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Research Paper

PREVALENCE OF ESBL AND AMP C BETA LACTAMASE PRODUCTION AMONG *Escherichia coli* AND *Klebsiella* ISOLATED IN URINARY TRACT INFECTION

Medical Science

KEYWORDS : UTI, ESBL &,AmpC β-lactamase producing E.coli & *Klebsiella* , Treatment.

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ABSTRACT

A study was under taken to know the occurrence of ESBL & Amp C β-lactamases producing strains and their antimicrobial susceptibilities to guide therapy for urinary tract infection. 500 samples were collected from MSU & in it, 150 showed significant growth of bacteria. E. coli 57% was the commonest organism isolated followed by Klebsiella 20%. The phenotypic tests reveal 55% , 60% were found to be ESBL producer in E.coli & Klebsiella respectively. Amp C β- lactamase producer was E. coli 12% , Klebsiella 23% . ESBL producing E. coli and Klebsiella spp showed 100 % sensitivity to Imipenam & Piperacillin-Tazobactum .And Amp C β- Lactamase producing species shows 100% sensitivity to Imipenam .

INTRODUCTION

Urinary Tract Inf.ections (UTIs) are one of the most common bacterial infections affecting approximately 150 million people worldwide and the incidence in India is 50,000 million persons per year[1]. Recurrent infection occurring in 20 – 30 % of the females results in high morbidity and mortality[2].More than 90% of acute UTI is caused by *Escherichia coli* and 10 – 20 % by CONS especially Staphylococcus saphrophyticus[3,4]. Among members of the Enterobacteriaceae family, resistance to β- lactams has been reported to be associated with ESBL and Amp C β- lactamase.

ESBL producing organisms hydrolyze oxyimino β- lactams like Cefotaxime, Ceftriaxone, Ceftazidime and Monobactams but have no effect on Carbapenems [5]. Plasmids responsible for ESBL and Amp C β- lactamase production frequently carry genes encoding resistance to other drugs also and therefore antibiotic options in the treatment of β- lactamases producing organisms are extremely limited[6]. It is necessary to investigate the prevalence of ESBL positive and Amp C β- lactamase strains in hospitals, so as to guide the clinician for better therapy. The present study is under taken to find out the frequency of the uropathogens, and ESBL , Amp C β- lactamase production in order to facilitate effective management of UTI.

REVIEW OF LITERATURE

In 1995, Quantitative bacterial counting over 105 bacteria per ml was regarded as significant bacteriuria by Kass concept[7]. Third generation Cephalosporins are most widelyprescribed & because of their extensive use they also developed resistance to many organismsespecially ESBL producing organism[8]. First plasmid mediated β-lactamase TEM-1 was described in early 1960's & isolated in *Escherichia coli* from a patient Temoniera in Greece and the gene was named after him. Another common plasmid mediated β-lactamase found in *Klebsiella* pneumoniae and *Escherichia coli* are SHV-1 (Sulph Hydryl in Variable). A new β-lactamase, called SHV-2 was derived from a mutation in the well-known SHV-1β-lactamase commonly found in *Klebsiella* and as they lead to resistance of extended spectrum cephalosporin they are called extended spectrum β-lactamases[9].

The β- Lactamase act by cleaving an amide bond of beta- lactam ring to form an acyl-enzyme complex. Any β-lactam antibiotic may be inactivated by these enzymes[10]. β -lactamase inhibitors structurally resemble β-lactam antibiotics which bind to β-lactam antibiotics protecting the antibiotics from destruction.They have weak antibacterial activity but are potent inhibitors of many β-lactamases. Three important β-lactamase inhibitors are Clavulanic acid, Sulbactam and Tazobactam. Enzymes capable of hydrolyzing major β-lactam antibiotics including third generation Cephalosporins are called as extended spectrum beta- lactamases.[11]

Detection methods of ESBL [11,12]

a.E test:

E test ESBL strips have 2 gradients, on one end Ceftazidime and on the opposite end Ceftazidime plus Clavulanic acid. MIC is the point of intersection of the inhibition ellipse with the E-test strip edge. Ratio of MICs of Ceftazidime and Ceftazidime Clavulanic acid is > 8 indicates presence of ESBLs.

Detection of Amp C β-lactamase[13]

b. Amp C disc test (Black et al., 2005)

The test is based on the use of Tris – EDTA to permeabilize a bacterial cell and release β-lactamases into the external environment. Amp C discs (i.e., filter paper disks containing Tris-EDTA) were prepared in house by applying 20 µl of 1:1 mixture of saline and 100 X Tris – EDTA to sterile filter paper discs allowing the discs to dry and storing them at 2- 8 °C. The surface of a MHA plate was inoculated with a lawn of Cefixitin- susceptible *E. coli* ATCC 25922 according to the standard disc diffusion method. Immediately prior to use, Amp C discs were rehydrated with 20 µl of saline and several colonies of each test organism were applied to a disc. A 30 µg Cefoxitin disc was placed on the inoculated surface of the MHA. The inoculated Amp C disc was then placed almost touching the antibiotic disc with the inoculated side of the disc is in contact with the agar surface. Overnight incubation at 35 °C in ambient air and plates were examined for either a distortion, indicating no significant inactivation of Cefoxitin (positive result), or the absence of a distortion, indicating significant inactivation of Cefoxitin (negative result).

Carbapenems are most effective and reliable as they are highly resistant to the hydrolytic activity of all ESBLs due to the Trans 6 – hydroxy ethyl group. Alternatively β- lactam and β-lactamase inhibitor combination may also be a further option to consider[13].

MATERIALS AND METHODS

The present study was conducted at the Department of Microbiology, Thanjavur Medical College Hospital. Ethical clearance was obtained from the college ethical committee before the commencement of the study & Informed consent was obtained from all patients .

20 ml of midstream urine samples were collected in 50ml wide mouth sterile container as per CLSI guidelines for urine Group16-A2. Immediately after collection, the samples were labelled and transported to the laboratory and processed within two hours[15]. After examined macroscopically for the presence of colour and turbidity,wet mount preparation and Gram staining were done with one drop of uncentrifuged urine and then

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inoculation was done using a calibrated loop (0.001) in Nutrient agar,Mac Conkey agar & Blood agar. After incubation for 24 hrs at 35-37°C, colonies were counted on each plate with the help of hand lens. The number of colonies was multiplied by 1000 to determine the number of microorganism per ml in the original specimen. More than 1, 00,000 CFU /ml is taken as Significant bacteriuria [16]. The isolated colony was identified by adopting the procedures of Gram staining, motility and routine biochemical reactions.

Using E-test strip contains Ceftazidime gradient at one end and Ceftazidime plus Clavulanate gradient on the opposite end ESBL production was detected .Amp C β lactamase production was detected by AmpC disc test The antimicrobial sensitivity pattern for all the isolates were done in Muller Hinton Agar by modified Kirby – Bauer disc diffusion method as per CLSI guidelines using antibiotic discs[17].

RESULTS

Among the samples collected from 500 patients, 150 samples shows culture positive. In it, the gram negative bacilli (GNB) with 129 isolates (86.0%) was the major cause for UTI while only 21 isolates were gram positive cocci (GPC). Among the 129 GNB, the *Escherichia coli* was 85 and *klebsiella* spp 30 & constituted 77 % of total isolates. ESBL production was found in Escherichia coli 47 in *Klebsiella* spp 18& total 65(56%). Amp C β- lactamase production was for Escherichia coli 10 and *Klebsiella* spp 7 total 17(15%) - Table I.

Antimicrobial susceptibility pattern of ESBL producer Amp C β Lactamase producer and non ESBL producer among isolated *E. coli* and *Klebsiella* spp. were compared in -Table II.

The ESBL producer shows multiple drug resistance than the non- ESBL producers. In case of *E. coli*, sensitivity of Gentamicin is reduced from (71% to 21%). Amikacin shows (17%) reduction in sensitivity. Sensitivity also reduced in Nalidixic acid (50 % to 9 %), Norfloxacin (43% to 9%),and Levofloxacin (96 % to 81%). Whereas, in *Klebsiella* spp the highest resistance was found for Gentamicin, sensitivity diminished from (80% to 17%). For Nalidixic acid (60 % to 22%), Norfloxacin (60% to 22%) and Ciprofloxacin (60% to 50%).

The antimicrobial susceptibility of ESBL pproducing *E. coli* and *Klebsiella* spp to Imipenem, Piperacillin-Tazobactum, Cefipime, Cefoxitin, Cefeparazone-Sulbactum, Amikacin, Levofloxacin and Nitrofurantoin were 100%/100%, 100%/94%, 91%/89%, 91%/89%, 91%/89%, 83/ 83%, 81/89% and 68/56% respectively and for Amp C β- Lactamase producing E.coli & *Klebsiella* spp are Imipenam (100%/100%), Cefipime (90%/71%), Levofloxacin(80%/86%), Amikacin (70%/86%) and Nitrofurantoin (80%/71%).

DISCUSSION

E test revealed that out of 85 isolates of *E. coli*, 47 (55%) were found to be ESBL producer, out of 30 isolates of *Klebsiella*, 18 (60%) were found to be ESBL producer.Whereas, lower occurrence of ESBL producer in urinary isolates of *E. coli* and *Klebsiella* was found to be 40 and 41 % respectively as reported by Baby Padmini and Appalaraju, 2004 Coimbatore[18] .In the present study, the prevalence of Amp C β- lactamase producer was lesser among *E. coli* (12%) as compared to the 23% prevalence among *Klebsiella*. This is in accordance with the findings of Shasikala et al., 2010))[14]from Tamil Nadu who had recorded lesser prevalence of Amp C β- lactamase in *E. coli* (9.9%) and more among *Klebsiella* (31.1%). The ESBL producer shows multiple drug resistance than the non- ESBL producers.

The present study revealed that the most effective antibiotic against ESBL producing *E. coli*, and *Klebsiella* spp. in UTI are Imipenam,Piperacillin-Tazobactum ,Cefipime , Cefeperazone-sulbactum , Cefoxitin , Amikacin , Levofloxacin and Nitrofurantoin. Baby Padmini and Appalaraju, (2004)18 and suggested Imipenam (100%), Nitrofurantoin (89%) and Amikacin (86%) is the drug of choice for ESBL producers which are similar to this study.Study conducted in Kerala by (Shasikala et al., 2010) showed Piperacillin-Tazobactum (96.8%), Cefeperazone-sulbactum (92.2%), were sensitive to ESBL producers[14].

The antibiotic of choice for Amp C β- Lactamase producing *E. coli* and *Klebsiella* spp in UTI are Imipenam (100/100%), Cefipime (90%/71%), Amikacin (70%/86%), Levofloxacin (80%/86%) and Nitrofurantoin (80%/71%). It is comparable with Bush, K., Jacoby G[11] and they suggest carbapenam are more effective than cefipime. Screening for ESBL and Amp C β- Lactamase production as a routine procedure in clinical laboratories gives valuable information to the clinician in appropriate selection of antibiotics.

CONCLUSION

Based on our study, we conclude that, there is a high prevalence rate of ESBL and Amp C β- Lactamase production seen among uropathogenic *E. coli* and *Klebsiella* spp. Even though all the isolates are 100% sensitive to Imepenam, it should be kept in reserve as the second line of drug. Other drugs which is most economic and orally effective like Nitrofurantoin and Levofloxacin can be given to outpatients. Amikacin, Cefipime and β lactamase inhibitors like Piperacillin-Tazobactum, Cefeperazone- Sulbatum can be given to inpatients. According to the prevalence rate of the ESBL and Amp C β- Lactamase production in a health care facility, institutional antibiotic policy can be tailored to achieve superior therapeutic outcome and bring about a reduction in healthcare costs. It also eliminates misuse of conventional cephalosporin in a significant proportion of patients.

Plate 20. E test

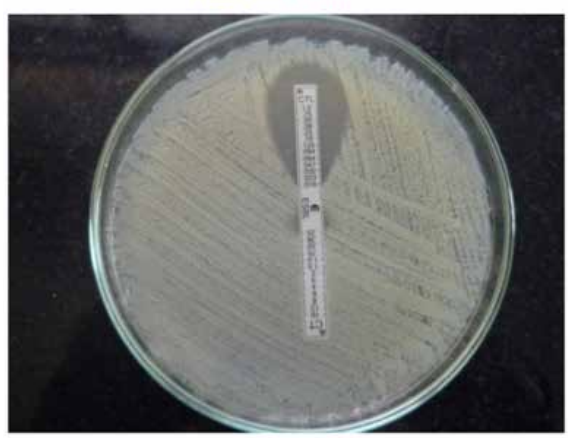

Table I: Prevalence ESBL and Amp C β - lactamase

Organism	ESBL	Amp C β -lactamase	No β -lactamase	Total(%)
E. coli (n=85)	47(55%)	10 (12%)	28(33%)	100
Klebsiella spp. (n=30)	18(60%)	7 (23%)	5(17%)	100
Total (n = 115)	65(56%)	17(15%)	33(29%)	100

Table II: Antimicrobial susceptibility pattern of ESBL producer Amp C β Lactamase producer and non ESBL producer among isolated *E. coli* and *Klebsiella* spp.

Organism		G	AK	A	NF	NA	NX	LE	CN	CA	CPM	AO	I	AC	CFS	PT
E. coli	ESBL producer n =47	10 (21)	39 (83)	0 0	32 (68)	4 (9)	4 (9)	38 (81)	43 (91)	9 (19)	43 (91)	12 (26)	47 (100)	20 (42)	43 (91)	47 (100)
	ESBL non producer n = 28	20 (71)	28 (100)	14 (50)	26 (93)	14 (50)	12 (43)	27 (96)	27 (96)	22 (79)	27 (96)	26 (93)	28 (100)	11 (39)	20 (71)	22 (79)
	Amp C β Lactamase producer n = 10	2 (20)	7 (70)	0 (0)	8 (80)	2 (20)	3 (30)	8 (80)	0 (0)	2 (20)	9 (90)	3 (30)	10 (100)	2 (20)	2 (20)	5 (50)
Klebsiella spp	ESBL producer n = 18	3 (17)	15 (83)	0 0	10 (56)	4 (22)	4 (22)	16 (89)	16 (89)	5 (28)	16 (89)	6 (33)	18 (100)	9 (50)	16 (89)	17 (94)
	ESBL non producer N =5	4 (80)	5 (100)	2 (40)	4 (80)	3 (60)	3 (60)	4 (80)	5 (100)	4 (80)	5 (100)	4 (80)	5 (100)	2 (40)	4 (80)	3 (60)
	Amp C β Lactamase producer n = 7	3 (43)	6 (86)	0 (0)	5 (71)	2 (29)	2 (29)	6 (86)	0 (0)	2 (29)	5 (71)	1 (14)	7 (100)	2 (29)	3 (43)	4 (57)

***. G-Gentamicin, AK-Amikacin, A-Ampicillin, N F-Nitrofurantoin, NA-Nalidixic acid, NX-Norfloxacin, LE-Levofloxacin, CN-Cefixitin, CA-Ceftazidime, CPM-Cefipime, AO-Aztreonam, I-Imipenam, AC- Amoxyclav, CFS-Cefeperazone-Sulbactum, PT- Piperacillin-Tazobactum**

REFERENCE

1. http: //www. India study channel.com/resources/57366 – How – Urinary – Tract – Infections – UTI –OC 12/2/10. Author : Dr. Sparsha. | 2. Topley Wilson, 2007. Microbiology and microbial infection.Vol.1. Bacterial infections of the urinary tract 10th Edition, pp: 672 – 683. | 3. Ferrel D.J., Morrisey, I., De Rubies, D., Robbins, M. and Felmingham, D. 2003. A UK multicentered study of antimicrobial susceptibility of bacterial pathogen causing UTI. J. Infect. 46: 94 -100. | 4. Akram, M., Shahid, M. and Khan, A. U. 2007. Etiology and antibiotic resistance patterns of community- acquired urinary tract infections in N N M C Hospital Aligarh, India. Annals of Clinical Microbiology and Antimicrobiols. 6 (4): 1- 7. | 5. Phillipon, A., Arlet, G. And Lagrange, P.H. 1996. *E. coli*. Frequency de resistance et evolution a divers antibiotiques urinaries don't la fosfomycine en milieu hospitalier. Med Mal Infect 26: 539 – 541. | 6. Paterson, D.L., Bonomo, R.A. 2005. Extended-spectrum β-lactamases: Clinical update. Clin. Microbiol. Rev. 18(4):657-686. | 7. Kass, E. 1955. Asymptomatic infection of the urinary tract. Trans Association of American Physician; 69: 56 – 63. | 8. Marple, C. 1941. Annals of internal medicine. 14: 2220 | 9. Hootan, T. M., Sholes, D., Staple, A.E. and Roberts P.L. and Winrawe, M.C.S. 2000. A prospective study of symptomatic bacteria in sexually active young women. New Eng. J. Med.:343(14): 992 – 7. | 10. Goodmann & Gillman's. 2006. The pharmacological basis of therapeutics 11th Edn:, 1095-1154. | 11. Bush, K., Jacoby G.A. and Medeiros, A.A. 1995. A functional classification scheme for β-lactamase and its correlation with molecular structure. Antimicrobial agents' chemotherapy. 39: 1211 – 1233. | 12.Collee. J.G., Fraser. A.G., Marimon, B.P. and A. Simmons. 2006. Mackie and Mc Cartney - Practical Medical Microbiology. 14th Edn. 84 – 90, 95 – 111; 151 – 78. | | 13.Black, J. A., Moland , E.S. and Thomson, K.S. 2005. Amp C disc test for detection of plasmid mediated Amp β- lactamase. J. Clinical Microbiology. 43(7): 3110 - 13. | 14.Shashikala, S., R. Kavitha, A. R., Gireesh, P.M. and Shamsul Karim. 2007. Routine screening for ESBL production N necessity of Today. The International Journal of Microbiology. 3(1). | 15.Clinical and Laboratory Standards Institute. Urine analysis approved guidelines. 2008. II Edn. CLSI document group 16 A2. Clinical and Laboratory Standards Institute, Wayne, Pa. USA. | 16. Bailey and Scott's Diagnostic Microbiology. 2007. XII edn. pp:172 – 214;842 -55 | 17.Clinical and Laboratory Standards Institute. 2008. Performance Standards for Antimicrobial Disc Susceptibility Tests. II Edn. CLSI document group - M2 A 10. Clinical and Laboratory Standards Institute, Wayne, PA. USA. | 18.. Baby padmini, S., and B. Appalaraju. 2004. ESBL in Urinary isolates of *E. coli* and *Klebsiella* pneumonia - prevalence and susceptibility pattern in a Tertiary Care Hospital, IJMM, 22(3): 172-174. |