabhati Mishra, Varsha Gupta. Prevalence of Clinical Strains to Various Beta lactams in a Tertiary Care Hospital in India. J. Infect Dis. 2004;57:146-149
33. P.Kanchanadevi, S.Chandra Sekaran. Importance of EDTA in the Detection of Metallo Beta Lactamase from Imipenem Resistant Gram Negative Bacilli. Int.J.Curr.Microbiol.App.Sci. 2016;5(11):702-706.
34. Clare Franklin, Lisa Liolios, and Anton Y. Peleg. Phenotypic Detection of Carbapenem-Susceptible Metallo-Beta-Lactamase-Producing Gram-Negative Bacilli in the Clinical Laboratory. Journal of Clinical Microbiology. 2006;44(9):3139-3144.
35. Irene Galani, Panagiota Danai Rekatsina, Despina Hatzaki, Diamantis Plachouras, Maria Souli, Helen Giamarellou. Evaluation of different laboratory tests for the detection of metallo-b-lactamase production in Enterobacteriaceae. Journal of Antimicrobial Chemotherapy. 2008;61:548–553.
36. Walsh T R, Bolmstrom A, Qwarnstrom A, Gales A. Evaluation of a New Etest for Detecting Metallo-β-Lactamases in Routine Clinical Testing. Journal of Clinical Microbiology. 2002;40:2755-9.
37. Carvalhaes C G, Picao R C, Nicoletti A G, Xavier D E, Gales A C. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in *Klebsiella pneumoniae*: be aware of false positive results. Journal of Antimicrobial Chemotherapy. 2010;65:249-51
38. Deshpande P, Rodrigues C, Shetty A, et al. New Delhi Metallo- beta lactamases (NDM-1) in Enterobacteriaceae: treatment options with carbapenems compromised. Journal of the Association of Physicians of India. 2010;58:147-9.
39. Chaudhary AK, Bhandari D, Amatya J, Chaudhary P, Acharya B. Metallo-Beta-Lactamase Producing Gram-Negative Bacteria among Patients Visiting Shahid Gangalal National Heart Centre. Austin Journal of Microbiology. 2016;2(1):1010.
40. Vikas Kumar, Malay Ranjan Sen, Shampa Anupurba, Pradyot Prakash, Richa Gupta. An Observational Study of Metallo Beta Lactamase production in Clinical Isolates of *Pseudomonas aeruginosa*: An Experience at a Tertiary care hospital in North India. Indian J. Prev. Soc. Med. 2011;42(2):173-176.
41. Miss. Deepali Shivajirao Kamble. Phenotypic detection of ESBL and MBL in Gram Negative bacilli isolated from clinical specimens. International Journal of Medical Research and Review. 2015;3(8):866-870
42. Mita D.Wadekar, K.Anuradha and D.Venkatesha. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. Int J Curr Res Aca Rev. 2013;1(3):89-95.
43. Hisaaki Nishio, Masaru Komatsu, Naohiro Shibata, Kouichi Shimakawa. Metallo-Lactamase-Producing Gram-Negative Bacilli: Laboratory-Based Surveillance in Cooperation with 13 Clinical Laboratories in the Kinki Region of Japan. J of Clinical Microbiology. 2004;42(11):5256–5263.
44. Prasanth Manohar, Thamaraiselvan Shanthini, Ramankannan Ayyanar,Bulent Bozdogan, Aruni Wilson,Ashok J. Tamhankar, Ramesh Nachimuthu, Bruno S. Lopes. The distribution of carbapenem- and colistin-resistance in Gram-negative bacteria from the Tamil Nadu region in India. Journal of Medical Microbiology. 2017;66:874–883.

45. Naveenkumar.C, Swathi.S, Srikumar.R. Metallo-beta lactamase (MBL) detection in Multi drug resistant Gram negative bacilli (MDR-GNB) isolates. Journal of Innovative Research and Solutions. 2014;1(1):-7
46. Surya Narayan Mishra, Seba Ranjan Biswal, Basanta Kumar Behera, Dipti Pattnaik. Detection of prevalence of metallo-beta lactamases in clinical isolates of imipenem resistant *Pseudomonas aeruginosa* from neonatal septicaemia cases in a tertiary hospital in Odisha, India. International Journal of Contemporary Pediatrics. 2018;5(1):61-66.
47. Chakraborty D, Basu S, Das S. A Study on Infections Caused By Metallo Beta Lactamase Producing Gram Negative Bacteria in Intensive Care Unit Patients. American Journal of Infectious Diseases. 2010;6:34-9.