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DETECTION OF EXTENDED SPECTRUM BETALACTAMASES AND THEIR ANTIBIOTIC SUSCEPTIBILITY AMONG URINARY ISOLATES

KEYWORDS: UTI, ESBL, AST,DDST

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ABSTRACT

Background : Urinary tract infections (UTI) are one of the most common bacterial infections in humans worldwide. A study on the changing antibiotic sensitivity pattern is pertinent for an appropriate treatment and for prevention and control of the various mechanisms of drug resistance.

Aims& Objectives: To perform antibiotic sensitivity of urinary isolates and to detect the extended spectrum betalactamase (ESBL) production among Escherichia coli, Klebsiella pneumoniae and Proteus by double disc synergy test (DDST) and Etest.

Materials & Methods : A total of 422 samples collected during a period of six months were processed in blood agar and MacConkey agar. Antibiotic susceptibility tests done and ESBL isolates were detected using double disc synergy test and E test.

Results : Escherichia coli was the most common isolate and also an ESBL producer (67.5%). ESBL isolates are more susceptible to Meropenem, Nitrofurantoin, and Amikacin.

Conclusion : E.coli was the predominant ESBL producer . Coresistance of non beta lactum antibiotics was also observed.

INTRODUCTION: Urinary tract infections(UTI) contribute to 35-40% of hospital acquired infections causing morbidity and increased mortality in hospitalized patients.[1] It has a spectrum of clinical entities with severity ranging from asymptomatic infection to acute pyelonephritis with sepsis.

The main causative agent of urinary tract infection is Escherichia coli. Other Gram negative organisms like Klebsiella, Proteus, Pseudomonas, Enterobacter and Gram positive organisms such as Enterococcus faecalis and Staphylococcus saprophyticus also play a major role .

Antimicrobial resistance is a growing threat worldwide, resistance mechanisms being found for almost every class of antibiotic agents. The production of beta lactamases are the important mechanism of resistance in Gram negative bacteria. The production of extended-spectrum β-lactamases (ESBL) is an important mechanism of resistance to third-generation cephalosporins which are widely used in urinary tract infections because of lesser nephrotoxicity effects.

Extended spectrum beta lactamases (ESBL) are enzymes capable of conferring bacterial resistance to the penicillins ,first, second and third generation cephalosporins and aztreonam(but not the cephamycins or carbapenems) by hydrolysis of these antibiotics. They are inhibited by beta lactamase inhibitors such as clavulanic acid, sulbactum and tazobactum They are thought to have evolved by mutation of the TEM and SHV genes. Organisms responsible for UTIs such as E.coli and Klebsiella produce these enzymes. These enzymes are plasmid borne and confer multi drug resistance. Disc diffusion methods have been proposed by CLSI for the detection of ESBLs in organisms such as Klebsiella, Escherichia coli and Proteus. The reduction in the zone diameters of antibiotics such as cefpodoxime, ceftriaxone, ceftazidime, cefotaxime or aztreonam indicate ESBL production. Phenotypic confirmatory methods, such as double disc synergy test, combined disk method and E test (epsilometry methods) are available which are based on in vitro inhibition of ESBLs by betalactamase inhibitors, Genotypic detection methods are useful in distinguishing between specific enzymes (TEM, SHV, CTX-M) responsible for ESBL production and for epidemiological purposes.

MATERIALS AND METHODS: This prospective study was conducted in a tertiary care Hospital in Coimbatore for a period of six months. During the study period 422 urine samples were collected. Macroscopic examination was done. Samples were centrifuged and examined microscopically by wet mount and Grams staining. Number of pus cells and organisms were noted.

Culture of the specimen : Fresh specimen (uncentrifuged urine) were inoculated directly on blood agar and MacConkey agar plates and incubated overnight at 37◦c .Plates were read on the next day.

4 mm loops were used and quadrant streaking method was followed for colony count. Bacterial identification was done using the following biochemical reactions. Indole test, Citrate test, Triple sugar iron agar test, Mannitol motility media, Bile esculin agar.

Antibiotic susceptibility testing

This test was done using modified Kirby-Bauer disc diffusion technique using Mueller Hinton agar[2].

Screening for extended spectrum betalactamase : According to CLSI guidelines, those organisms showing zone inhibitory size less than 27mm for cefotaxime was considered as potential ESBL producers and were subjected to confirmatory tests. Organisms showing resistance were subjected to Double disc synergy test and Etest.

Double disc synergy test :

Test organism was inoculated in peptone water and lawn culture was made on Mueller-Hinton agar as recommended for a standard disk diffusion susceptibility test. Discs containing 30µg of ceftazidime and 30µg cefotaxime were placed 15 mm apart (centre to centre) to the disc containing Amoxicillin/Clavulanic acid (20/10µg). Plates were incubated at 37◦C overnight.

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Enhancement of zone of inhibition between the clavulanate discs and any one of the β lactam antibiotic discs were interpreted as an indicator of ESBL producers.

E test : EM079A

It is a phenotypic ESBL detection strip which is coated with mixture of three different cephalosporins with and without clavulanic acid on a single strip in a concentration gradient manner. The upper half has Ceftazidime, Cefotaxime and Cefepime + Clavulanic acid and Tazobactum with concentration gradient tapering downwards. The lower half is coated with Ceftazidime, Cefotaxime and Cefepime in a concentration gradient in reverse direction. Inoculum was prepared from pure culture, MHA agar was streaked with swab dipped in the inoculum. E strip was applied on the agar plate and incubated, read on the next day according to the manufacturer's instruction.

RESULTS

In our study among the 422 (100%) samples, 281 (66.6%) samples yielded no growth and organisms isolated from 141(33.4%) samples.

Out of 141 isolates , Gram negative organisms predominate showing 108 (76.60%) . The Gram positive organisms and Candida species were isolated at a rate of 20 (14.18%) and 13 (9.22%) respectively. The overall sensitivity and resistance pattern of uropathogens were shown in **Table 1**.All the organisms were 128(100%) sensitive to Meropenem. The highest sensitivity was shown to Amikacin 119(92.97%), and the lowest sensitivity to Ampicillin 21(16.41%). Sensitivity to all other antibiotics were , Nitrofurantoin 111 (86.72%), Cefoperazone sulbactum 105 (82.03%), Gentamycin 89 (69.53%), Ciprofloxacin 63(49.22%), Norfloxacin 44(34.38%),, Cefotaxime 35 (27.34%), Cotrimoxazole 32 (25%) and Ampicillin 21 (16.41%).

1. Overall sensitivity and resistance pattern of uropathogens to antibiotics (n=128)

Antibiotics	Sensitivity		Resistance	
	No	%	No	%
Ampicillin(A)	21	16.4	107	83.6
Gentanycin(G)	89	69.5	39	30.5
Ciprofloxacin(CIP)	63	49.2	65	50.8
Amikacin(AK)	119	93.0	9	7.0
Cotrimoxazole(COT)	32	25	96	75
Nitrofurantoin(NIT)	111	86.7	17	13.3
Norfloxacin(NX)	44	34.4	84	65.6
Cefotaxime(CTX)	35	27.34	93	72.7
Cefoperazone sulbactum(CFS)	105	82.0	23	18
Amoxyclav(AMC)	60	46.9	68	53.1
Meropenem (MRP)	128	100	0	0

2. Percentage of ESBL organisms(n=67)

Name of the organism	Total number	ESBL positivity	Percentage
E.coli	77	52	67.5
Klebsiella pneumonia	15	10	66.7
Proteus species	9	5	55.6

All the Cefotaxime resistant isolates of E. coli , Klebsiella and Proteus were subjected to confirmatory tests by double disc synergy test and E tests. The results were shown in **Table 2 .** Among the 67 ESBL positive isolates, Escherichia coli constituted 52 (67.5%), Klebsiella 10 (66.7%) and Proteus 5(55.6%) respectively.

3. Comparison of resistance pattern of ESBL and non ESBL E.coli

Antibiotics	Total no of resistant organisms	ESBL E. coli(n=52)	Non –ESBL E. coli(n=25)
Ampicillin	62	50 (96.2%)	12 (48%)
Gentamycin	15	12 (23.1%)	3 (12%)
Ciprofloxacin	47	34 (65.9%)	13 (52%)
Amikacin	4	4 (7.7%)	0
Cotrimoxazole	59	44 (84.6%)	15 (60%)
Nitrofurantoin	1	1 (1.9%)	0
Norfloxacin	60	43 (82.7%)	17 (68%)
Cefotaxime	54	52 (100%)	2 (8%)
Cefoperazone Sulbactum	3	3 (5.8%)	0
Amoxicillin clavulanic acid	39	31 (59.6%)	8 (32%)

The resistance pattern of ESBL and non ESBL E. coli were compared in **Table 3.** The ESBL Escherichia coli isolates showed highest resistance towards Cefotaxime 52(100%), Ampicillin 50(96.2%), Cotrimoxazole 44(84.6%), Norfloxacin 43(82.7%) and Ciprofloxacin 34(65.9%) compared to non ESBL stains.

4.Comparison of resistance pattern of ESBL and non ESBL Klebsiella

Antibiotics	Total number of resistant organisms	ESBL Klebsiella (n=10)	Non ESBL Klebsiella (n=5)
Ampicillin	14	10 (100%)	4 (80%)
Gentamycin	3	2 (20%)	1 (20%)
Ciprofloxacin	4	4 (40%)	0
Amikacin	2	2 (20%)	0
Cotrimoxazole	11	9 (90%)	2 (40%)
Nitrofurantoin	4	3 (30%)	1 (20%)
Norfloxacin	9	6 (60%)	3 (60%)
Cefotaxime	10	10 (100%)	0
Cefoperazone Sulbactum	-	-	-
Amoxicillin clavulanic acid	8	7 (70%)	1 (20%)

The resistance pattern of ESBL and non ESBL Klebsiella were compared in **Table 4** The ESBL Klebsiella strains were highly resistant towards Cefotaxime 10 (100%), Ampicillin 10(100%), Cotrimoxazole 9(90%), Amoxy clavulanic acid 7(70%) and Norfloxacin 6(60%)

DISCUSSION:

Current knowledge of the organisms that cause urinary tract infection and their antibiotic susceptibility pattern is mandatory, to ensure appropriate therapy.

The overall antibiotic sensitivity pattern of the uropathogens in our study showed higher sensitivity to Meropenem(100%) ,Amikacin (93%) and Nitrofurantoin(86.7%). There was a higher degree of resistance towards Ampicillin(83.6%) and Cotrimoxazole (75%). This was in accordance with the study of **Sandhiya R et al.,**[3] where the uropathogens showed a higher degree of sensitivity towards nitrofurantoin and Amikacin. **Chaudhary U et al .,**[4] have proved that uropathogens were 100% sensitive to Meropenem.Another study by **Durgesh D et al.,**[5] showed that there was a high degree of resistance towards Ampicillin(79%), similar to our study.

Another study by **Gaurav Dalela et al.,**[6] showed that there was a high degree of resistance towards Cotrimoxazole (78.9%), Cefotaxime (78.2%), Amoxy-clavulanate (82.4%) and Norfloxacin (67.6%). This is similar to our study where the resistance was 75%, 72.7% ,53.1% and 65.6% for Cotrimoxazole, Cefotaxime, Amoxy-clavulanate and Norfloxacin respectively.

In our study , ESBL sceening was done using Cefotaxime disc which was similar to the study done by **N M Suryawanshi et al.,**[7]**.** In this, they have proved that all the ESBL producers were uniformly resistant to all three third generation cephalosporins (Cefotaxime, Ceftriaxone and Ceftazidime). Confirmation was

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done by double disc synergy test and Etest.[8].

According to our study 67.5% of Escherichia coli, 66.7% of Klebsiella and 55.6% of Proteus were confirmed as ESBL producers. A study by **Anbumani Narayanasamy et al.,**[9] indicated that the prevalence of ESBL varies from 28% to 84%. In our study high rate of ESBL was seen in Escherichia coli isolates followed by Klebsiella and Proteus. Our study correlates with the Studies **by S Baby padmini et al., Rugved Kulkarni et al .**, **S.Krishna kumar et al. ,**and **Venkatadri Babu et al.,** which showed that Escherichia coli was the commonest ESBL producer.[10,11,12,13] In contrast, other study by **Omar B Ahmed et al.,**[15] 2013 showed that Klebsiella pneumoniae was the common ESBL producer.

The ESBL percentage of Escherichia coli in our study coincides with the study by **Omar B Ahmed et al.,**[15] and **Abdulghani et al.**[21], which showed 65% of Escherichia coli were ESBL producers. The study by **Rugved Kulkarni et al**[11]., and **S.Krishnakumar et al.,**[12] also showed a lower incidence of 40.7% and 44.4% of ESBL producing Escherichia coli respectively. Anotherther study by **Umadevi S et al** .,[14] showed a higher prevalence of ESBL Escherichia coli (81%). Studies by **Rugved Kulkarni et al.,**[11] (15.9%) and **S.Krishnakumar et al** .[12], (37.0%) isolated lower percentage of ESBL producing Klebsiella similar to our study whereas study in Coimbatore by **BabyPadmini et al.,**[10] showed 41% of ESBL E.coli and 40% of ESBL producing Klebsiella . The rate of ESBL production in Proteus was 48.9% and 33.3% respectively by the studies conducted by **Jitendra Kumar Pandy.,**[16] **and Omar B Ahmed** .,[15] which was slightly lower than in our study.

The sensitivity pattern of Escherichia coli revealed that maximum sensitivity was shown towards Nitrofurantoin, Cefoperazone sulbactum and Amikacin. Other than beta lactum antibiotics, ESBL strains showed maximum resistance towards Gentamycin, Ciprofloxacin, Norfloxacin and Cotrimoxazole which is statistically significant when compared to non ESBL isolates.
This showed that ESBL strains exhibit co resistance for fluroquinolones , aminoglycosides and cotrimoxazole.. The study by **Swaminathan ranjan.,**[17] also showed that ESBL E.coli strains were highly resistant to Gentamycin, Norfloxacin, Cotrimoxazole and Ciprofloxacin. ESBL E.coli strains showed high sensitivity towards Nitrofurantoin and Amikacin. The ESBL Klebsiella was more resistant to Cotrimoxazole and Norfloxacin apart from beta lactum antibiotics. This is similar to a study by **Anil Chander et al.,**[18]

The study by **S.Shafiyabi et al .,**[19] showed that there was an increased resistance of results towards Amikacin . This was contradictory to our study and many other studies which showed that there was high sensitivity towards Amikacin. In a study conducted by **Mohammed Rashid et** al.,[20] Escherichia coli and Klebsiella isolates were highly resistant against nitrofurantoin This is in contrast to our study where the sensitivity towards nitrofurantoin was high.

Since Proteus strains (mirabilis&vulgaris) did not show multidrug resistance and are sensitive to most of the antibiotics, our discussion is limited to the ESBL producing strains of E.coli & Klebsiella.

Our study suggests Nitrofurantoin as the first line drug against UTI before culture and sensitivity is done. It is cost effective and readily available and also safer in pregnancy.

Considering aminoglycosides and fluroquinolones, Gentamycin and Norfloxacin were highly resistant. Ciprofloxacin also showed resistance in ESBL isolates compared to non ESBL isolates. This can be explained due to co-resistance of ESBLs with aminoglycosides and fluroquinolones because of plasmid mediated gene transfer.[10]

Nitrofurantoin can be considered in case of un complicated UTI cases and Amikacin along with cefoperazone sulbactum can be used in treating hospitalized patients with complicated UITs with Meropenem as a reserve drug where response to all other drugs are inadequate..

CONCLUSION

Escherichia coli is the predominant isolate from urine specimens. It is also the most common ESBL producer in our study. Though ESBL isolates are more susceptible to Meropenem, other drugs like Nitrofurantoin and Amikacin can also be prescribed for urinary isolates so that indiscriminate use of carbapenems may be avoided. There is also associated co resistance of non beta lactam antibiotics like Norfloxacin, Cotrimoxazole and Gentamycin which has been observed among the ESBL positive isolates. Hence they can no longer be prescribed as an empirical therapy. Strict infection control policy in hospitals along with antibiotic stewardship programs can limit the spread of these multi drug resistant organisms.

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