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ORIGINAL RESEARCH

Study on the Prevalence of Beta Lactamases in Gram Negative Clinical Isolates

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ABSTRACT

Background: Antimicrobial resistance is really a threat to clinical practice. Bacteria develop various mechanisms of resistance to different group of antibiotics. Antimicrobial resistance is the resistance of a microorganism to an antimicrobial drug that was originally effective for treatments of infections caused by it, by acquiring genes coding for it the consequences of antibiotic resistance lead to longer duration of illness, treatment with expensive drugs, higher mortality and increased burden on the health system. The predominant mechanism for resistance to beta lactam antibiotics is production of beta lactamases. Based on this aim of our study is to find out the various β lactamases producing gram negative bacteria from the samples received from outpatients and inpatients admitted in our hospital.

Methods: A prospective study was conducted for a period of one year in a tertiary care teaching hospital. Those who were resistant to one of the 3rd generation cephalosporin were selected as suspicious beta lactamase producer. These isolates were subjected to confirmatory test for ESBL, Amp C and Carbapenamases and MBL.

Results: 492 gram negative samples were obtained during the study period among which 204 isolates showing resistance to one of the III generation cephalosporins, were taken for beta lactamase detection. The antibiotic susceptibility profile of our study revealed that susceptibility of beta lactamase producers to Imipenem and Amikacin were found to be 87.8%, and 83.3% respectively. Majority of the isolates found to have decreased susceptibility to Ampicillin and Amoxyclav drugs. The prevalence of beta lactamase production in GNB in our study was found to be 41.4%.Total ESBL producer was 32.94%. The occurrence of beta lactamases producers in various isolates in this study showed, that E.coli was the common ESBL producer. Total Amp C production was 14.22%. The major Amp c producer was Klebsiella species. Total MBL production was 5.48%. There were few combinations too.

Conclusion: The study conducted in our hospital highlights the emerging prevalence of beta lactamases. The consequences of antibiotic resistance lead to longer duration of illness, treatment with expensive drugs, higher mortality and increased burden on the health system.

Keywords: beta lactamase, gram negative, resistance

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 01, 2023

INTRODUCTION

Antimicrobial resistance is really a threat to clinical practice. Bacteria develop various mechanisms of resistance to different group of antibiotics. Antimicrobial resistance is the resistance of a microorganism to an antimicrobial drug that was originally effective for treatments of infections caused by it, by acquiring genes coding for it. Antimicrobial resistance is a global concern, as new resistance mechanism evolve, making the treatment of even common infections difficult resulting in disability or death of individual.

β lactamases production is one of the most important mechanisms of microbial resistance to β-lactam antibiotics which consist of four major groups such as ,Penicillins, Cephalosporins, Monobactams, and Carbapenems. Excessive use of antibiotic results in selective pressure, enabling resistant bacteria to thrive, and the beta lactamase gene undergoes continuous mutation[1] Certain acquired mechanism such as plasmids help in exchange of resistant determinants which has become a threat to clinicians as these cause rapid dissemination of genes between species.

The enzymes produced by bacteria are usually distinguished into four types ,out of which 3 form major group ,such as extended spectrum β-lactamases (ESBL), class C Cephalosporinases (Amp C)) and β-lactamases with carbapenemase activity in which metallo -β-lactamases (MBL) is of great concern today.[2]*E.coli* and *Klebsiella* are leading causes of pneumonia, UTI, diarrhea, cholecystitis, meningitis. β lactam antibiotics has been the drug of choice in treating these conditions. However multiple antibacterial resistances in recent decades among enterobacteriaceae are commonly met and the above conditions and pose a problem in management.[3]

Over the past few years the occurrence of multidrug-resistant Gram-negative bacteria has increased due to multiple mechanism of resistance, which is a continuous phenomenon. The major concern is the production of AmpC beta-lactamases and/or extended-spectrum beta-lactamase in bacterial strains. ESBL's confer resistance to, Cephalosporins, Oxyimino-cephalosporins (e.g., Ceftriaxone, Cefotaxime, and ceftazidime) Amino- penicillins and AmpC beta-lactamases in addition are resistant to cephamycin (e.g., cefoxitin) and monobactams.[4]

ESBLs are still a threat to clinicians as they are coded by plasmid and can be easily transmitted between species. ESBL producing organisms are highly effective in inactivating Pencillins, most Cephalosporins and Aztreonam

Amp C beta lactamases are of either chromosomal, or plasmid mediated. The transfer of chromosomal genes onto plasmids results in Amp C β lactamases called as inducible Amp c lactamases. Hence isolates of *Enterobacter aerogenes, Salmonella, Escherichia coli, Klebsiella, and Citrobacter* have acquired plasmid mediated Amp C beta lactamases.[5]

Various risk factors are involved for the infection or colonization with the ESBL producing organisms. Prolonged stay in the hospital or ICU, Persons with vascular or urinary catheters, and those undergoing hemodialysis or emergency abdominal surgery also fall in this group. The prior antibiotic therapy to any antibiotic such as Aminoglycoside, Quinolones, Trimethoprim-Sulfamethoxazole, and Metronidazole or gut colonization with resistant bacteria also predisposes to acquisition of a resistant microbe.[6]

With the introduction of carbapenem, there came an end to war against antimicrobial resistance till carbapenamase enzymes emerged. These were useful drugs as they had broad spectrum of activity to combat infection caused by Pencillin and Cephalosporin resistant bacteria resistant. Resistance to carbapenem is predominantly mediated by metallo-betalactamases, a class B type of betalactamases that require bivalent metal ions for activation. Hence detection of these β lactamases producing organisms becomes important for effective therapeutic approach and enhanced infection control. This study aims at detecting the prevalence of ESBL, Amp C,

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 01, 2023

Carbapenamases production and their combinations {ESBL +Amp C; ESBL +MBL; Amp C +MBL} in the isolates.

MATERIALS AND METHODS

A prospective study was conducted for a period one year in the department of Microbiology in a tertiary care teaching hospital. The present study aims to find out the various β lactamases producing gram negative bacteria from the samples received from outpatients and inpatients, who were admitted to different wards in our hospital. 492 Gram negative bacilli (GNB) were isolated from various clinical specimens during study period.

Isolates obtained from various clinical samples sent to the microbiology laboratory were identified by standard microbiological techniques. The antimicrobial susceptibility testing was carried out by the disc diffusion method by Kirby Bauer according to CLSI guidelines. Antimicrobial discs [Himedia Mumbai] used the isolates showing resistance to any of the 3GC were stored in stock vials for further processing. Media and disks were tested for quality control with standard strains.

All gram negative clinical isolates from both sexes of all age group Enterobacteriaceae and nonfermenters showing resistant to any of the third generation cephalosporins were tested for beta lactamase production (ESBL, Amp C Carbapenamase) were included in the study. Whereas Non Enterobacteriaceae isolates, those samples which showed mixed growth and Isolates sensitive to all antimicrobial disc tested were excluded

The isolates stored in the stock vials were sub cultured at the time of testing. The purity and viability of the isolates was checked. They were subjected to various phenotypic methods of β lactamases detection along with MIC determination. Agar dilution technique was used for MIC was determination. Inoculum was prepared and those specimens which showed resistance to one of III generation cephalosporins was subjected to other tests for detection of ESBL, Amp C, and Carbapenamase production.

Phenotypic tests were done to confirm ESBL i.e. combined disc test which uses Ceftazidime [30µg] and Ceftazidime-clavulanic [30/10µg discs). Cefoxitin disc was used for Amp C detection among the isolates. Those which showed Cefoxitin zone of inhibition ≤18 mm were taken as Amp C positive. Then resistant isolates (Cefoxitin resistance) was subjected to Amp C disk test. The AmpC production is indicated by an indentation or a flattening of the zone of inhibition.

The carbapenamase production was detected by the Modified Hodge test and Imipenem – EDTA combined disc test. Clover leaf pattern in Modified Hodge test was taken as positive. The organisms were considered to be MBL producers if the increase in the zone of inhibition between Imipenem and Imipenem –EDTA (10µg/750µg) {Himedia} disc was ≥ 7mm in combined disc test.The observations of the study were recorded and analyzed. The results were compared and discussed by using SSPS software and Chi Square test.

RESULT

Among 492 specimens 204 showed resistance pattern. The specimens received were from various age groups, predominantly falling in the age group of 40 -60 years (44.12%), the clinical isolates were obtained from 97 male patients and 107 female patients.

The various clinical specimens from which these isolates were obtained are as follows- urine (100), pus (58), sputum(30),wound swab (4), cervical swab (2), high vaginal swab(2), aural swab(1), catheter tip (3), tracheal tip(1) ,tissue(2) and blood (1). In our study the urinary tract infections were predominant 49.01%, (100/204), followed by skin and soft tissue infections

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 01, 2023

30.4%% {pus samples 58/204 and wound swab 4/204} and respiratory infections was 14.7%. (30/204)

Among the 204 isolates, 161 isolates belong to family enterobacteriaceae and nonfermenters constituted 43 isolates. Out of the 161, 105 samples showed growth of *E.coli,* 51 had *Klebsiella* species, 02 were *Enterobacter* species and 03 were *citrobacter* species. Among the nonfermenters, 37 were *Pseudomonas* species and 06 belong to *Acinetobacter* species.

Table 1

Organism	Number	Percent
E.coli	105	51.47%
Klebsiella spp.	51	25%
Pseudomonas spp.	37	18.14%
Acinetobacter spp.	6	2.94%
Citrobacter spp.	3	1.47%
Enterobacter spp.	2	0.98%
Total	204	100%

E.coli was the common organism isolated among the samples from various wards. In the medical ward the common organism isolated was E. coli (54.3%) followed by Pseudomonas (25.7%). It was observed that E .coli was also common organism among the specimens of surgical wards (38.6%) and ICU (48.9%). Klebsiella species was the second common isolate in ICU specimens (33.3%) and in surgical wards (29.8%).Pseudomonas was almost equal among the specimens from various wards in IPD except in Pediatric ward which showed the least number of isolates.

The antibiotic susceptibility test was done by Kirby Bauer technique to determine the susceptibility pattern of isolates the interpretation was done according to CLSI guidelines. Sensitivity to Quinolones, Cotrimaxole, Nitrofurantoin and Gentamicin varied from 20% - 44%. Most of the species were highly sensitive to Amikacin, and Imipenem, 83.3%and 87.8% respectively.

Most of the species were resistant to Ampicillin. Among the E.coli(n =105) resistance to Ciprofloxacin was observed in 83 cases , Cotrimaxole resistance in 85 cases , Nitrofurantoin resistance in 40 cases, and resistance to and Amikacin was 11 and Imipenem was12 cases..

Of the 51 isolates of Klebsiella species included in the study resistance to Ciprofloxacin was observed in 34 cases, Cotrimaxole resistance in 34 cases, Nitrofurantoin resistance in 48 cases, and resistance to Imipenem and Amikacin was observed in 08 and 13 cases respectively.

Of the 37 isolates of Pseudomonas, 12 were resistance to Ciprofloxacin, 34 were Cotrimaxole resistance, resistance to Amikacin and Imipenem was 13 and 07 respectively.

Next we analyzed ESBL production in clinical isolates and 162 among 204 specimens was combined disc positive. Amp C production (cefoxitin resistance) was tested by disc diffusion method according to CLSI guidelines strains exhibiting zone diameter ≤ 18mm were taken as resistant, ≥ 21 mm as sensitive and 18 – 21 as intermediate sensitive , 129 were sensitive and rest 75 were resistant . And those 75 isolates which showed resistance to cefoxitin was subjected to AmpC disc test, 50 of them showed indentation, and 20 showed flattening, while 5 isolates did not give positive results.

Next the sensitivity test was done for Imipenam, Meropenam by disc diffusion test. Isolates were considered sensitive if in the range of zone diameter ≥23 mm, resistant if zone diameter ≤19mm and intermediate if between 20 -22mm diameter.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 01, 2023

Meropenem sensitivity was detected by disc diffusion method and MIC determination was done by Agar dilution technique. Isolates were considered sensitive if zone diameter was ≥ 23mm, intermediate sensitive if in range of 20- 22 mm, resistant if zone diameter was ≤ 19mm, among which 179 were sensitive and rest 25 were intermediate and resistant for meropenam, whereas 176 were sensitive and rest 28 were either intermediate or resistant.

Next MBL detection by phenotypic methods: All the strains were subjected to MBL detection by Modified Hodge test and combined disc test. Among 25 imipenam resistant cases hodges test was positive in 16 and among 28 meropenam resistance specimens Hodge test was positive in 18 specimens.

In agar dilution method all those strains which have MIC ≤1μg/ml are sensitive, 2-4μg /ml are intermediate strains and which have MIC range ≥4μg/ml are resistant. Of the 204 isolates, 179 isolates had resistance to Ceftazidime, while 25 isolates were resistant to other third generation Cephalosporins (Cefotaxime, Ceftriaxone).

Table 2: Detection of resistance genes by PCR

Organism	No. tested	TEM positive	SHV positive	IMP positive
Escherichia coli	4	4	4	0
Klebsiella pneumoniae	4	4	4	1
Pseudomonas	2	2	2	1

Randomly selected 10 study isolates were tested for resistance genes (TEM, SHV, IMP) by PCR. Out of 10, TEM and SHV genes were positive in all isolates, 2 isolates were positive for IMP gene. TEM, SHV gene were identified in Klebsiella pneumoniae, Escherichia coli and Pseudomonas species. IMP gene was detected in Klebsiella spp, and Pseudomonas spp.

The beta lactamases occurred either singly or in combination .ESBL was common beta lactamase produced. About 118 isolates produced ESBL alone, 27 isolates Amp C alone, 13 isolates MBL only. Combination of various enzymes was also noted. ESBL and Amp C found in 31 cases , ESBL and MBL in 3cases ,Amp C and MBL in 2 cases, all three enzymes in 10 cases. Thus total ESBL production was in 162 cases, total Amp C production seen in 70 cases, and MBL in 28 cases.

Most of the E.coli isolates showed predominant production of ESBL while AmpC was common in Klebsiella spp, and Pseudomonas spp. AmpC and ESBL were found in OP specimens also. Only 1 isolate showed resistance to all three enzymes.

DISCUSSION

Antimicrobial resistance is a growing threat worldwide. Increasing resistance to third generation cephalosporins has become a cause for concern among Enterobacteriaceae.[7]The efficacy of beta lactam antibiotics has been reduced by production various types of beta lactamases. The indiscriminate use of the beta-lactam antibiotics offers selective pressures which lead to development of selected mutated forms of β-lactamases such as the ESBLs, AmpC β-lactamases and metallo-β-lactamases which have emerged as the most important resistance mechanism. This poses a therapeutic challenge to the health care settings.[8]Since Amp C production is accompanied by multidrug resistance, therapeutic options become limited and failure to identify them, results in inappropriate treatment. The occurrence of extended spectrum β lactamases (ESBLs) and AmpC among members of Enterobacteriaceae are common. Nowadays therapeutic failure is common among beta lactam antibiotics.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 01, 2023

The members of Enterobacteriaceae are the most frequent human pathogen in causing infections and isolated many a times from clinical samples. In the present study, a total of 204 samples showing resistance to Ceftazidime, Cefotaxime or Ceftriaxone were selected from 492 GNB samples. These consisted of urine constituting 49.01% (100/204), pus 28.43% ((58/204) and sputum 14.7% (30/204).

Our study showed that among the isolates, E. Coli was predominant accounting to 51.47% (105/204) followed by Klebsiella spp about 25% (51/204), Pseudomonas 18.13% (37/204), Acinetobacter 2.94% (6/204) Citrobacter 1.47% (3/204)and Enterobacter0.98% (2/204). This finding was on par with other studies by Metri et al[9] and Mathur et al[10] where the E.coli was the predominant organism isolated.

The antibiotic sensitivity pattern of beta lactamase producers revealed that maximum susceptibility was seen for Imipenem and Amikacin 87.8%, and 83.3% respectively. Sensitivity to Quinolones, Cotrimaxole, Gentamicin and Nitrofurantoin was 32.4%, 21.1%, 44.62%, and 40.2% respectively. Most of the isolates showed decreased susceptibility to Ampicillin and Amoxyclav drugs, 2.45% and 6.37% respectively.

In our present study, 492 gram negative clinical isolates were collected to study the prevalence of beta lactamases. Out of 492 isolates, 204 showed resistance to one or more of third generation cephalosporins. The prevalence of beta lactamases production was 204/ 492 isolates accounting for 41.4%. A study by Vijaya Doddaiaha et al[11] accounts a prevalence of 52.13 % correlating to our study.

In our study, expressions of various β-lactamases occurred, either singly(ESBL ,Amp C , MBL) or in combinations like (ESBL +Amp C , ESBL + MBL, Amp C + MBL , ESBL +AMP C +MBL). The combination of ESBL/MBL/ AmpC β- lactamases was observed in 46 isolates 46 / 204. (Fig 5) Presence of multiple classes of β-lactamases in a single organism may pose a serious challenge in therapeutics.

It was found that the total ESBL producers were 32.93 %(162/492). Among the 162 organisms producing ESBL, we found that 118 strains produced only ESBL (118/162), and further their combinations (ESBL +AmpC) were found in 31 strains (31/162),(ESBL +MBL)was in three strains (03/162) , and ESBL with both AmpC and carbapenemase was in 10 strains (10/ 162) . These results were comparable with the study from Karnataka by Vijay Doddehia et al[11] where ESBL production was34.04%. 30.8% isolates were ESBL positive in a study by Rajesh Bareja at Haryana[12], Babypadmini et al[13] studies on ESBL showed prevalence to be 40% which are comparable to our study.

ESBL production was highest in E.coli (61.9%) isolates and followed by Klebsiella species 18.6%. Since the number of isolates of Enterobacter, Citrobacter and Acinetobacter are less, they are statistically not significant. Detection rates across the country vary from 27.2% to 63.7% in E. coli for ESBL and 14% to 97.1% in K. pneumoniae. Sreekanth et al, report that among ESBL producers, 72.6% isolates of E. coli, and 22.6% isolates were Klebsiella spp.[14]correlating with our study .Rudresh et al from Bangalore too reported ESBL production is highest in E .coli about 65%.[15] In Pseudomonas spp. ESBL production is 12.7% which is lesser when compared to Enterobacteriaceae. This decreased incidence in Pseudomonas spp may be due other bacterial resistance mechanism such as the lack of drug penetration, the loss of certain outer membrane proteins and due to mutations in the porins and efflux pumps.

Amp C:

In the present study, prevalence of Amp C was 14.22 % (70/492).27 isolates produced only Amp C, 31 isolates of AmpC coexisted with ESBL. 2 isolates of MBL coexisted with AmpC and all

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 01, 2023

three enzymes occurred in 10 isolates. Rajesh Bareja et al[12] study showed a prevalence of AmpC 15.35% was similar as our study. A study from North India by Vikas Manchanda et al[16] found AmpC enzyme producers among Gram negative bacteria was 20.7%(33) and another study from South India by Mathur et al reported 22.2% of AmpC producers both are concordant with our study.[10] Amp C production was predominant in Klebsiella spp, (34.8%) followed by E.coli (19.45%) in our study.

The only drug of choice in ESBL and Amp C resistant isolates was carbapenems, but due to their irrational use the carbapenem resistance is increasing and mostly due to MBL production. Most isolates were susceptible to Imipenem except 25 isolates (25/204).Resistance to Meropenem was also detected and it was found in 28 isolates (28/204). Except 3, all isolates which showed resistance to Meropenem were also resistant to Imipenem. This was confirmed by MIC, where 28 isolates showed MIC >4µg. In the current study, we majority of the isolates were sensitive to Imipenem, which was concordant with other studies. Similar findings were seen in another study from Coimbatore.[17]

In the current study production of MBL was seen in 5.69 % (28/492). A study from North India [16] shows MBL production to be7.86 %. (68)They attribute this low prevalence of MBL to stringent antibiotic stewardship program. Another study by Nagdeo et al[18] also showed the prevalence of MBL as 7.44%. Results of both studies are similar to our study. The prevalence is low in our study. It may be due to using Carbapenem as a reserve drug for the treatment of multidrug-resistant Gram-negative pathogens only.

The production of all three enzymes (ESBL +Amp C +MBL) was 2.03%. (10/492) All three enzymes were simultaneously detected in (4.21%) of cases in a study by Nagdeo et al which very well correlates with our study. [18]The distribution of the three enzyme classes among all the species shows a characteristic pattern. When resistance was conferred by producing a single enzyme, it was commonly the ESBL producer but AmpC and MBLs were more commonly seen when the multiple enzyme production was observed.

This study results indicate that ESBL production is a major mechanism of resistance to cephalosporins among Gram-negative bacteria. Our study of phenotypes indicative that Enterobacteriaceae produce ESBL, AmpC, and MBL, but with varying frequencies. Over three fourth of strains produce ESBL's; one third of them produce AmpC and about a minimal number of strains produced MBL. While most of the Gram-negative organisms tend to produce ESBL more frequently, Klebsiella spp produces AmpC, and non-fermenters tend to produce AmpC and MBLs.

In our study also we observed that the ESBL and MBL producing isolates were often resistant to other classes of drugs like quinolones and Aminoglycosides. This could be due to co-existence of genes encoding drug resistance to those antibiotics on the plasmids carrying ESBL and MBL.

Considering PCR as the gold standard, we selected few strains to detect the genes for ESBL and MBL. All the ten isolates selected showed the presence of TEM and SHV genes, and 2 isolate showed the presence of IMP gene. The strains which were negative for IMP gene could harbor other carbapenamase genes.

CONCLUSION

The study conducted in our hospital highlights the emerging prevalence of beta lactamases. The consequences of antibiotic resistance lead to longer duration of illness, treatment with expensive drugs, higher mortality and increased burden on the health system. We must have a functional hospital infection control committee appropriate antibiotic policy and regular updates, to prevent

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 01, 2023

the spread of, Amp C, ESBL and MBL, Each and every antibiotic usage in hospital should be documented which will help us to develop dosing strategies. In order to select of appropriate therapeutic schemes additionally, detection and surveillance of beta lactamases production becomes a matter of major importance and implementation of infection control measures.

REFERENCES

1. M J C Noyal GA Marz B N Harish S Sujatha P C Parija .Simple screening tests for detection of carbapenamases in clinical isolates of non fermenting gram negative bacteria. Indian Journal of Medical research June 2001; 129 : 707 -712
2. N. Fam, M. Diab, H. Helmi and I. El-Defrawy. Phenotypic Detection of Metallo-β-Lactamases and Extended Spectrum β-Lactamases Among Gram Negative Bacterial Clinical Isolates. Egyptian Journal of Medical Microbiology, October 2006; Vol. 15: (4) 719- 725.
3. David L. Paterson1* and Robert A. Bonomo. Extended-Spectrum β-Lactamases: a Clinical Update. Clinical Microbiology Reviews. Oct. 2005, Vol. 18(4) : 657–686
4. S.Peter G Etzhalf, S Pols Fuss M. Poledica .Detection of AMP C beta lactamases in E.coli. Comparison of 3 phenotypic confirmatory assay and genetic analysis. Journal of Clinical Microbiology 2011; volume 49 : 2929-2932
5. Neena V. Nagdeo, Navinchandra M. Kaore1, Vilas R. Thombare. Phenotypic method of detection of various beta lactamases in gram negative clinical isolates – need of the Hour. Chronicles of young scientist Oct-Dec 2012 ; Vol. 3(4)
6. Gaurav Dalela. Prevalence of Extended Spectrum beta Lactamase (ESBL) Producers among Gram Negative Bacilli from Various Clinical Isolates in a Tertiary Care Hospital at Jhalawar, Rajasthan. India Journal of Clinical and Diagnostic Research. 2012 April; Vol-6(2): 182-187
7. Mita D.Wadekar*, K.Anuradha and D.VenkateshaPhenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. International Journal of current Research and Review, 2013;1(3)89-95.
8. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum -lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. Indian J Med Microbiol 2006; 24:208-11.
9. Metri Basavaraj, Jyothi P, Peerapur Basavaraj. V The Prevalence of ESBL among Enterobacteriaceae in a tertiary Care Hospiyal in North Karnataka ,India,Journal of Clinical and Diagnostic Research 2011 June; 5(3):470-475.
10. S Singhal, T Mathur, S Khan, DJ Upadhyay, S Chugh, R Gaind, *A Rattan. Evaluation of methods for Amp C β-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian Journal of Medical Microbiology 2005; 23 (2): 120-124.
11. Vijaya Doddaiah , Dhanalakshmi Anjaneya , Prevalence of ESBL, AmpC and carbapenemase among gram negative bacilli isolated from clinical specimens American Journal of Life Sciences 2014; 2(2): 76-81.
12. Rajesh Bareja1, Shinu Pottathil2, Rajesh Kumar Shah3, Prem Singh Grover4, Varsha Ashok Singh5. Simultaneous detection of Extended-spectrum-β-lactamase, AmpC-β-lactamase and Metallo-β-lactamase in gram negative clinical isolates on a single plate. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) Mar.- Apr. 2013; 6(2):74-77
13. Baby padmini S, Appalaraju B. Extended spectrum β-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae -prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol. 2004;22:172–4.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 01, 2023

14. Bhavanarushi Sreekanth, Gogi Suresh Dattaraya detection of extended spectrum β-lactamases (ESBLS) in clinical isolates, International Journal of Medical Science and Public Health 2014; 3 (9).
15. Rudresh SM , Nagarathnamma T Extended spectrum β lactamase producing Enterobacteriace and antibiotic co resistance . Indian journal of medical Microbiology
16. Vikas Manchanda and Narendra P. Singh .Occurrence and detection of AmpC β-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi. India Journal of Antimicrobial Chemotherapy 2003; 51: 415–418.
17. Baby PS, Appala RB, Mani KR. Detection of Enterobacteriaceae producing CTX-M extended spectrum beta-lactamases from a tertiary care hospital in south India. Indian J Med Microbiol 2008; 26:163-6.
18. Neena V. Nagdeo, Navinchandra M. Kaore1, Vilas R. Thombare. Phenotypic method of detection of various beta lactamases in gram negative clinical isolates – need of the Hour. Chronicles of young scientist Oct-Dec 2012 ; Vol. 3(4).