Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 5| Issue 1|JAN-MAR|2015|100-108

Research Article
Biological Sciences

STUDY OF PREVALENCE OF β-LACTAMASE IN CLINICAL SAMPLES

Shivangi H.Bhatt[1*] & Neepa D. Pandhi[2]

[1]Department of Biochemistry, Shree M & N Virani Science College, Rajkot

[2]Department of Microbiology, Shree M & N Virani Science College, Rajkot

*Corresponding Author Email: shivangibhatt23@gmail.com

ABSTRACT

Background: The development of drug resistance is inevitable following the introduction of a new antibiotic in pathogens. Initial levels of resistance to new drugs are normally less. However, irrational uses of antibiotics have caused a huge increase in the number of resistant bacteria. Often, nosocomial resistance problems get more attention than community-based problems. Objective: A study was conducted to know the prevalence of various types of β-lactamases in hospitals and community associated clinical samples.Material & Methods: Clinical samples were collected and bacterial strains were identified according to standard microbiological investigations approved by CLSI guideline. To determine the prevalence of drug resistance among samples phenotypic detection methods were used.Result: Out of 155 samples that had shown the significant growth, 63 isolates are found to be resistant towards 2nd generation and onwards β -lactam group of drugs. Overall 40.64% of the isolates showed resistance towards 2nd and 3rd generation β -lactam antibiotics.Interprertation & conclusion: Antibiotic resistance is really a serious concern. If emergence of resistant strains will continue then antibiotics will be no more effective to treat bacterial infections. Resistant is not only more prominent in hospital samples but it is also widely prevalent in community acquired samples.

KEY WORDS

Antibiotic resistance, β-lactam drugs, various β-lactamase.

INTRODUCTION

Antibiotics have had a profound impact on human health and belong to one of the largest-selling class of drugs worldwide. However, the accelerated emergence of bacteria that are resistant to multiple antibiotic types now appears as the most serious threat to continuing success in the treatment of infectious diseases. [1] Infectious diseases have been an ever-present threat to mankind. Over the past two decades, the reports of multi-drug resistant organisms and explosive epidemics of unidentified and re-emerging diseases have given the world a warning call against irrational use of medicines.[2] Infectious diseases continue to be a leading cause of mortality the world over, more so in developing countries with poorly accessed health services. [3] Conjugational transmission of antibiotic resistance genes across bacterial species and genera has amplified the problem of antibiotic resistance in pathogens. [4]

Members of the family *Enterobacteriaceae* are among the most important bacterial human pathogens accounting for the majority of bacteria isolated from clinical samples. That these Gram negative bacilli (GNB's) are rapidly acquiring resistance to one or more antimicrobial agents traditionally used for treatment is a matter of concern.[5] Coliforms have changed their susceptibility pattern extensively; most tertiary care hospitals are faced with extensive resistance problems in their *E-coli* and *Klebsiella* as well as

other multi resistance *Enterobacteriaceae* members.[6], particularly to oral β-lactams, emphasizing the need for more effective compounds.[7] β- lactams remain the most widely utilized antibiotics owing to their comparatively high effectiveness, low cost, ease of delivery and minimal side effects.

The most common mechanism is the production of enzymes that degrade or modify the antibiotic before it can reach the appropriate target site. The β -lactamase families of enzymes degrade β -lactam antibiotics and are found widely disseminated amongst Gram-positive and Gram-negative bacteria. [8] Amongst the mechanisms of resistance to third generation cephalosporin, production of ESBLs and AmpC β -lactamases are the most common. [9] Extended spectrum β-lactamase (ESBL)-producing organisms pose unique challenges to clinical microbiologists, clinicians, infection control professionals and antibacterial-discovery scientists. ESBLs are enzymes capable of hydrolyzing penicillin, broad-spectrum cephalosporins and monobactams, and are generally derived from TEM and SHV-type enzymes. ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species.

Although the prevalence of ESBLs is not known, it is clearly increasing, and in many parts of the world 10–40% of strains of *Escherichia coli* and *Klebsiella pneumoniae* express ESBLs. [10] Both ESBL and AmpC β lactamase are encoded on plasmids and confer a selective advantage to strains harboring these in a hospital setting. It is important to know the occurrence of ESBL and AmpC producing strains as well as their antibiotic susceptibilities to newer agents to guide empirical therapy for various infections. A study conducted in 2002 overall 26.6 per cent of urinary isolates was ESBL producers [11].

MATERIALS AND METHODS

Specimen collection:

The samples (blood, urine, tracheal aspirates, stool, pus and sterile body fluids), collected from various hospitals and private pathological laboratory of Rajkot city were processed for isolation and identification of bacterial pathogens according to standard microbiological techniques.

Isolation and identification:

Organisms were grown in pure culture and the samples which showed significant growth were considered for further study. Bacterial strains were identified by standard biochemical tests using Hi-25 enterobacteriaceae identification kit as shown in figure-1. This kit contain 25 different biochemical test including sugar fermentation reactions used to detect various strains of pathogenic bacteria.

Antibiotic assay:

Antibiotic susceptibility test was done by disc diffusion method. Antibiotic susceptibility was determined by Kirby Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines. Antibiotics were chosen depending on the organism and the sample and results were interpreted as sensitive or resistant as per CLSI recommendations. [12, 13]

Various generation of β -lactam group of drugs were chosen by using multidisc as shown in figure -2 for screening the resistance towards β- lactam group of drugs. These drugs were including **1st generation**- cefalexin and cefadroxil, **2nd generation**- cefaclor and cefoxitin, **3rd generation**- ceftriaxone, cefotaxime, ceftazidime and cefoperazone, **4th generation**- cefepime, **carbapenems**- ertapenem and imipenem, **aztreonam, oxacillin**, **b-lactam/b-lactamase inhibitor-** cefotaxime and clavulanic acid, ceftazidime and clavulanic acid, ticarcillin and clavulanic acid, pipracillin and tazobactam, cefoperazone and salbactam, ampicillin and salbactam.

Phenotypic determination of various β-lactamases:

Phenotypic detection of various types of β-lactamase was done as per CLSI guideline which was shown in figure-3. ESBLs were detected by the confirmatory method of National Committee for Clinical Laboratory Standards (NCCLS) now known as Clinical and Laboratory Standards Institute (CLSI) using cefotaxime (30 mcg) and ceftazidime (30 mcg) and a disc of cefotaxime plus clavulanic acid (30 and 10 mcg) and ceftazidime and clavulanic acid (30/10 mcg) placed at a distance of 20 mm on a lawn culture (0.5 McFarland inoculum size) of suspected ESBL producing clinical isolate on Mueller-Hinton Agar (MHA, Hi-Media,) ESBL production was inferred if the inhibition zone increased by 5 mm towards the cefotaxime plus clavulanic acid disc or ceftazidime plus clavulanic acid disc in comparison to the third generation cephalosporin disc alone.

Cefepime and Cefoxitin disks were used to detect AmpC beta lactamases. AmpC strains are resistant to the cephamycins (i.e.; cefoxitin and cefotetan) and are susceptible to cefepime. High level AmpC producers cause resistance to all 1st, 2nd and 3rd generation cephalosporins, the beta lactam-inhibitor drugs and the monobactams.

For detection of K1 b-lactamase, aztreonam, ceftazidime, cefotaxime and ceftriaxone disks were used. Cefotaxime Sensitive and Ceftriaxone Resistance are considered as K1 b-lactamase producing strain.

Detection of Carbapenemase, (ertapenem and imipenem) disks were used to screen for carbapenemase resistance). Strains which are Imipenem – Sensitive and Ertapenem – Resistance were considered as Carbapenemase producing strains.

RESULT

Nosocomial infection caused by multi drug resistant bacteria poses a serious threat due to limited therapeutic option against them and it is also found to be spreading in community.

β-lactam antibiotics are a broad class of antibiotics commonly used in treatment of bacterial infections. In this study the susceptibility of community and hospital acquired strains were tested against different classes of β lactams.

Total 270 clinical samples were collected from various hospitals and private microbiology laboratories of Rajkot city. Out of that 155 samples showed the significant growth. 63 isolates were found to be resistance towards 2nd generation and onwards b-lactam. Overall 41.835% resistance was found.

Figure: 1- It shows biochemical test for identification of bacterial pathogens by using Hi-25 Enterobacteriaceae identification kit which is a combination of 25 tests for identification of Enterobacteriaceae species.

Figure: 2- The above figure shows screening of clinical samples for 2nd generation and onwards b-lactam resistance by using multidisc containing various generations of b-lactam drugs.

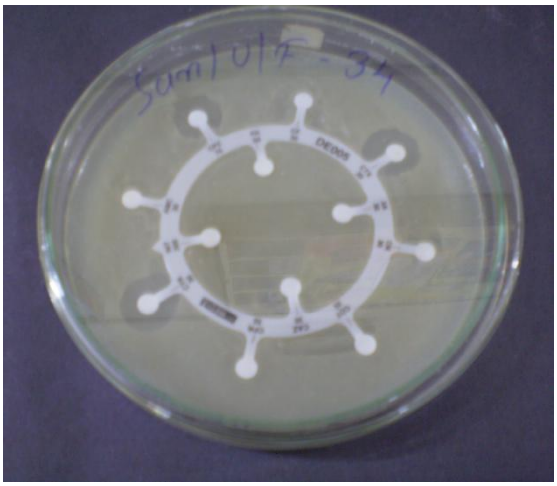
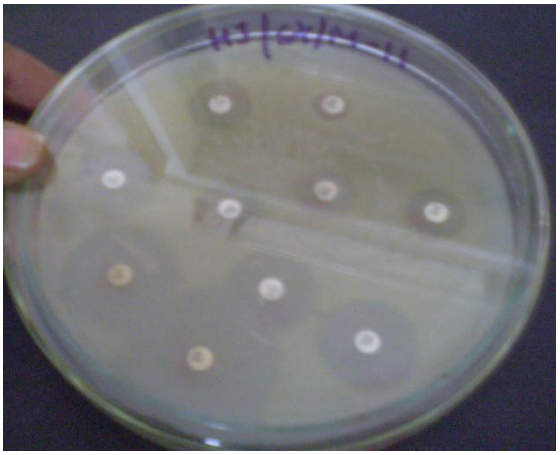


Figure: 3- Phenotypic confirmation of different types of β- lactamases were done according to CLSI guidelines by placing various β - lactam group of drugs.



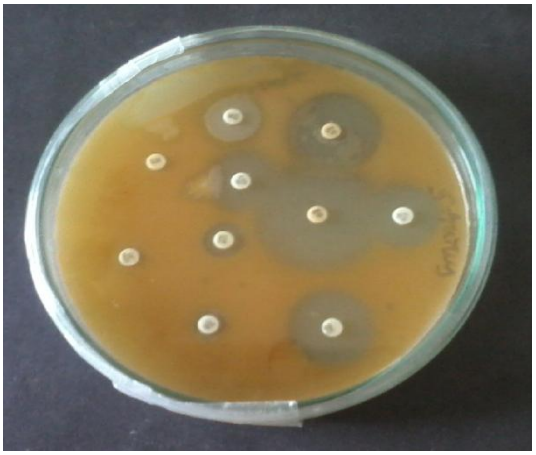


Figure: 4- The graph shows from total number of sample collected and from that how many samples have shown significant growth and out of that how many samples shows resistant strains isolated from various hospitals and private labs.

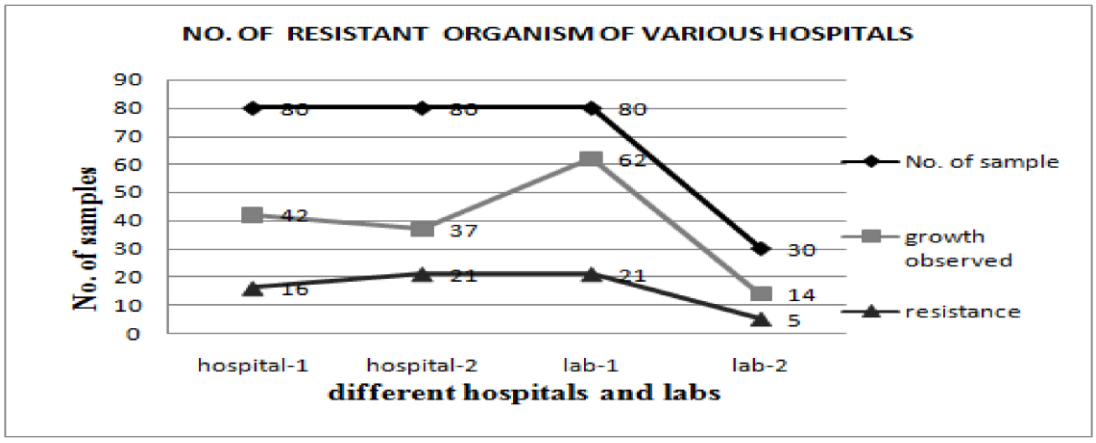
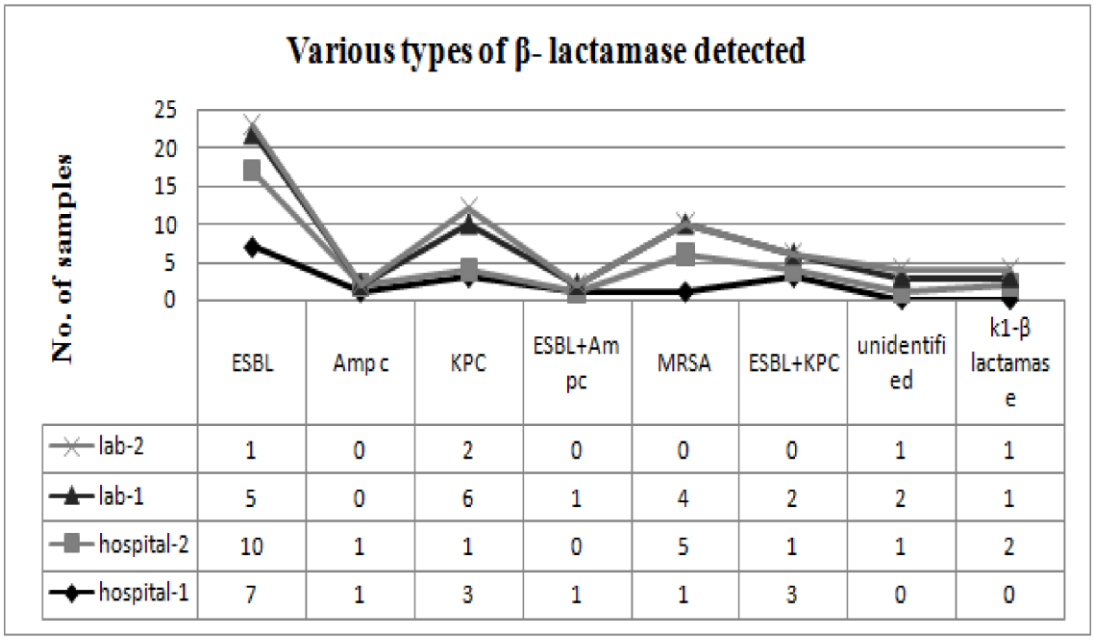


Figure: 5 – Various types of β – lactamases were detected by phenotypic methods from various hospitals and private labs.



	ESBL	Amp c	KPC	ESBL+Ampc	MRSA	ESBL+KPC	unidentified	k1-β lactamase
lab-2	1	0	2	0	0	0	1	1
lab-1	5	0	6	1	4	2	2	1
hospital-2	10	1	1	0	5	1	1	2
hospital-1	7	1	3	1	1	3	0	0

Figure: 6 – Number of ESBL were detected from various hospitals and labs by placing 3rd generation of β – lactam drug along with 3rd generation plus clavulanic acid (30/10 mcg) concentration. Zone of inhibition was increased 5mm or more than that towards 3rd generation plus clavulanic acid in comparison to the third generation cephalosporin disc alone were consider as ESBL producing strains.

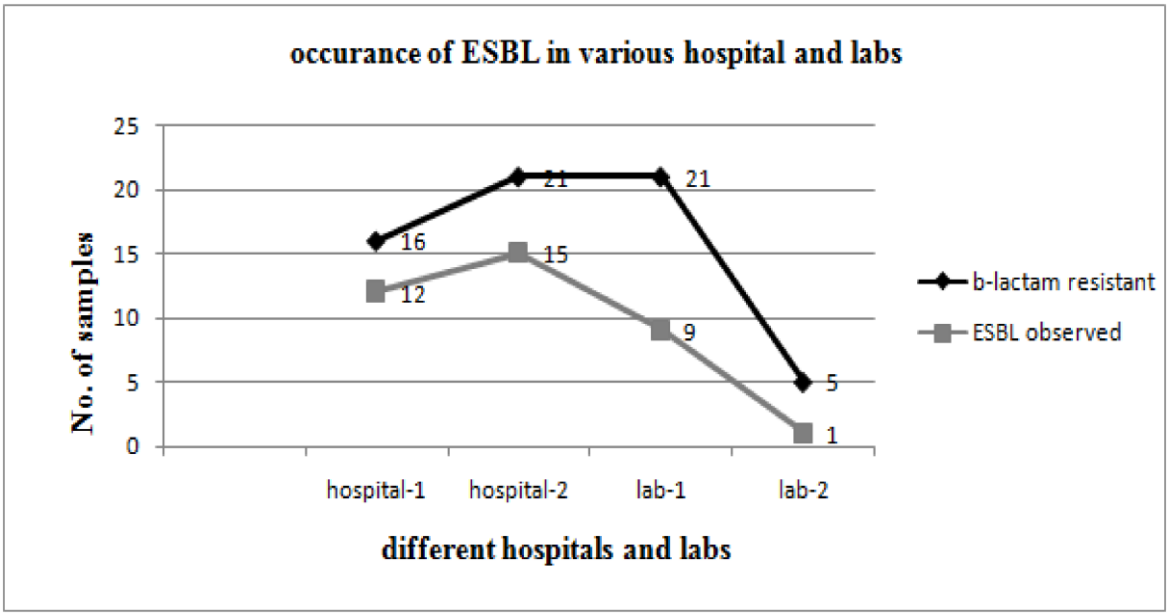


Figure: 7 – The above graph shows Amp-c β- lactamase producing strains which were sensitive to 4th generation cefepime and resistant to cephamycins (cefoxitin) detected from various hospitals and laboratories.

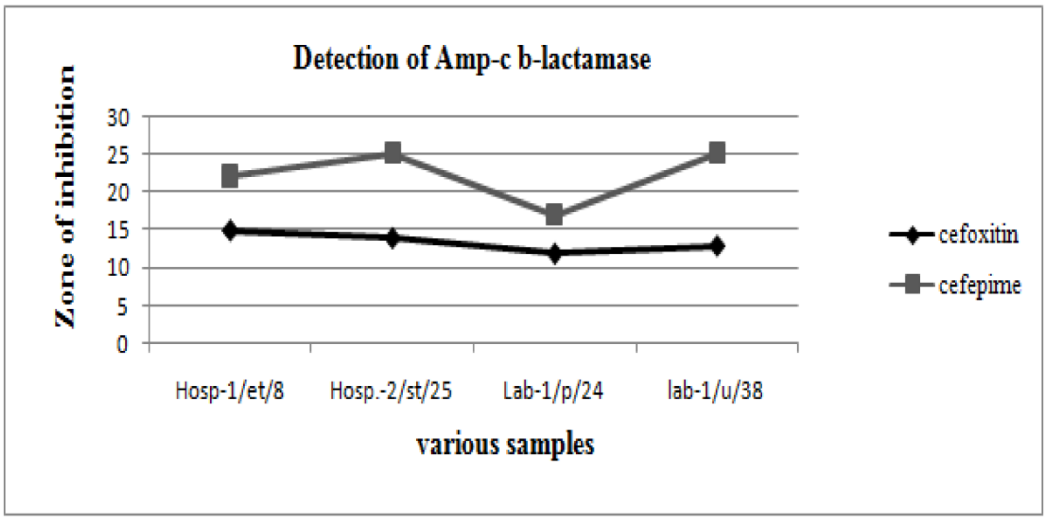
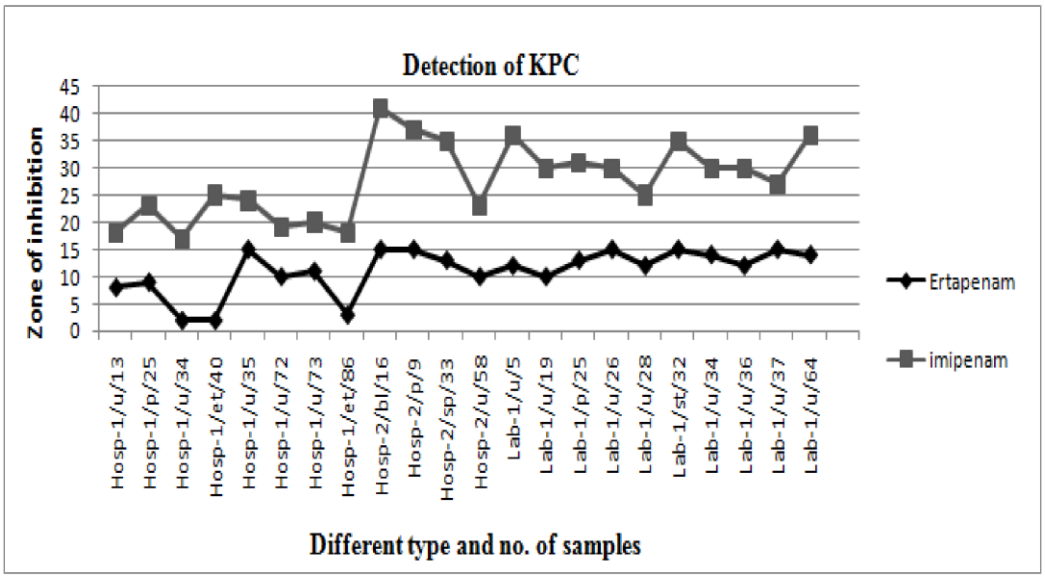


Figure: 8– The above graph shows KPC β- lactamase producing strains which were sensitive to imipenam and resistant to ertapem detected from various hospitals and labs.



DISCUSSION

Detecting the presence of β-lactam drug resistance in clinical samples from patient is important in clinical decision making as well as it also guide in infection control measure by preventing misuse of antibiotics. In our study as we have shown in figure-4 we observed that the ratio of resistance towards β -lactam group of drug is slightly higher in hospitals than in laboratories where community samples were usually collected, indicating that resistance towards β -lactam is equally widespread in community as well. The percentage of resistance in hospital-1 was 38.09% and hospital-2 was 56.75%. While the resistance in samples of lab-1 was 33.87% and lab-2 was 35.71%.

In this study as shown in figure -5 various types of β- lactamase were detected. The percentage of strains showing co-resistance to ESBL+AmpC in hospital-1 was 6.25% and in lab-1 it was 4.76 %. Co-resistance due to ESBL+KPC in hospital-1 was 18.75 %, hospital-2 was 4.76 % and in lab-1 it was 9.52 %.

6.34% strains were totally resistant to all the classes of β-lactams including 4th generation as well as β-lactam-β-lactamase inhibitor drugs.

Study from China shows the percent of ESBL producers varies between 25-40 %. [14] The percentages of non ESBL producers are KPC- 15.62%, AmpC- 3.12%, MRSA- 9.37% and K1- 6.25%.

In our study as shown in figure- 6 the ratio of ESBL was quite higher than all other type of β-lactamase.

Among all the β-lactam resistant strains isolated, 37.5% were ESBL producers and 40.62% were non ESBL producers. Few of the strains were found to be having co-resistance between ESBL and KPC, ESBL and AmpC.

Another study in 2005 from New Delhi showed 68.78 % of the strains of Gram negative bacteria to be ESBL producers. [15] ESBLs have been reported from community isolates from north India as well. [16]

A study from Coimbatore, Tamil Nadu, showed the presence of ESBL to be 40 per cent while from Nagpur this figure was 50 per cent in urinary isolates. [17, 18]

A similar study from Lucknow showed high levels of ESBL production 63.6- 86.6 %. [19]

In our study as mentioned in figure -7 the resistance towards Amp-c β-lactamase was 6.34%.

Many bacterial species like *Enterobacter, S. marcescens, E. coli, P. aeruginosa* and *C. freundii* possess β-lactamases of the Amp C type. [20] The product of Amp C gene is an enzyme that is broadly active against cephalosporins but is not inhibited by clavulanate. This differentiated Amp C enzymes from ESBLs.

Amp C enzyme has also been described in 3.3 per cent of isolates from Karnataka. [21]

Transferable Amp C beta lactamases were detected in 3.3 per cent of *K. pneumonia* isolates. [22]

In our study as shown in figure-8 occurrence of KPC was observed individually as well as co resistance along with ESBL. Total KPC resistance was found to be 28.57%. Out of that individual resistance was 19.04% while co-resistance was found to be 9.52%.

Antibiotic resistance is really a serious and big concern. If such emergence of superbugs will continue then antibiotics will be no more as 'magic bullets' to treat bacterial infections. Many bacteria have emerged that are resistant to each of the antibiotics currently on the market. Major causalities have occurred as a result of untreatable bacterial infections. This trend will be continuing until and unless we take the problem of antibiotic resistance very seriously and take major actions to curb it. [23, 24]

CONCLUSION

- Increasing levels of resistance are being found among common community-acquired pathogens, and outpatient.
- Antibiotic regimes which traditionally have been effective are now failing at increasing rates.
- Often, nosocomial resistance problems garner more attention than community-based problems.
- We are facing the global threat of superbugs. Continued irresponsible and irrational use of antimicrobials will see few more lethal 'Superbugs' in future until and unless we learn from our past and implement it in our present and future.
- Emergence of superbugs is common problem of our healthcare system and whoever is the part of this system are responsible for it. Rather than playing a blame game over it, we all need to work together to curb this global concern. [2]

REFERENCES

1. Anderssona, A. C. Terwisscha van Scheltingaa and K. Valegårdb. *Towards new b-lactam antibiotics.* CMLS, Cell. Mol. Life Sci. *58*, 1897–1906. (2001)
2. Reema Thomas and Mukesh Nandave. *Emergence of 'Super Bug' and Use of Antibiotics: What We Learned, What We Have Yet to Learn?* Journal of Pharmacy Research. *4(7),* 2064-2066. (2011)
3. World Health Report. *A safer future* (Geneva: World Health Organization) (URL: *http://www.who.int/whr/2003/en/*). (2007)
4. Bhattacharjee, MR Sen, P Prakash, A Gaur, S Anuprabha, G Nath. *Observation on integron carriage among clinical isolates of Klebsiella pneumonia producing extended spectrum ß-lactamases.* Indian journal of medical microbiology. *28(3),* 207-10. (2010)
5. Eisenstein BI, Zaleznik DF. Enterobacteriaceae. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*, 5th ed. Philadelphia, Pa: Churchill Livingstone; p. 2294-310. (2000)
6. Jain A and Mandal R. *Prevalence of antimicrobial resistance pattern of extended-spectrum beta-lactamase producing Klebsiella species isolated from cases of neonatal septicemia.* Indian J Med, Res; *125*, 89-94. (2007)
7. F W. Goldstein, 1y. Pe´an, 2 j. Gertner, 2 and the vigil'roc study group. *Resistance to Ceftriaxone and Other b-Lactams in Bacteria Isolated in the Community.* antimicrobial agents and chemotherapy, p. 2516–2519. (1995)
8. Mark S Wilke1, Andrew L Lovering1 and Natalie CJ Strynadka. 2005. *b-Lactam antibiotic resistance. a*

current structural perspective. Current Opinion in Microbiology, *8*:525–533. (*1995*)

9. Black JA, Moland ES, Thomson KS. *AmpC. disk test for detection of plasmid-mediated AmpC- b lactamases in Enterobacteriaceae lacking chromosomal AmpC- b lactamases.* J Clin Microbiol; *43,* 3110-3. (2005)
10. Mark E. Rupp and Paul D. Fey Drugs. *Extended Spectrum ß-Lactamase (ESBL)-Producing Enterobacteriaceae Considerations for Diagnosis, Prevention and Drug Treatment.* Drugs. *63 (4),* 353-365. (2003)
11. Khurana S, Taneja N, Sharma M. *Extended spectrum beta lactamase mediated resistance in urinary tract isolates of family Enterobacteriaceae*. Indian J Mel Res; *116*, 145-48.(2002)
12. National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A5 and informational Supplement M100-S10.* Wayne, PA. (2000)
13. National Committee for Clinical Laboratory Standards (NCCLS). *Performance standards for antimicrobial disk susceptibility tests, 8th ed. Approved standards. NCCLS Document M2-A8*, Wayne PA. (2003)
14. Yu Y, Zhou W, Chen Y, Ding Y, Ma Y. *Epidemiological and antibiotic resistant study on extended-spectrum betalactamase- producing Escherichia coli and Klebsiella pneumoniae in Zhejiang Province*. Chin Med J (Engl); *115*, 1479-82. (2002)
15. Mohanty S, Singhal R, Sood S, Dhawan B, Das BK, Kapil A. *Comparative in vitro activity of beta-lactam/beta-lactamase inhibitor combinations against Gram negative bacteria.* Indian J Med Res*; 122,* 425-8. (2005)
16. Gupta V, Datta P. *Extended-spectrum beta-lactamases (ESBL) in community isolates from North India: frequency and predisposing factors.* Int J Infect Dis*; 11*, 88-9. (2007)
17. Babypadmini S, Appalaraju B. *Extended spectrum beta lactamases in urinary isolates of E.coli & K.pneumoniae– prevalence and susceptibility pattern in a tertiary care hospital.* Indian J Med Microbiol*. 22,* 172-4. (2004)
18. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. *Evaluation of extended spectrum beta lactamase in urinary isolates.* Indian J Med Res*; 120:* 553-6. (2004)
19. Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. *Prevalence of extended-spectrum beta-lactamase-producing Gram negative bacteria in septicaemic neonates in a tertiary care hospital.* J Med Microbiol*; 52,* 421-5. (2003)
20. Pfaller MA, Segreti J. *Overview of the epidemiological profile and laboratory detection of extended-spectrum beta lactamases.* Clin Infect Dis*; 42 (Suppl) 4*: S153-63. (2006)
21. Ratna AK, Menon I, Kapur I, Kulkarni R. *Occurrence & detection of Amp C b-lactamases at a referral hospital in Karnataka.* Indian J Med Res*; 118, 29*-32. (2003)
22. Moland ES, Hanson ND, Black JA, Hossain A, Song W, Thomson KS. *Prevalence of newer beta-lactamases in Gramnegative clinical isolates collected in the United States from 2001 to 2002.* J Clin Microbiol*; 44*, 3318-24. (2006)
23. Therrien C, Levesque RC. *Molecular basis of antibiotic resistance and beta-lactamase inhibition by mechanism-based inactivators: perspectives and future directions.* FEMS Microbiol Rev.*; 24(3):*251-262. (2000)
24. Dye D, Croize J, Brambilla C. *Mechanism of antibiotic resistance in bacteria responsible for respiratory infections.* Rev Mal Respir. *12(5)*, 415-27. (1995).

***Corresponding Author:**
shivangibhatt23@gmail.com,
neepa.pandhi@gmail.com