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Phenotypic Detection of Various β-Lactamase from Urinary Isolates

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ABSTRACT

Background: Resistance to antibiotics and other drugs has emerged in most bacterial infections, which constitutes a significant proportion of the burden of health care and disease management in developing countries. The incidence of infections due to organisms resistant to β - lactam agents due to production of various enzymes has increased in recent years. The extensive use of antibiotics in the community and hospitals has fuelled this crisis. **Methods:** Urine samples were collected from various hospitals and private pathological laboratories. Bacterial strains were identified according to standard microbiological investigations approved by CLSI guideline. Isolated pathogens were undergone for screening test to find out the prevalence of β -lactam resistance towards various generations of β-lactam drugs. Resistant isolates were classified by phenotypic detection method. **Results & discussions:** The study was conducted to evaluate the prevalence of various types of β -lactamase among urinary isolates. Out of 175 samples 152 pathogens were isolated in pure culture. 42.10% resistance were observed among this urinary isolates against various generation of β-lactam drugs. **Conclusion:** Increasing levels of resistance are being found among common community-acquired urinary pathogens, and nosocomial pathogens. The increasing resistance has made empirical treatment more difficult. UTIs complicated by ESBL organisms tend to lead in our study.

KEY WORDS: β -lactam resistance, phenotypic detection, urinary isolates

1. INRODUCTION

Urinary tract infections (UTIs) are the most common infectious diseases occurring in nosocomial and community setting.[1] Antibiotic-resistant organisms that cause complicated UTI include Gram positive *Staphylococcus aureus,* Gram negative organisms particularly those species that produce AmpC enzymes or extended-spectrum β-lactamases (ESBLs) includes, *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas*, and *Serratia* spp. Urea-splitting organisms such as *Proteus spp., Morganella morganii* and *Providencia stuartii* are often found in patients with Indwelling devices.[2]

The antimicrobial resistance among uropathogenic bacteria across the globe are emerging. In recent years, spread of plasmid mediated ESBL and various other β -lactamase in gram negative bacteria has risen. This results in an increasing incidence of resistance to third generation of cephalosporin among the urinary pathogens and causing a number of therapeutic problems.[3-5]

Penicillins and cephalosporins (the β-lctams) are one of the most valuable groups of antibiotic used in human medicine. The excessive application of these antibiotics is believed to have been a selective force in the emergence of resistance. The emergence and spread of resistance particularly into *E. coli* (a common cause of gut and urine infection) is a very serious challenge.

The development of extended spectrum cephalosporin in the early 1980s was regarded as a major addition to our therapeutic armamentarium in the fight against β-lactamase mediated bacterial resistance. The emergence of *Escherichia coli and Klebsiella pneumoniae* resistant to ceftazidime & other cephalosporins seriously compromised the efficacy of these life saving antibiotics.[6]

ESBL producing organisms are often resistant to several other classes of antibiotics, as the plasmids with the gene encoding ESBLs often carry other resistance determinants. Initially ESBL producing organisms were isolated from nosocomial infections but these organisms are now also being isolated from community. [7]

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So, the present study was undertaken to detect the prevalence of various β-lactamase by phenotypic method, which provides a base line survey of drug resistance pattern.

2. MATERIALS AND METHODS

2.1. Specimen collection:

The mid stream urine samples were collected from hospitals and private pathological laboratories. Urine samples were undergone for isolation and identification of bacterial pathogens according to standard microbiological techniques.

2.2. Isolation and identification:

Samples were inoculated on Nutrient and MacConkey's agar for isolation of pathogens. Organisms were grown in pure culture and the samples which have shown significant growth were considered for further study. Isolates were subjected to genus and species level identification by using Hi25- *Enterobacteriaceae* identification kit based on Bergey's Manual for Systematic Bacteriology. This kit contains 2 different strips for each isolates. One strip has media for IMViC test as well as others biochemical test and second strip contain various media for testing the results of sugar fermentation. For oxidase test, oxidase reagent disc were used.

2.3. Antibiotic assay:

Antibiotic susceptibility test for β-lactam drugs were done by disc diffusion method. Antibiotic susceptibility was determined by Kirby Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines. Various generation of β -lactam group of drugs were chosen for screening of β -lactam drug resistance as shown in figure -2 for screening the resistance towards β- lactam group of drugs. These drugs were including **1st generation**-cefalexin(30 mcg) and cefadroxil(30 mcg), **2nd generation**-cefaclor(30 mcg) and cefoxitin(30 mcg), **3rd generation**-ceftriaxone(30mcg), cefotaxime(30 mcg), ceftazidime (30mcg) and cefoperazone(75mcg), **4th generation**- cefepime(30 mcg) and **combination drugs** like Augmentin (Amoxy-clav) (30 mcg) & ampicillin/salbactam (10/10 mcg).

2.4. 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 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.

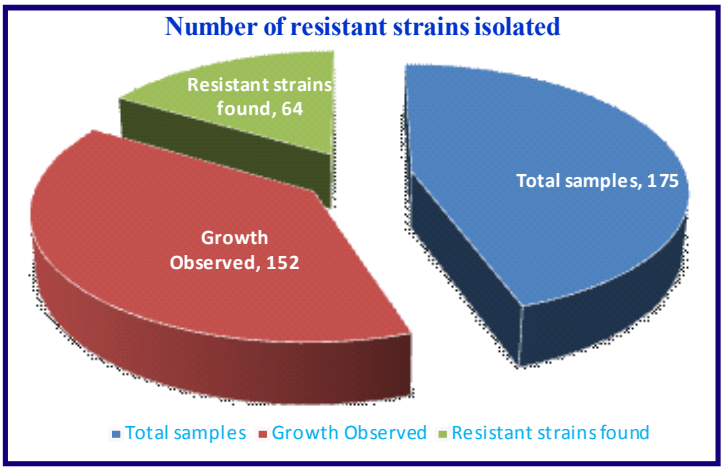
Cefepime (30 mcg) and cefoxitin (30 mcg) disks were used to detect AmpC β- lactamases. AmpC strains are resistant to the cephamycins (i.e.; cefoxitin and cefotetan) and are susceptible to cefepime. For detection of K1 β-lactamase, aztreonam (30 mcg) and ceftazidime (30 mcg) disks were used. Ceftazidime sensitive and aztreonam resistance were considered as K1 β-lactamase producing strain. To screen carbapenemase resistance, ertapenem (10mcg) and imipenem (10mcg) disks were used. Strains which are imipenem – sensitive and ertapenem – resistance were considered as carbapenemase producing strains.

3. RESULT & DISCUSSION

Total 175 urine samples were collected from hospital and laboratories. Samples were inoculated on Nutrient agar and MacConkey's agar for isolation of pathogens. 152 out of 175 samples have shown significant growth on media and organisms were identified by standard biochemical tests as shown in figure-1.

Figure -1: Identification of urinary isolates by Hi25- *Enterobacteriaceae* identification kit

As shown in figure-2, various generation of β- lactam drugs including 1st to 4th generation as well as combination β- lactam drugs were placed to screen the resistance towards β- lactam group of drugs. According to graph 1, out of 152 screened isolates 64 urinary isolates were resistance to 2nd generation and onwards β- lactam resistant.



Graph: 1: Screening of urinary isolates to detect the β-lactam resistance.

So 42.10% resistance were observed among this urinary isolates. Out of 64 urinary isolates, 36 isolates were from hospital and 28 were community urinary isolates.

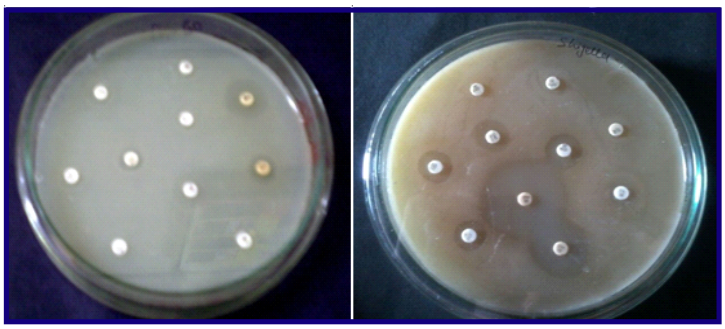
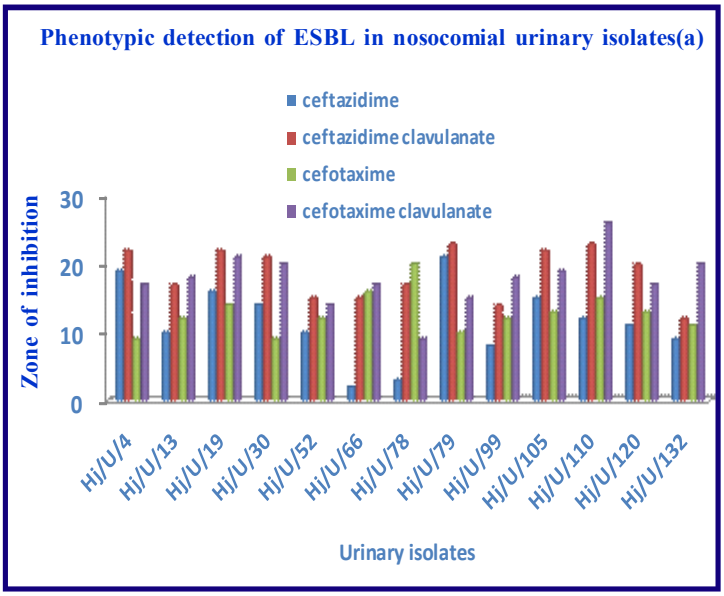
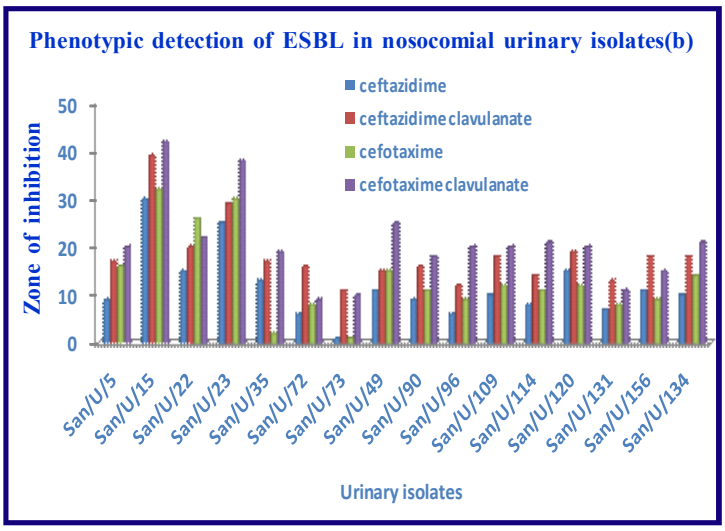


Figure -2: Screening of isolates for evaluation of β lactam resistance

As shown in figure-3, these resistant isolates were undergone for phenotypic detection of various types of β-lactamase according to CLSI guidelines.



Graph: 2: Phenotypic detection of ESBL from hospital isolates.

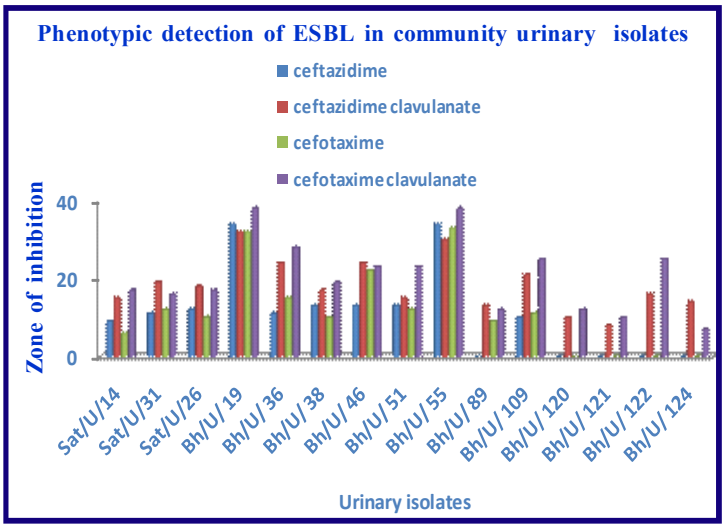


Graph: 3: Phenotypic detection of ESBL from hospital isolates.

Graph 2 and 3 were showing the ESBL prevalence in nosocomial urinary isolates. Out of 36 screened resistant nosocomial urinary isolates, 29 were showing presence of extended spectrum β -lactamase activity. Some of them have also shown co- resistance with KPC.

ESBLs have been reported from all parts of the world. However, prevalence varies widely even in closely related regions. The true incidence is difficult to determine because of the difficulty in detecting ESBL production & due to inconsistencies in testing & reporting. [8]

In 2007 in Asia pacific region was found to harbour plasmid borne ESBLs 62% and 75% in *E. coli* and *Klebsiella* spp. Respectively.[9] ESBL production rate was 43%, 73.8%, 96% and 70% in *E. coli* and 60% in *Klebsiella* spp. in Pakistan, Iraq, Iran and India respectively in 2009, 2011 and last two were in 2010.[10-12]



Graph: 4: Phenotypic detection of ESBL from community isolates.

According to graph 4, out of total 28 resistant community isolates 15 were phenotypically detected to be ESBL producing isolates. There were a limited number of studies on prevalence of ESBL showing a high rate in Bangladesh, where *E. coli* and *K. pneumoniae* were 43.2% and 39.5% respectively in 2004 [13] and at Rajshahi in Bangladesh it was 57.89% in *Klebsiella* spp. followed by *Proteus* spp. 50.0%, *E. coli* 47.83% and *pseudomonas* spp. 31.35% in 2010. [14] In France CTX-M-1, CTX-M-3 and CTX-M- 14 lactamases from *Entero bacteriaceae* was isolated in 2002. [15]

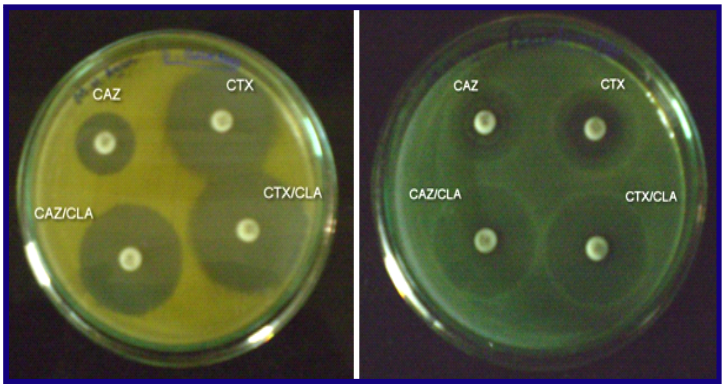
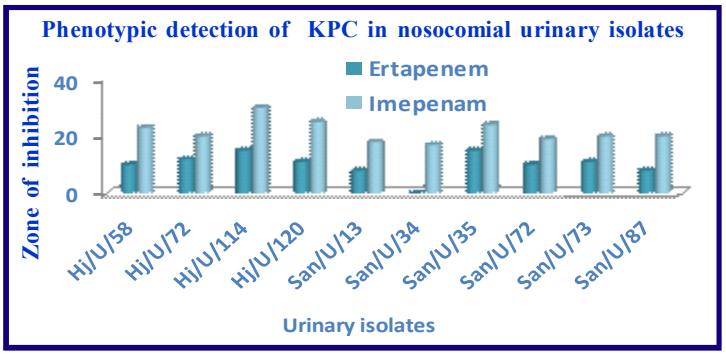
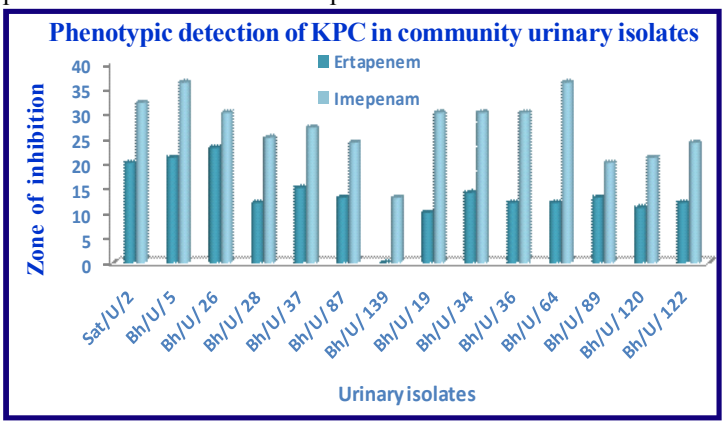


Figure -3: Phenotypic detection of ESBL



Graph: 5: Phenotypic detection of KPC from hospital isolates.

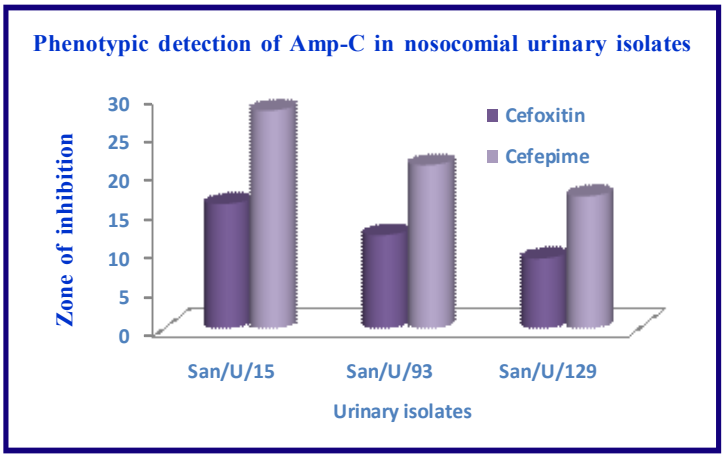
According to graph-5, prevalence of KPC among hospital isolates was 27.7 %. Nosocomial outbreaks of carbapenemase-producing *Enterobacteriaceae* infection have also been reported in Greece. [16] Dissemination of carbapenem resistance among *Enterobacteriaceae* poses a considerable threat to public health.



Graph: 6: Phenotypic detection of KPC from community isolates.

Prevalence of KPC in community, according to graph -6 was quite higher than nosocomial isolates. In community urinary isolates the prevalence of KPC was 50% in our study.

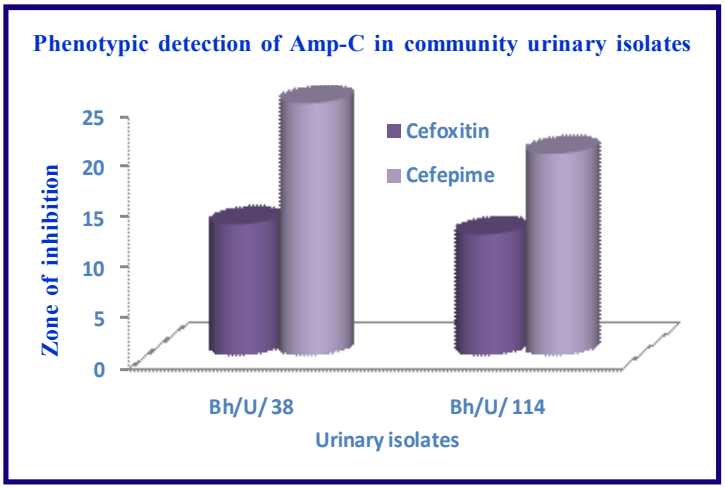
Carbapenems are considered the last line of effective therapy available for the treatment of severe infections. [17, 18] Resistance to these agents reduces clinical therapeutic choices and frequently leads to treatment failure.



Graph: 7: Phenotypic detection of Amp-C from hospital isolates.

In Gram-negative organisms, the detection of AmpC-mediated resistance is problematic as phenotypic techniques may give misinterpreted results and, consequently, treatment failures. [19] Moreover, there are no guidelines of Clinical and Laboratory Standards Institute (CLSI) for phenotypic techniques to investigate AmpC-producing organisms. [20] According to graph-7, occurrence of Amp-C among hospital isolates was only 8.33%.

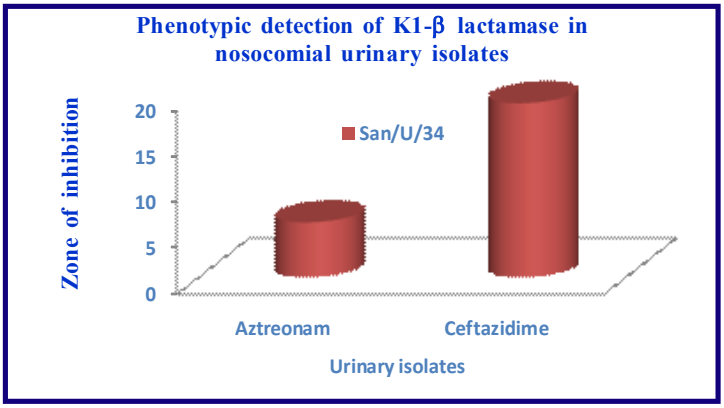
A number of detection methods for AmpC β-lactamases have been proposed. These screening tools include resistance to cephamycins and/or ceftazidime [21], retaining cefepime susceptibility. [22]



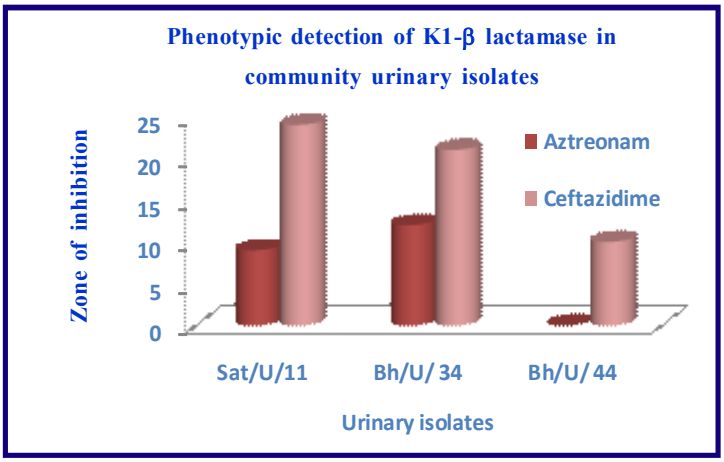
Graph: 8: Phenotypic detection of Amp-C from community isolates.

According to graph- 8, the incidence of Amp-C production in community isolates was 7.14% , which is parallel to the resistance found among hospital isolates. Resistance due to plasmid mediated AmpC enzymes is less common than ESBL production in most parts of the world but may be both harder to detect and broader in spectrum. [23] High AmpC production level results in high clinical treatment failures with broad-spectrum cephalosporins[22]. The exact prevalence of AmpC β-lactamases is unknown and this may be due to the absence of simple and reliable detection methods in clinical laboratories.

Phenotypic detection of KPC and Amp-C β - lactamase were shown in figure-4. Hyper production of the K1 enzyme can occur by mutation. The K1 enzyme is predominantly a penicillinase that can also significantly hydrolyze aztreonam, cefuroxime and ceftriaxone and has weak activity against cefotaxime or ceftazidime.



Graph: 9: Phenotypic detection of K1- β lactamase from hospital isolates.



Graph: 10: Phenotypic detection of K1- β lactamase from community isolates.

Very few isolates were found to be K1 β-lactamase producing strains. As shown in graph-9, the K1- β lactamase production among hospital urinary isolates were 8.33 %, while according to graph-10, community isolates were found to be only 3.57% resistant.

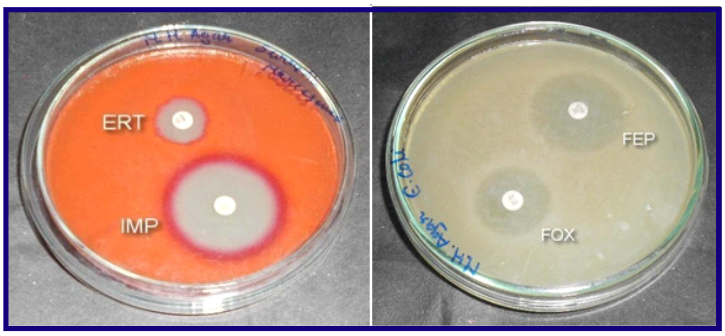


Figure -4: Phenotypic detection of KPC and Amp-C β - lactamase

4. CONCLUSION

Increasing levels of resistance are being found among common community-acquired urinary pathogens, and nosocomial pathogens. Antibiotic regimes which traditionally have been effective are now failing at increasing rates. Over the past few years the incidences of ESBL producing strains were not only restricted to hospitals but they have also been isolated from infections in outpatients.

Proper use of antibiotics is very important for various reasons. It reduces unnecessary expenses, reduces development of resistance to useful and life saving antibiotics, and minimizes many side effects. Knowledge of resistance pattern of bacterial strains in a geographical area will help to guide the appropriate and judicious antibiotic use. The correct management of such infections is extremely important for the future, particularly in term of reducing the incidence of new antibiotic resistance patterns.

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REFERENCES

1. Nicolle L. Complicated urinary tract infection in adults. Can J Infect Dis Med Microbiol 2005; 16: 349–60.
2. Nicolle LE. Catheter-related urinary tract infection. Drugs Aging 2005; 22: 627–39.
3. Rosenthal VD, Maki DG, Jamulitrat S, Medeiros EA, Kumar TS, Yepes Gómez D, et al. International Nosocomial Infection Control Consortium (INICC) report, data summary for 2003-2008. Am J Infect Control. 2010; 38:95-106.
4. Gaspari RJ, Dickson E, Karlowsky J, Doern G. Antibiotic resistance trends in pediatric uropathogens. International Journal of Antimicrobial Agents. 2005; 26:267–271.
5. McLoughlin TG Jr, Matar MJ. Antibiotic resistance patterns of uropathogens in pediatric emergency department patients. Emerg Med. 2003; 10(4):347-351.

6. Perez F, Endimiani A, Hujer KM, Bonomo RA. The continuing challenge of ESBLs. Current Opinion in Pharmacology. 2007, vol. 7, no. 5, pp. 459-469.
7. Pitout JDD, Laupland, KB. Extended Spectrum Lactamase producing Enterobacteriaceae: an emerging public health concern. Lancet infect Dis. 2008, vol. 8, pp.159-66.
8. Yushau MM, Kumurya AS, and Suleiman L. Prevalence of Extended spectrum lactamases among Enterobacteriaceae in Murtala Mohammed specialist hospital, Kano,N, Journal of Pure and Applied Sciences. 2010, vol.3, no.1, pp.169- 172.
9. Bell JM, Chitsaz M, Turnidge JD, Barton M, Walters LJ, and Jones RN. Prevalence and Significance of a Negative Extended-Spectrum -Lactamase (ESBL) Confirmation Test Result after a Positive ESBL Screening Test Result for Isolates of *Escherichia coli* and *Klebsiella pneumoniae*: Results from the SENTRY Asia-Pacific Surveillance Program, Journal of Clinical Microbiology. 2007, vol. 45, no. 5, pp-1478-1482.
10. Ali AM. Frequency of Extended Spectrum Beta Lactamase (ESBL) Producing Nosocomial Isolates in a Tertiary Care Hospital in Rawalpindi, A journal of Army medical corps. 2009, 3 ISSN 0030-9648.
11. Al- Charrakh, AH Yousif, SY, and Al- Janabi HS. Occurence and detection of Extended Spectrum -lactamases in Klebsiella isolates in Hilla, Iraq, African Journal of Biotechnology, 2011, vol. 10 no. 4, pp- 657-665.
12. Sharma J, Sharma M, and Roy P. Detection of TEM and SHV genes in Escherichia coli and Klebsiella pneumoniae isolates in a tertiary care hospital from India, Indian J Med, 2010, Res 132, pp.332-336.
13. Rahman MM, Haque JA, Hossain MA, Sultana R, Islam F, AHM, Islam S. Prevalence of extended spectrum beta lactamase-producing Escherichia coli and Klebsiella pneumoniae in an urban hospital in Dhaka Bangladesh, International Journal of antimicrobial agents. 2004, vol.24, no. 5, pp. 508-510.
14. Haque R, Salam MA. Detection of ESBL producing nosocomial gram negative bacteria from a tertiary care hospital in Bangladesh, Pk J Med Sci. 2010, vol. 26, no. 4, pp.887-891.
15. Dutour C, Bonnet R, Marchandin H, Boyer M, Chanal C, Sirot D, and Sirot J. CTXM-1,CTX-M-3 and CTX-M-14 -lactamases from Enterobacteriaceae Isolated in France, Antimicrobial Agents and Chemotherapy, 2002, vol. 46, no. 2, pp. 534-537.
16. Poirel L, Pitout JD, Nordmann P. Carbapenemases: molecular diversity and clinical consequences. Future Microbiol. 2007, 2:501–512.
17. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, Nordmann P, Patel JB, Paterson DL, Pitout J, Villegas MV, Wang H, Woodford N, Quinn JP. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. Lancet Infect Dis. 2013; 13:785–796.
18. McLaughlin M, Advincula MR, Malczynski M, Qi C, Bolon M, Scheetz MH. Correlations of antibiotic use and carbapenem resistance in *Enterobacteriaceae*. Antimicrob Agents Chemother. 2013; 57:5131–5133.
19. Rand K. H., Turner B., Seifert H., Hansen C., Johnson J. A., and Zimmer A., "Clinical laboratory detection of AmpC β-lactamase: does it affect patient outcome?" The American Journal of Clinical Pathology. 2011, vol. 135, no. 4, pp. 572-576.
20. Clinical and Laboratory Standards Institute CLSI, Performance standards for antimicrobial susceptibility testing, 24 th informational supplement. 2014, M100-S24.
21. Moland E. S., Black J. A., Ourada J., Reisbig M. D., Hanson N. D., and Thomson K. S., "Occurrence of newer β-lactamases in Klebsiella pneumoniae isolates from 24 U.S. Hospitals," Antimicrobial Agents and Chemotherapy. 2002, vol. 46, no. 12, pp. 3837-3842.
22. Doi Y. and Paterson D. L., "Detection of plasmid-mediated class C β-lactamases," International Journal of Infectious Diseases. 2007, vol. 11, no. 3, pp. 191–197.
23. Jacoby GA. AmpC β-lactamases.Clin Microbiol Rev. 2009; 22: 161-82.

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