Investigation of the phenotypes of aerobic gram-negative bacteria with emphasis on AmpC β-lactamases and ESBL

Abstract

β-lactamases are a type of β-lactam resistance that is most common. Expanded spectrum β-lactamases (ESBLs), which are now being identified in large numbers all over the world, are an important category of β-lactamses along with inducible AmpC β-lactamases and derepressed mutants. The goal of the current study was to precisely analyze beta-lactamase production in medical isolates, ESBLs, and AmpC β-lactamases, by reordering the traditional discs employed in reporting susceptibility. Klebsiella pneumoniae and Escherichia coli are the two most prevalent bacteria identified in mid stream urine (MSU) samples from all across the world. The primary producers of the extended spectrum β-lactamase, which significantly limits the therapeutic treatment of urinary tract infections, are these uropathogens. A rising problem in the world is bacterial strains that are resistant to antibiotics. Urinary tract infections (UTIs) rank among the most common bacterial diseases in humans in both the community and hospital settings. Klebsiella pneumoniae and Escherichia coli are the two pathogens found in urine that are most frequently seen. Gram-negative microbes continue to gain resistance to β-lactam antibiotic mostly due to β-lactamases. In this study, screening tests and confirmatory tests were conducted along with antibiotic sensitivity testing using the Kirby-Bauer method. Confirmatory test and Screening test for AmpC beta was also done.

Key words: Beta –lactamases, Klebsiella pneumoniae, Escherichia coli, Antibiotic resistance, Kirby- Bauer method.

Introduction

The Gram-negative bacteria that are aerobic majority of bacterial discovered variants in clinical collections, with gram-positive bacteria making up a smaller portion [1], [2]. They can be found in any kind of infectious disease, and have been linked to antibiotic resistance. They are found in both human and animal big intestines and can be found on the outside, within, and in the environment of man [3], [4],[5],[6]. The metabolic pathways used by non-fermentative bacterial species to break down carbohydrates do not include fermentation [7],[8],[9]. Beta-lactamases hydrolyze beta-lactam antibiotics, rendering them inactive and giving rise to inactive substances. Some bacteria develop beta-lactamases, making them resistant to beta-lactam antibiotics [10],[11],[12]. The main cause of gram-negative microorganisms developing resistance to beta lactam medicines is beta lactamases. Early in the 1980s, cephalosporins were utilized for the first time in clinical settings to fight bacteria that had developed antibiotic resistance brought on by beta-lactamases [13],[14],[15]. This was heralded as a breakthrough in the fight over germs that are resistant to medicines because of β-lactamases [16], [17], [18]. Beta lactamases, such as ESBLS, have evolved due to the widespread use of newer-generation cephalosporins [19],[20],[21]. Transferrable conjugative plasmids used to make ESBLs often contain resistance genes for other antimicrobial drugs, leading to the development of additional Gram-negative bacteria resistant in hospitals and the general population [22], [23], [24],[25]. Because they are frequently missed by routine susceptibility testing techniques, ESBL-producing strains are probably more common than is currently recognized. Recent publications have found bacteria that produce ESBL with an unusually broad spectrum of antibiotic resistance [26],[27],[28],[29]. The bacterial enzymes known as beta lactamases deactivate beta lactam antibiotics through hydrolysis, producing inactive molecules [30],[31],[32]. Some bacteria develop beta-lactamases, which make them resistant to β lactam antibiotics like, Cephalosporin, Cephamycin, Penicillin and Carbapenems [33]. These antibiotics all share the same component in common with one [34],[35]. In view of the high prevalence of beta-lactamase production, prompt action is required on the point of infection control and therapeutic perspective in clinic isolates caused by numerous mechanisms. When taken orally, beta lactamases may have clinical advantages in maintaining the normal intestinal flora during parenteral antibiotic therapy [36],[37],[38]. A wide variety of nosocomial pathogens may be protected against by this. The rise of broadened spectrum cephalosporin bacterial resistance in gram-negative species has been a major source of worry [39],[40]. The

penicillin, cephalosporin, carbapenem, and monobactam families of antibiotics, which are also known as beta-lactam antibiotics, are the main groups that include the beta-lactam ring [41],[42],[43]. These antibiotics function by preventing bacteria from synthesising cell walls. Bacteria, especially Gram-positive ones, are fatally affected by this [44],[45],[46]. However, by producing beta-lactamase, bacteria can develop resistance to beta-lactam antibiotics. In this investigation, Isolates from clinical trials with a gram-negative organism from higher care facilities were simultaneously screened for extended-spectrumbeta-lactamases (ESBL) and ampCbeta-lactamases [47].

Materials and Methods

Materials and equipments: Peptone water, Simmons citrate water, Urease agar, Triple Sugar iron agar medium, Mueller Hinton agar plates, Mannitol motility medium, Antibiotic discs, Inoculation loop, Incubator, Microscope.

Study design and clinical isolates: Over the course of six months (April 2018 to October 2018), 200 aerobic gram-negative bacilli were identified in a clinical microbiology lab from a variety of clinical samples including urine, sputum, pus, and other body fluids. The Saveetha medical college and hospital in Chennai expressed appreciation for the study's design. On both mac conkey agar and nutritional agar plates, each sample was streaked, and it was then left to sit for 24 hours at 37 degrees. Following incubation, regular biochemical assays allowed for the identification of all the gram-negative bacilli.

Development of Extended Spectrum Beta Lactamase test

Test of double disk synergy

The creators of ESBL were found by screening Enterobacteriaceae cells which showed moderate or resistant properties to third generation cephalosporins.

Using a sterilized cotton swab, the test inoculums (0.5Mc Farland) were applied to Mueller hinton agar. The ceftazidime (30 mg) and cefatoxime (30 mg) disks were positioned on either side of the amoxicillin plus clavulanic acid (20 mg+10 mg) disc at an angle of 15 mm center to center relative to the amoxicillin plus clavulanic acid disc. Samples were left to incubate for 18–20 hours at 35 degrees Celsius to identify the area of resistance pattern.

Likely ESBL producers were identified and selected for verification as ESBL producers among isolates which displayed a distinctive shape and size with potency toward amoxicillin + clavulanic disks [48].

Study for phenotypic verification using diffusion of disks

Phenotypic assays amongst samples that might produce ESBLs verified ESBL formation. Sensitivity disks with a third-generation cephalosporin, aztreonam (30 micrograms), clavulanic acid (10 micrograms), and cefatoxime (10 milligrams) are available. Following NCCLS guidelines, a disk diffusing assay was conducted, and changes in zone widths between disks containing and excluding clavulanic acid where noted.

If the diameter of the zone of the ceftazidime/clavulanic disc increases by 5 mm compared to the zone width of the ceftazidime disc by itself, the living thing will be regarded to be an ESBL generator.

As negative and positive controls, accordingly, Klebsiella pneumonia strain and Escherichia coli ATCC25922 48188 will be utilized [48].

Detection of AmpC Beta Lactamases

Altered double disk estimation technique

Using a sterile cotton swab, the experiment an inoculum (0.5Mc Farland turbid) was applied to Mueller hinton agar. Ceftazidime (30 microgram), cefatoxime (30 microgram), and cefoxitin (30 microgram) disks have been placed on both sides of the amoxicillin plus clavulanic discs at an offset of 20 mm center to center from each other. Plates were incubated for 18 to 20 hours at 35 degrees Celsius to identify the region of inhibition patterns.

Ceftazidime or cefotaxime-resistant isolates and cefotixin-resistant isolates were assessed to be potential AmpC growers, and the latter was validated by an AmpC disks analysis [49].

Testing for AmpC disc

AmpC disk testing was used in order to verify the isolates. Escherichia coli ATCC25922 grass culture was established on an MHA plate. Several colonies of the test organism were injected onto sterile disks (6 mm) that had been moistened using sterile saline (20 ml). On the inoculated plate, the inoculated disk was next to a cefoxitin disk, practically touching. The dishes were left to incubate at 35 degrees Celsius for the entire night. A flattening or indentation of the cefoxitin inhibition zone close to the test disk indicated a successful test. The zone was undistorted in a negative test.

As positive and negative controls, accordingly, Klebsiella pneumoniae strain 48188 and Escherichia coli ATCC25922 will be utilized [49].

Results and Discussion

Different specimens were collected over the course of the seven-month investigation from April 2010 to October 2010 such as urine, pas, wound swab, exudates, cereberospinal fluid, pleural fluid, synovial fluid, stool sample, and catheter tips were taken from the inpatients and outpatients attending all departments in Saveetha Medical College and Hospital. From the aforementioned samples, 200 aerobic Gram negative bacilli have been identified.

Table 1 displays the abundance of aerobic Gram negative bacilli that have been collected from diverse collections.

Table No. 1 Distribution of aerobic gram negative bacilli isolated from various samples.

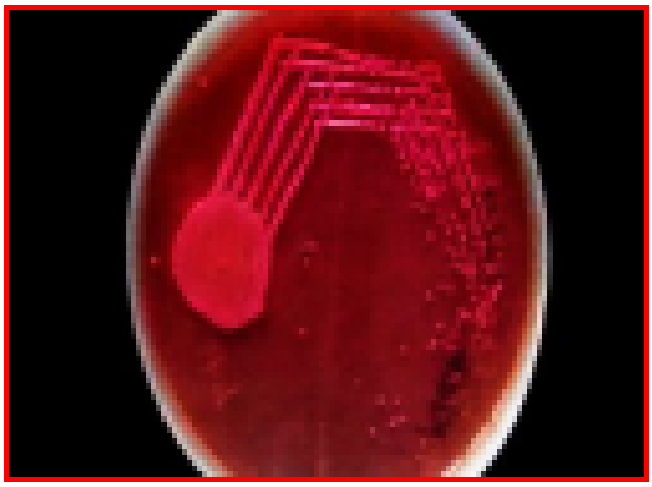
Organism	Urine	Pus	Sputum	Blood	Total
Escherichia Coli	103	6	3	0	112
Klebsiella pneumoniae	15	5	8	0	28
Pseudomonas aeruginosa	0	7	4	0	11

Citrobacter SPP	6	5	0	2	13
Enterobacter Cloacae	7	8	0	8	15
Proteus SPP	2	2	0	8	4
Acinetobacter Baumanni	4	3	1	0	8
Non fermentative gram negative bacilli	5	4	0	8	9
Total	142	32	16	10	200

Ratio of sensitivities Table 2 displays how Gram negative Bacilli respond to various antibiotics.

Antibiotics	**E.coli**	**K.pneumonia**	**P.aeruginosa**	**Proteus spp**	**Citrobac. spp**	**A.baumanii**	**E.cloacae**
Imipenem	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**
Ampicilin	**17.8%**	**17.8%**	**36.4%**	**------**	**30.7%**	**---------**	**20%**
Amikacin	**80.4%**	**82.2%**	**63.6%**	**50%**	**100%**	**50%**	**100%**
Gentamycin	**43.7%**	**53.6%**	**81.8%**	**50%**	**69.3%**	**62.5%**	**80%**
Nitrofuran	**95.5%**	**35.7%**	**-----**	**25%**	**61.5%**	**----------**	**60%**
Norfloxacin	**27.6%**	**39.3%**	**-----**	**25%**	**61.5%**	**37%**	**40%**
Cefuroxime	**16.9%**	**10.7%**	**-----**	**-----**	**23%**	**34.6%**	**20%**
Ceptazimidime	**17.8%**	**28.5%**	**18.9%**	**-------**	**38.5%**	**25%**	**20%**
Cefotaxime	**23.2%**	**53.6%**	**-----**	**25%**	**61.5%**	**37.5%**	**40%**
Ciprofloxacin	**49.1%**	**39.3%**	**81.8%**	**75%**	**46.3%**	**62.5%**	**53.4%**

In this study, out of two hundred isolates (200) of gram negative organisms, one twenty six (126) 63% of isolates have been moderately susceptible to three classes of antibiotics and sensitive or opposed to three groups of antibiotics and were moderately sensitive or resistant With any third-generation cephalosporin antibiotic (3GC- ceftazidime, ceftriaxone, cefotaxime) remaining seventy four (74) of the isolates were sensitive to all antibiotics (37%).



Mac Conkey Agar with mucoid lactose fermenting colonies of Klebsiella pneumoniae

Biochemical test for identification of Escherichia coli.

Distribution of multidrug resistant strains among the aerobic gram negative bacilli shown in Figure 1.

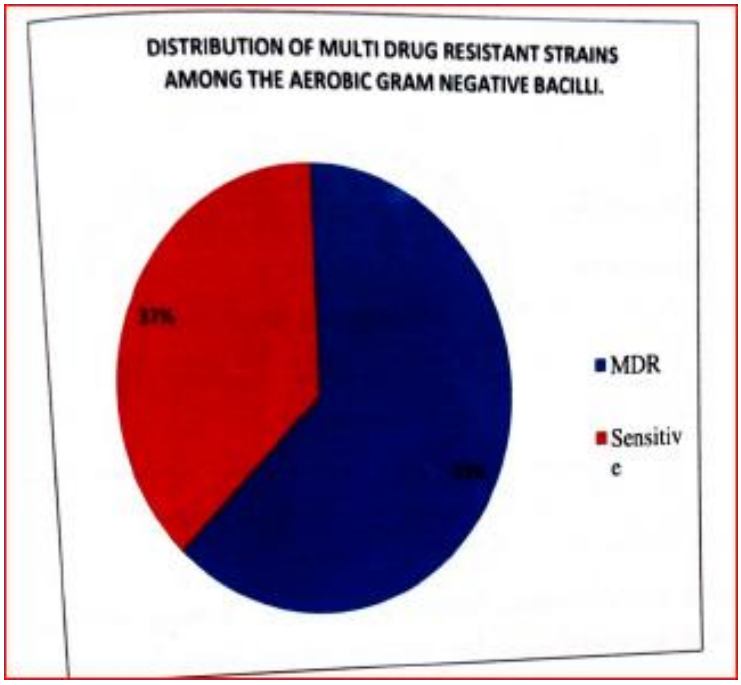


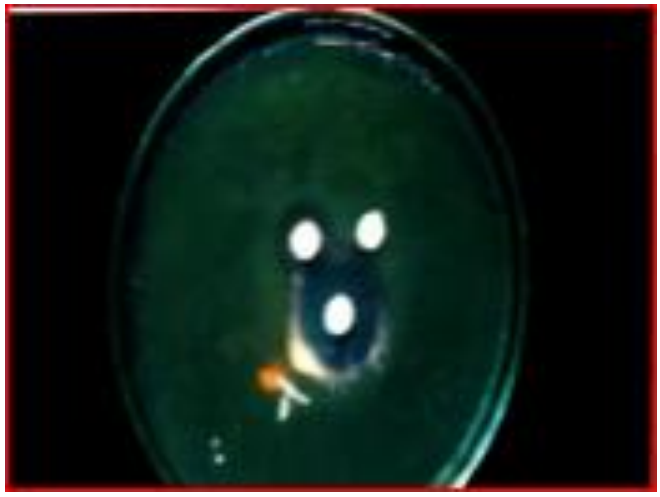
Figure 1

200 isolates were discovered, and 126 (63%) of those were multidrug resistant strains. A total of 118 people (59%) produced ESBLs, of which 54 (27%) additionally generated derepressed mutants (resistant to cefoxitin and cefotaxime, blunting the zone toward inducer, and increasing the zone width by >5 mm), and the remaining 64 (32%) were simple ESBL producers. Three of these isolates, or 1.5% of them, produced induced AmpC beta lactamases. In 3 (1.5%) of the isolates, ampC-mediated beta lactamase synthesis was found. AmpC mediated beta lactamase was seen in two Klebsiella pneumoniae which are also the ESBL producers and one in Pseudomonas aeruginosa which is a non ESBL producer. Remaining 5 (2.5%) resistant strains were neither ESBL nor AmpC beta lactamase producers.

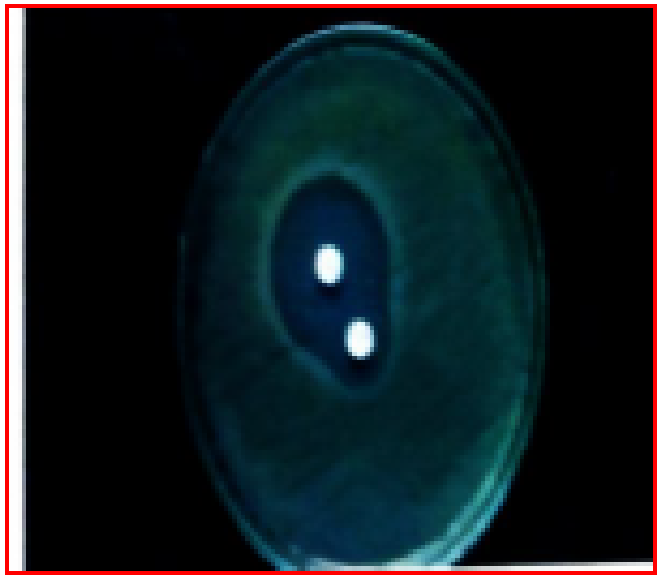
Figure -2

Kirby-Bauer technique of determining sensitivity to antibiotics tests, including screening tests and confirmatory tests

Kirby bauer method showing multi drug resistant strains of Escherichia coli



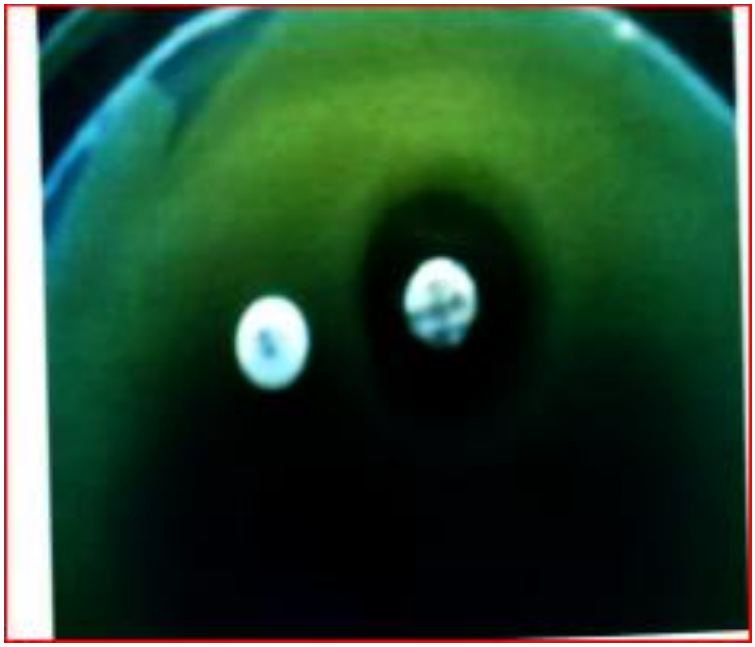
Screening test for detection of ESBL organism showing enhanced zone between Ca/Ce and Amoxicillin/clavulanic acid (ESBL producer).



Zone width between Cac and Ca increased by 5 mm in phenotypic confirmatory test (ESBL) organism

Screening test and confirmatory test for AmpC beta lactamases is shown in figure 3.

Figure-3



Modified double disk approximation method: ceftazidime inhibitory zone blunting next to cefoxitin discs

AmpC disc test: displaying flattening of zone of inhibition

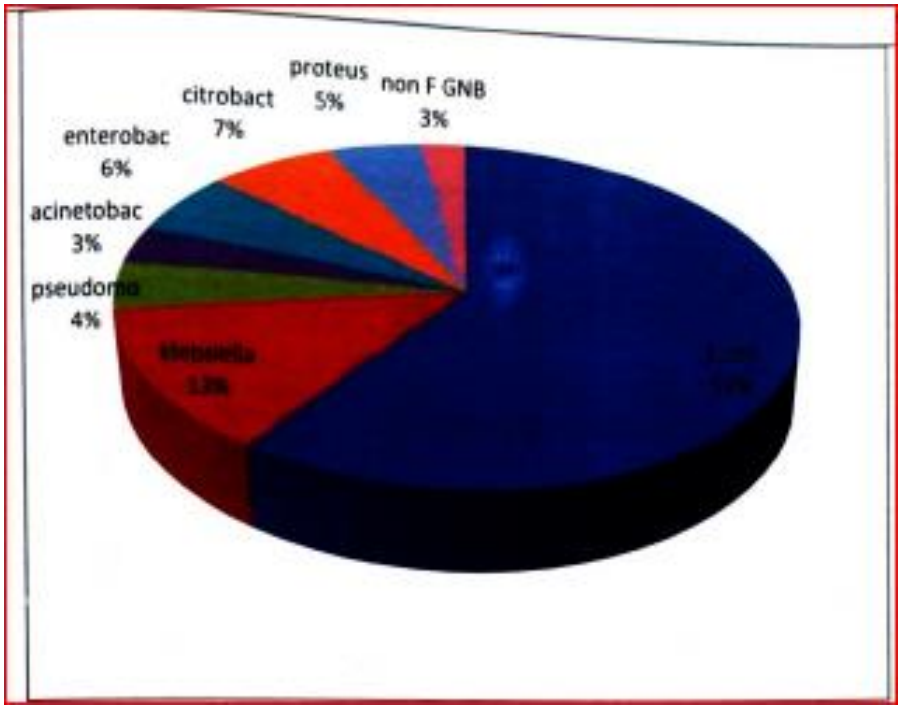
Table 3 displays the abundance of AmpC beta and ESBL Lactamase in several aerobic gram negative bacilli.

TABLE No. 3 AmpC and ESBL Beta Lactamase distribution in several aerobic gram-negative bacteria.

Sl. No.	Organism	Extended spectrum beta lactamase (ESBL)	AmpC spectrum beta lactamase (AmpC)
1	Escherichia coli	70	0
2	Klebsiella pneumonia	15	2
3	Pseudomonas aeruginosa	5	1
4	Acinetobacter baumannii	4	0
5	Enterobacter cloacae	7	0
6	Citrobacter spp	8	0
7	Proteus spp	6	0
8	Non fermentative gram negative bacilli	3	0
	Total	118	3

Majority of ESBL producers were Escherichia coli (59%) and klebsiella pneumonia (13%) followed by Citrobacter spp (7%), Enterobacter cloacae (6%), Proteus mirabilis (5%), Pseudomonas aeruginoosa (4%), Acinetobacter baumannii (3%) and non fermentative gram negative bacilli (3%). The abundance of ESBL in different microbes is displayed in figure 8.

Distribution of ESBL in different organisms



AmpC mediated beta lactamase was seen in two (1%) Klebsiella Pneumoniae which are also found to be an ESBL producers and one Pseudomonas Aeruginosa (0.5%) Which is a non ESBL producer.

Discussion

Multiple illnesses are brought on by aerobic gram-negative bacteria. A significant issue with isolates from patients has been the growth of multidrug susceptibility within aerobic gram-negative microbes. The development of beta lactamases is the most frequent reason for microbial resistance to the beta lactam medicines [50]. A number of both the second and third generations cephalosporins and penicillins were created expressly to withstand the hydrolytic effects of powerful beta lactamases. Extended spectrum beta lactamases are the newest addition to this group of enzymes. The ability to effectively hydrolyze oxyimino cephalosporins confers resistance to third generation cephalosporins like ceftazidime, cefotaxime, monobactams and ceftriaxome, like aztreonam. These enzymes are frequently generated by numerous species of the enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumoniae [51]. Extensively spectral cephalosporin resistant gram negative bacteria are frequently used to isolate ampC beta lactamases.

Particularly within enterobacteriaceae responsible for nosocomial infections, rising third generation cephalosporin resistance has become a source of worry. The predominance of wider spectrum beta lactamases amongst enterobacteriaceae members poses a severe danger to the efficacy of present beta lactam treatment, and which will result in an increase in prices [51].

Sixty-three percent (63%) of the 200 isolates in the current investigation were determined to be multidrug resistant pathogens. One hundred eighteen (118) were the producers of ESBL (59%) out of which fifty-four (27%) also consisted of derepressed mutants, while sixty-four (32%) were plain ESBL providers and three (1.5%) were AmpC β lactamase producers.

Similarly to the current investigation, Rodrigues et al.'s research on the detection of lactamases found that 150 of the lactamases detected were makers of ESBL (53%). The other 20 (7%) were plain ESBL growers, leaving 131 (45.8%) of the 53% producers of ESBL who were also derepressed mutants. 19 (7%) of the isolates had inducible AmpC beta lactamase production, which is higher than expected based on the results of our investigation [52].

Escherichia coli has been the most prevalent producer of ESBLs (53.6%) in the majority of studies on the prevalence of ESBLs between gram-negative bacilli, ahead of Klebsiella pneumonia (19.2%). This finding is almost identical to that of another study by Rodrigues et al. In comparison to our analysis, their study reported an AmpC beta lactamase concentration of 7%, which is rather high.

Various Indian studies which have the different percentage of AmpC and ESBL beta lactamases part of the aerobic gram negative bacilli in shown in table 4 [52], [53],[50].

Study done and year	**Percentage of ESBL Isolates**	**Percentage of AmpC beta lactamase isolates**
C.Rodrigues et al 2004	53%	7%
S.Singhal et al 2005	64%	8%
V. Hemalatha et al 2007	45%	9.2%
Present study	59%	1.5%

Some are sporadic observations regarding wider spectrum beta lactamases from important hospitals in India, and a few of these cases indicate the incidence to be as much as 60–68% [51]. During the past 20 years, this group of beta lactamases has undergone fast evolution. In our study

also we observed almost similar findings regarding the prevalence of ESBL in gram negative organism that is 59%. In the current investigation, evaluation of the 118 confirmed ESBL samples showed that ESBLs appeared most frequently found in Escherichia coli (59%), followed by klebsiella pneumoniae (13%) and other enterobacteriaceae such as Proteus mirabilis and Proteus vulguris 9% , Citrobacter diversusand Citrobacter fruendii 7%, Enterobacter cloacae 6%, Pseudomonas aeruginosa 4%, Acinebacter baunmannii 3%.

All the ESBL and AmpC producing and chromosomal mediated and moreover they are found to be multi drug resistant organisms. In present study all the strains were sensitive to imipenem and Amikacin. Among the non beta lactum antibiotics second most effective drug in Ciproflooxacin and its sensitivity varies between 58.2% to 62.98%. Sensitivity to Nitrofurantoin, Cefotaxime and Norfloxacin are 36.67%, 34.4% and 32.2% respectively and Ampicillin and Cefuroxime are 17.53% and 15.03% sensitive to all strains respectively.

Conclusion

Sixty-three percent of the 200 isolates of gram-negative bacteria were multidrug resistant strains, whereas the remaining 37 percent were antibiotic sensitive. Imipenem and Amikacin were the only antibiotics that were 100% effective against all gram-negative bacteria, preceding Ciprofloxacin (62.98%), Nitrofurantoin (36.67%), Cefotaxime (34.40%) and Norfloxacin (32.96%), Ampicillin (17.53%), and Cefuroxime (15.03%). The predominant AmpC beta Lactamase producers among the aerobic Gram negative bacilli is Klebsiella pneumonia (1%) which is also a ESBL producer followed by Pseudomonas aeruginosa (0.5%) which is a non ESBL producer. Ideal empirical treatment for gram negative bacilli is Imipenem and Amikacin.

References

1. Elmer, W. K., Stephen, D. A., William, M. J., Paul, C. S., & Washington, C. W. (1997). A Color Atlas and Text Book of Diagnostic Microbiology.

2. Collee JG, Mackie TJ, McCartney JE. Mackie & McCartney practical medical microbiology. Harcourt Health Sciences; 1996.

3. Raghunath D. Emerging antibiotic resistance in bacteria with special reference to India. Journal of biosciences. 2008 Nov;33(4):593-603.

4. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical microbiology reviews. 2001 Oct 1;14(4):933-51.

5. Rice LB, Bonomo RA. β-Lactamases: which ones are clinically important?. Drug Resistance Updates. 2000 Jun 1;3(3):178-89.

6. Batchelor M, Threlfall EJ, Liebana E. Cephalosporin resistance among animal-associated Enterobacteria: a current perspective. Expert review of anti-infective therapy. 2005 Jun 1;3(3):403-17.

7. Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended-spectrum β-lactamase-producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. Journal of Medical Microbiology. 2003 May;52(5):421-5.

8. Ali T, Ali I, Khan NA, Han B, Gao J. The growing genetic and functional diversity of extended spectrum beta-lactamases. BioMed research international. 2018 Mar 26;2018.

9. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical microbiology reviews. 1998 Oct 1;11(4):589-603.

10. Rawat D, Nair D. Extended-spectrum β-lactamases in Gram Negative Bacteria. Journal of global infectious diseases. 2010 Sep;2(3):263.

11. De Rosa M, Verdino A, Soriente A, Marabotti A. The Odd Couple (s): An overview of Beta-Lactam antibiotics bearing more than one pharmacophoric group. International Journal of Molecular Sciences. 2021 Jan 9;22(2):617.

12. Page MG. Beta-lactam antibiotics. InAntibiotic discovery and development 2011 Nov 19 (pp. 79-117). Boston, MA: Springer US.

13. Parry CM. Antimicrobial drug resistance in Salmonella enterica. Current opinion in infectious diseases. 2003 Oct 1;16(5):467-72.

14. Steward CD, Rasheed JK, Hubert SK, Biddle JW, Raney PM, Anderson GJ, Williams PP, Brittain KL, Oliver A, McGowan Jr JE, Tenover FC. Characterization of clinical isolates of Klebsiella pneumoniae from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum β-lactamase detection methods. Journal of clinical microbiology. 2001 Aug 1;39(8):2864-72.

15. Akhtar A, Fatima N, Khan HM. Beta-lactamases and their classification: an overview. Beta-Lactam Resistance in Gram-Negative Bacteria: Threats and Challenges. 2022 Apr 21:25-33.

16. Poirel L, Nordmann P. Acquired carbapenem-hydrolyzing beta-lactamases and their genetic support. Current pharmaceutical biotechnology. 2002 Jun 1;3(2):117-27.

17. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-lactamases: the quiet before the storm?. Clinical microbiology reviews. 2005 Apr;18(2):306-25.

18. Livermore DM. Clinical significance of beta-lactamase induction and stable derepression in gram-negative rods. European journal of clinical microbiology. 1987 Aug;6:439-45.

19. Bush K. Proliferation and significance of clinically relevant β-lactamases. Annals of the New York Academy of Sciences. 2013 Jan;1277(1):84-90.

20. Moland ES, Kim SY, Hong SG, Thomson KS. Newer β-lactamases: clinical and laboratory implications, Part II. Clinical Microbiology Newsletter. 2008 Jun 1;30(11):79-85.

21. Steward CD, Rasheed JK, Hubert SK, Biddle JW, Raney PM, Anderson GJ, Williams PP, Brittain KL, Oliver A, McGowan Jr JE, Tenover FC. Characterization of clinical isolates of

Klebsiella pneumoniae from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum β-lactamase detection methods. Journal of clinical microbiology. 2001 Aug 1;39(8):2864-72.

22. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clinical microbiology reviews. 2005 Oct;18(4):657-86.

23. Jacoby GA. AmpC β-lactamases. Clinical microbiology reviews. 2009 Jan;22(1):161-82.

24. Rawat D, Nair D. Extended-spectrum β-lactamases in Gram Negative Bacteria. Journal of global infectious diseases. 2010 Sep;2(3):263.

25. Brun-Buisson C, Philippon A, Ansquer M, Legrand P, Montravers F, Duval J. Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant Klebsiella pneumoniae. The lancet. 1987 Aug 8;330(8554):302-6.

26. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clinical microbiology reviews. 2005 Oct;18(4):657-86.

27. Livermore DM. Beta-lactamases in laboratory and clinical resistance. Clinical microbiology reviews. 1995 Oct;8(4):557-84.

28. Gold HS, Moellering Jr RC. Antimicrobial-drug resistance. New England journal of medicine. 1996 Nov 7;335(19):1445-53.

29. Jacoby GA, Medeiros AA. More extended-spectrum beta-lactamases. Antimicrobial agents and chemotherapy. 1991 Sep;35(9):1697-704.

30. MA W. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. Clsi (Nccls). 2006;26:M7-A7.

31. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer AW, Levine DP, Murray BE, Rybak MJ. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children. Clinical infectious diseases. 2011 Feb 1;52(3):e18-55.

32. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. International journal of nanomedicine. 2012 Dec 5:6003-9.

33. Wang Z, Koirala B, Hernandez Y, Zimmerman M, Park S, Perlin DS, Brady SF. A naturally inspired antibiotic to target multidrug-resistant pathogens. Nature. 2022 Jan 27;601(7894):606-11.

34. Shah IH, Ashraf M, Sabir IA, Manzoor MA, Malik MS, Gulzar S, Ashraf F, Iqbal J, Niu Q, Zhang Y. Green synthesis and Characterization of Copper oxide nanoparticles using Calotropis procera leaf extract and their different biological potentials. Journal of Molecular Structure. 2022 Jul 5;1259:132696.

35. Thomson KS, Sanders CC. Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests. Antimicrobial agents and chemotherapy. 1992 Sep;36(9):1877-82.

36. Coudron PE, Moland ES, Sanders CC. Occurrence and detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae at a veterans medical center: seek and you may find. Journal of clinical Microbiology. 1997 Oct;35(10):2593-7.

37. Babay HA. Detection of extended-spectrum b-lactamases in members of the family enterobacteriaceae at a teaching hospital, Riyadh, Kingdom of Saudi Arabia. Saudi medical journal. 2002 Feb 1;23(2):186-90.

38. Garrec H, Drieux-Rouzet L, Golmard JL, Jarlier V, Robert J. Comparison of nine phenotypic methods for detection of extended-spectrum β-lactamase production by Enterobacteriaceae. Journal of clinical microbiology. 2011 Mar;49(3):1048-57.

39. Emery CL, Weymouth LA. Detection and clinical significance of extended-spectrum beta-lactamases in a tertiary-care medical center. Journal of clinical microbiology. 1997 Aug;35(8):2061-7.

40. Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: review and bench guide. Clinical Microbiology and Infection. 2008 Jan;14:90-103.

41. Wiegand I, Geiss HK, Mack D, Sturenburg E, Seifert H. Detection of extended-spectrum beta-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. Journal of clinical microbiology. 2007 Apr;45(4):1167-74.

42. Tzelepi E, Giakkoupi P, Sofianou D, Loukova V, Kemeroglou A, Tsakris A. Detection of extended-spectrum β-lactamases in clinical isolates of Enterobacter cloacae and Enterobacter aerogenes. Journal of clinical microbiology. 2000 Feb 1;38(2):542-6.

43. Babay HA. Detection of extended-spectrum b-lactamases in members of the family enterobacteriaceae at a teaching hospital, Riyadh, Kingdom of Saudi Arabia. Saudi medical journal. 2002 Feb 1;23(2):186-90.

44. Pfaller MA, Segreti J. Overview of the epidemiological profileand laboratory detection of extended-spectrum β-Lactamases. Clinical Infectious Diseases. 2006 Apr 15;42(Supplement_4):S153-63.

45. Fang H, Ataker F, Hedin G, Dornbusch K. Molecular epidemiology of extended-spectrum β-lactamases among Escherichia coli isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. Journal of Clinical Microbiology. 2008 Feb;46(2):707-12.

46. Schilcher K, Horswill AR. Staphylococcal biofilm development: structure, regulation, and treatment strategies. Microbiology and Molecular Biology Reviews. 2020 Aug 19;84(3):10-128.

47. Amin Yavari S, Castenmiller SM, van Strijp JA, Croes M. Combating implant infections: shifting focus from bacteria to host. Advanced Materials. 2020 Oct;32(43):2002962.

48. Wiegand I, Geiss HK, Mack D, Sturenburg E, Seifert H. Detection of extended-spectrum beta-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. Journal of clinical microbiology. 2007 Apr;45(4):1167-74.

49. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, Rattan A. Evaluation of methods for AmpC β-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian journal of medical microbiology. 2005 Apr 1;23(2):120-4.

50. Hemalatha V, Padma M, Sekar U, Vinodh TM, Arunkumar AS. Detection of Amp C beta lactamases production in Escherichia coli & Klebsiella by an inhibitor based method. Indian journal of medical research. 2007 Sep 1;126(3):220-3.

51. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum ß-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. Indian journal of medical microbiology. 2006 Jul 1;24(3):208-11.

52. Rodrigues C, Joshi P, Jani SH, Alphonse M, Radhakrishnan R, Mehta A. Detection of β-lactamases in nosocomial gram negative clinical isolates. Indian journal of medical microbiology. 2004 Oct 1;22(4):247-50.

53. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, Rattan A. Evaluation of methods for AmpC β-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian journal of medical microbiology. 2005 Apr 1;23(2):120-4.

.