PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCTION AMONG URINARY ISOLATES IN A TERTIARY CARE SETUP.....A BITTER TRUTH ABOUT THE SUPERBUGS....!!!!!!!

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ABSTRACT

BACKGROUND

Urinary Tract Infection (UTI) occurs as a result of interaction between bacterial virulence and host biological and behavioural factors. Worldwide, about 150 million people are diagnosed with UTI each year, which costs the global economy. UTI although treatable, is now becoming increasingly tough to control, because of rampant antimicrobial resistance in the Enterobacteriaceae family, particularly in *E. coli*. ESBL (Extended Spectrum Beta Lactamase) producing organisms are distributed worldwide and their prevalence is increasing. Organisms responsible for UTI such as *E. coli*, *Klebsiella* species and *Enterobacter* species have the ability to produce ESBLs in large quantities. The World Health Organisation (WHO) has called antibiotic resistance an emerging disease. In almost all cases of UTI, empirical antimicrobial treatment initiated before the laboratory results of urine culture are available, thus, antibiotic resistance may increase in uropathogens due to frequent use of antibiotics. Therefore, the present study was conducted to study the bacterial spectrum causing UTI in our Institute and to find out the antibiotic response to these organisms with special emphasis on ESBL production by these organisms.

Aims and Objectives - 1) To study the bacterial spectrum causing UTI in our Institute; 2) To study the prevalence of drug resistance in Gram negative organisms to different urinary antibiotics with special emphasis on ESBL production.

MATERIALS AND METHODS

The prospective study was conducted over a period of three months (From October 2016 to December 2016) at Department of Microbiology, Dr. Vasantrao Pawar Medical College and Hospital, Nashik, Maharashtra, India. Total 955 urine samples received at Microbiology Laboratory were included in the study. Antibiotic Sensitivity Testing (AST) was performed using Kirby-Bauer Disc diffusion method on Mueller-Hinton agar by following the guidelines given by CLSI 2012. The antibiotic panel used for AST included - Gentamycin, Amikacin, Piperacillin, Cefotaxime, Cotrimoxazole, Ampicillin, Polymyxin B, Ceftazidime, Cefadroxil, Amoxiclav, Norfloxacin, Nitrofurantoin, Cefuroxime, Cefepime, Piperacillin-Tazobactam and Imipenem. All the isolates which showed resistance to third generation cephalosporins were further tested for confirmation of beta-lactamase production by phenotypic methods. Confirmation of ESBL production was done by using E-test (Hi-Media Laboratory, Mumbai).

Screening of ESBL production - This was done by two methods, a) Phenotypic Confirmatory Disc Diffusion Test (PCDDT); b) Double Disc Synergy Test (DDST).

Confirmation of ESBL production - This was done by using Ezy-MIC strips (Hi-Media Laboratories Pvt. Ltd.).

RESULTS

Total 955 urine samples were processed. Out of 955 samples, Gram Negative bacilli were isolated from 433 samples and Gram positive organisms were isolated from 54 samples. Out of 433 Gram negative organisms, 205 (47.3%) were *E. coli* and 82 (18.9%) were *Klebsiella pneumoniae*, 50 (11.5%) were *Proteus* species, 55 (12.7%) were *Pseudomonas* species and 41 (9.4%) were *Enterobacter* species. Out of 433 Gram negative organisms, 158 were ESBL producers. Out of 158 ESBL producers *E. coli* were 45.5%, *Klebsiella pneumoniae* were 30.4%, *Proteus* species were 24%, *Pseudomonas* species were 25.45% and *Enterobacter* species were 31.7%. Most of the organisms were resistant to third generation cephalosporins. ESBL producers showed resistance to Cefotaxime (81.01%), Cefoperazone (82.2%) and Ceftazidime (81.01%). Resistance to aminoglycosides such as Amikacin (24.05%) was found to be on lower side as compared to Gentamicin (62.02%). Also resistance to Tetracycline was found to be on higher side (77.8%). Sensitivity towards Piperacillin, Polymyxin B, Cefepime, Piperacillin + Tazobactam and Imipenem turned out to be satisfactory. *E. coli* shows higher percentage of resistance to third generation cephalosporins, Cefotaxime (85.1%), Ceftazidime (88.2%) and Cefoperazone (87.2%). *Klebsiella pneumoniae* shows unsatisfactory results as compared to *E. coli*. Also, *Klebsiella pneumoniae* show higher amount of resistance to third generation cephalosporins such as Cefotaxime (96%), Ceftazidime (96%) and Cefoperazone (92%). But resistance to higher antibiotics like Cefepime, Piperacillin + Tazobactam and Imipenem shows satisfactory level of sensitivity among all ESBL producers.

CONCLUSION

Increasing prevalence of ESBL producers among Gram negative organisms is nowadays becoming a great threat. This has become a major clinical problem in treating infections caused by ESBL producers. Increasing rate of resistance to commonly used antibiotics is an alarming sign for future of healthcare sector. Our study reveals that commonly used antibiotics are almost of no use for

treatment of UTI patients. But special drugs like Cefepime, Piperacillin + Tazobactam and Imipenem could be kept as reserved drugs to treat infections. If not used rationally resistance to these reserved drugs will emerge, which will ultimately take us to situation like "NO ANTIBIOTIC ERA".....and which is a bitter truth about the Superbugs.....!!!

KEYWORDS

Urinary Tract Infections (UTI), ESBL, Drug Resistance.

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BACKGROUND

Urinary Tract Infection (UTI) occur as a result of interaction between bacterial virulence and host biologic and behavioural factors.¹ "UTI" is a broad term that encompasses either asymptomatic microbial colonisation of urine or asymptomatic infection with microbial invasion and inflammation of urinary tract.^{2,3} UTI remains the most common bacterial infection in human population and is one of the most frequently occurring nosocomial infections.^{4,5} Worldwide, about 150 million people are diagnosed with UTI each year, which costs the global economy.^{6,7,8} Complicated UTIs are associated with comorbid conditions that prolong the need for treatment or increase the chances for therapeutic failure.^{9,10,11} UTI is presently the most commonly diagnosed infectious syndrome despite of the extensive availability of antimicrobial agents. So UTI although treatable, is now becoming increasingly tough to control because of rampant antimicrobial resistance in the Enterobacteriaceae family, particularly in *E. coli*.^{12,13,14}

ESBL (Extended Spectrum Beta Lactamase) producing organisms are distributed worldwide and their prevalence is increasing.¹⁵ So Gram negative pathogens harbouring ESBLs have occurred numerous outbreaks of infections and are becoming an increasing therapeutic problem in many countries.¹⁶ Organisms responsible for UTI such as *E. coli*, *Klebsiella* species and *Enterobacter* Species have the ability to produce ESBLs in large quantities. These 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.^{13,17} But the plasmid responsible for ESBL production carry resistance to many antibiotics like aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and cotrimoxazole.^{18,19,20} It has been found that with each new class of antibiotic, a new beta-lactamase emerged that caused resistance to that class of drug. Presumably, the selective pressure imposed by the use and overuse of new antibiotics has resulted in emergence of new variants of beta-lactamase.²¹

Majority of ESBL producing organisms are *E. coli* and *Klebsiella pneumoniae*. The major risk factors implicated are

long-term exposure to antibiotics, prolonged ICU stay, nursing home residency, severe illness, etc.^{21,22} *E. coli* has been reported as the commonest isolate causing UTI, but few authors have reported changing pattern in prevalence of uropathogens.^{23,24,25} The World Health Organisation (WHO) has called antibiotic resistance an emerging disease.²⁶ In almost all cases of UTI, empirical antimicrobial treatment initiates before the laboratory results of urine culture are available, thus antibiotic resistance may increase in uropathogens due to frequent use of antibiotics.^{27,28} The prevalence of antimicrobial resistance in patients with UTI is increasing and can vary according to geographical and regional location.²⁹

Therefore, the present study was conducted to study the bacterial spectrum causing UTI in our Institute and to find out the antibiotic response to these organisms with special emphasis on ESBL production by these organisms.

Aims and Objectives

- To study the bacterial spectrum causing UTI in our Institute.
- To study the prevalence of drug resistance in Gram negative organisms to different urinary antibiotics with special emphasis on ESBL production.

MATERIALS AND METHODS

The prospective study was conducted over a period of three months (From October 2016 to December 2016) at Department of Microbiology, Dr. Vasantrao Pawar Medical College and Hospital, Nashik, Maharashtra, India.

Total 955 urine samples received at Microbiology Laboratory were included in the study. The samples were inoculated on CLED (Cystine Lactose Electrolyte Deficiency) agar to study their cultural characteristics. Identification was done next day by using standard conventional, biochemical tests. Antibiotic Sensitivity Testing (AST) was performed using Kirby-Bauer Disc diffusion method on Mueller-Hinton agar by following the guidelines given by CLSI 2012. The antibiotic panel used for AST included - Gentamycin, Amikacin, Piperacillin, Cefotaxime, Cotrimoxazole, Ampicillin, Polymyxin B, Ceftazidime, Cefadroxil, Amoxycylav, Norfloxacin, Nitrofurantoin, Cefuroxime, Cefepime, Piperacillin-Tazobactam and Imipenem.

All the isolates which showed resistance to third generation cephalosporins were further tested for confirmation of beta-lactamase production by phenotypic methods. Confirmation of ESBL production was done by using E-test (Hi-Media Laboratory, Mumbai).

Screening of ESBL Production

This was done by two methods -

- a) Phenotypic Confirmatory Disc Diffusion Test (PCDDT).
- b) Double Disc Synergy Test (DDST).

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Phenotypic Confirmatory Disc Diffusion Test (PCDDT)

The suspected ESBL producing isolates were inoculated on Mueller-Hinton agar plates by lawn culture. Ceftazidime and Ceftazidime-Clavulanic acid discs were placed at a distance of 2.5 cm (centre to centre). Plates were incubated at 37°C overnight. Readings were taken next day. An increase in zone diameter for Ceftazidime-Clavulanic acid by ≥ 5 mm was considered positive for ESBL production (CLSI 2010).

Double Disc Synergy Test (DDST)

A single disc of Amoxycylav was placed at centre on Mueller-Hinton agar plate pre-swabbed with respective culture. Four antibiotics - Aztreonam, Cefotaxime, Ceftriaxone and Ceftriaxone discs were placed at a distance of 1.5 cm from centrally placed Amoxycylav disc. Plates were incubated at 37°C for 24 hours. Enhancement of zone of inhibition towards Clavulanic acid disc was considered indicative of ESBL producer.⁶

Confirmation of ESBL Production

This was done by using Ezy-MIC strips (Hi-Media Laboratories Pvt. Ltd.). These strips contained Ceftazidime on one side in two-fold gradient and Ceftazidime + Clavulanic acid combination on other side. The tests were done as per the manufacturer's instructions.

Reference strains used in the study were E. coli ATCC 25922 as negative control and Klebsiella pneumonia ATCC 700603 as positive control.

RESULTS

Name of Organism	N = 433	N = 54	%
	Gram Negative	Gram Positive	
E. coli	205		47.3
Klebsiella Species	82		18.9
Proteus Species	55		12.7
Pseudomonas Species	50		11.5
Enterobacter Species	41		9.4
Enterococcus Species		14	25.9
Staphylococcus aureus		40	74.0
Total Gram Negative/ Positive Organisms	433	54	

Table 1. Spectrum of Organisms Isolated in Urine Samples

Name of Organism (n)	Number of ESBL Producers	% of ESBL Producers
E. coli (205)	94	45.5
Klebsiella Species (82)	25	30.4
Proteus Species (55)	12	24
Pseudomonas Species (50)	14	25.45
Enterobacter Species (41)	13	31.7
Total	158	

Table 2. Incidence of ESBL Production among Gram Negative Bacilli Isolated in Urine Samples

Name of Antibiotic	% Resistance among ESBL Producers
Gentamicin	62.02
Amikacin	24.05
Ofloxacin	74.05
Tetracycline	77.8
Ciprofloxacin	81.01
Chloramphenicol	48.1
Piperacillin	25.3
Cefotaxime	81.01
Cotrimoxazole	75.3
Ampicillin	81.01
Polymyxin B	15.1
Ceftazidime	81.01
Cefoperazone	82.2
Cefadroxil	84.1
Amoxycylav	48.1
Norfloxacin	82.9
Nitrofurantoin	41.1
Cefuroxime	84.8
Cefepime	10.1
Piperacillin + Tazobactam	5.06
Imipenem	3.1

Table 3. Antibigram showing Resistance Pattern of ESBL Producers regarding different Antibiotics

Organism	G	Ak	Of	Te	Cp	C	Pip	Ce	Co	A	Pb
E. coli	58.5	6.3	80.8	85.1	86.1	42.5	19.1	85.1	79.7	86.1	11.7
Klebsiella pneumoniae	72	44	92	96	92	68	52	96	92	100	20
Proteus spp	66.6	83.3	41.6	66.6	83.3	66.6	33.3	83.3	75	66.6	25
Pseudomonas spp	57.1	28.5	57.1	57.1	64.2	50	14.2	78.5	50	50	0
Enterobacter spp	69.2	30.7	38.4	23	38.4	30.7	23	23	38.4	53.8	38.4

Organism	Ca	Cs	Cfd	Ac	No	Nf	Cu	Cpm	Pt	I
E. coli	88.2	87.2	88.2	43.6	90.4	24.4	89.3	2.1	3.1	2.1
Klebsiella pneumoniae	96	92	96	64	80	72	96	12	8	4
Proteus spp	75	83.3	91.6	75	91.6	66.6	91.6	33.3	8.33	8.33
Pseudomonas spp	64.2	64.2	50	42.8	64.2	57.1	57.1	21.4	7.1	0
Enterobacter spp	23	46.1	61.5	30.7	46.1	61.5	53.8	30.7	7.6	7.6

Table 4. Percent Resistance Pattern of ESBL Producers is Plated from Urine Samples with respect to Individual Antibiotics

G- Gentamicin, Ak- Amikacin, Of- Ofloxacin, Te- Tetracycline, Cp- Ciprofloxacin, C- Chloramphenicol, Pip- Piperacillin, Ce- Cefotaxime, Co- Cotrimoxazole, A- Ampicillin, Pb- Polymyxin B, Ca- Ceftazidime, Cs- Cefoperazone, Cfd- Cefadroxil, Ac- Amoxicillin + clavulanic acid, No- Norfloxacin, Nf- Nitrofurantoin, Cu- Cefuroxime, Cpm- Cefepime, Pt- Piperacillin + Tazobactam, I- Imipenem

Total 955 urine samples were processed. Out of 955 samples, Gram negative bacilli were isolated from 433 samples and Gram positive organisms isolated from 54 samples [Table 1].

Out of 433 Gram negative organisms 205 (47.3 %) were *E. coli*, 82 (18.9%) were *Klebsiella pneumoniae*, 50 (11.5%) were *Proteus* species, 55 (12.7%) were *Pseudomonas* species and 41 (9.4%) were *Enterobacter* species [Table 1]. Out of 433 Gram negative organisms, 158 were ESBL producers. Out of 158 ESBL producers *E. coli* were 45.5%, *Klebsiella pneumoniae* were 30.4%, *Proteus* species were 24%, *Pseudomonas* species were 25.45% and *Enterobacter* species were 31.7% [Table 2].

Most of the organisms were resistant to third generation cephalosporins. ESBL producers showed resistance to Cefotaxime (81.01%), Cefoperazone (82.2%) and Ceftazidime (81.01%) [Table 3]. Also, most of the ESBL producing organisms showed resistance to Fluoroquinolones, i.e. Ofloxacin (74.05%), Ciprofloxacin (81.01%) and Norfloxacin (82.9%) [Table 3]. Ampicillin resistance was shown by 81.01% of ESBL producers [Table 3]. Resistance to aminoglycosides such as Amikacin (24.05%) was found to be on lower side as compared to Gentamicin (62.02%). Also resistance to Tetracycline was found to be on higher side (77.8%) [Table 3]. Sensitivity towards Piperacillin, Polymyxin B, Cefepime, Piperacillin + Tazobactam and Imipenem turned out to be satisfactory [Table 3].

All ESBL producers were further confirmed for ESBL production by using Ezy-MIC strips. Out of 158 suspected ESBL producers, all were positive for confirmatory test for ESBL production.

E. coli shows higher percentage of resistance to third generation cephalosporins Cefotaxime (85.1%), Ceftazidime (88.2%) and Cefoperazone (87.2%). Also, higher amount of resistance is shown by *E. coli* to Fluoroquinolones such as Ofloxacin (80.8%) and Norfloxacin (90.4%) [Table 4]. Sensitivity of *E. coli* to Amikacin is quite satisfactory (Resistance 6.3%).

Klebsiella pneumoniae shows unsatisfactory results as compared to *E. coli*. Also, *Klebsiella pneumoniae* show higher amount of resistance to third generation cephalosporins such as Cefotaxime (96%), Ceftazidime (96%) and Cefoperazone (92%). Also, resistance to fluoroquinolones is much higher in percentage as compared to other organisms (Ofloxacin 92% and Ciprofloxacin 92%). Resistance to Tetracycline is also quite high (96%) [Table 4]. Resistance to aminoglycoside is also on higher side in *Klebsiella pneumoniae* as compared to *E. coli* [Table 4]. *Proteus* species shows higher percentage of resistance to third generation cephalosporins and so to fluoroquinolones (Ciprofloxacin 83.3% and Norfloxacin 80%). In *Pseudomonas* species resistance to cephalosporins, aminoglycosides as well as fluoroquinolones was found to be on lower side as compared to other organisms. *Enterobacter* species was found to be least resistant organisms as compared to others. Resistance ratio to cephalosporins was low such as Cefotaxime was 23%, Ceftazidime was 23% and Cefoperazone was 46.1%. But *Enterobacter* species shows higher resistance to Gentamicin among all organisms (69.2%) [Table 4].

But resistance to higher antibiotics like Cefepime, Piperacillin + Tazobactam and Imipenem shows satisfactory level of sensitivity among all ESBL producers [Table 4].

DISCUSSION

Increasing prevalence of ESBL producers among Gram negative organisms is nowadays becoming a great threat. This has become a major clinical problem in treating infections caused by ESBL producers. Resistance rates vary from country to country.³⁰ In present study among all uropathogens, *E. coli* is the predominant organism (47.3%). Also among ESBL producers, *E. coli* is a dominant pathogen. Our finding is supported by findings from similar studies by S. S. Tankhiwale et al who found *E. coli* as dominant uropathogen (49.8%).³¹ Also Monsour Amin et al have reported *E. coli* as dominant uropathogens.³² Similar findings were reported by K. Aruna et al and other studies.^{33,34} A study from Vinita Dogra and B. A. Tetry et al shows same results.^{35,36}

Klebsiella pneumoniae were found to be second most common pathogen (18.9%) in our study. And also second most common pathogen as ESBL producer in our study. Our this finding matches with findings from study by S. S. Tankhiwale who reported it as 37.8%.³¹ Monsour Amin et al reported it as 11.6%.³²

In our study incidence of *Klebsiella pneumoniae* was followed by *Proteus* species (12.7%), *Pseudomonas* species (11.5%) and *Enterobacter* species (9.4%). But percentage of ESBL production was higher among *Enterobacter* species. Our findings are similar to findings from study done by K. Aruna et al.⁶ Percentage of ESBL production in our study was found to be 24% in *Proteus* species, 25.45% in *Pseudomonas* species and 31.7% in *Enterobacter* species. Our findings go hand in hand with studies done by different people.^{37,38,39,40}

As evident from results, the study demonstrates *E. coli* to be predominant organisms (47.3%) amongst Gram negative organisms and *Staphylococcus aureus* (74%) amongst Gram positive organisms. These findings are similar with other studies.^{41,42} In our study most of the isolates showed resistance to commonly used drugs like third generation cephalosporins and fluoroquinolones. These findings are similar with findings from other studies.^{43,44}

In our study, we found *Klebsiella pneumoniae* as predominant drug resistant organism amongst all other organisms. This finding is contrary to findings from studies done by K. Aruna et al,⁶ study by Mohammad Akram⁷ and study by A. Bora^{45,46,47} who found *E. coli* as predominant drug resistant pathogen. But the results match with other similar studies.^{31,48,49,50} This could be due to difference in local prevalence of a particular organism.

It is also revealed that Ciprofloxacin and Norfloxacin, which are very commonly prescribed drugs for treatment of Urinary Tract Infections (UTI) are also showing higher percentage of resistance (81.01% and 82.9%). These findings are similar with findings from K. Aruna et al⁶ and M. Eshwarappa et al.³⁶

In present study, *E. coli* shows higher degree of resistance to third generation cephalosporins (almost above 80%). This finding goes hand in hand with findings from Mohammad Akram et al.⁷ But *E. coli* shows satisfactory degree of sensitivity to drugs like Cefepime (2.1% resistance), Piperacillin + Tazobactam (3.1% resistance) and Imipenem (2.1% resistance). This finding is similar with findings from study from Aligarh by Mohammad Akram et al.⁷ So it is evident that these drugs can be kept as reserved drugs to treat complicated UTIs.

Klebsiella pneumoniae showed much higher degree of resistance to third generation cephalosporins and

fluoroquinolones (above 90%). But *Klebsiella pneumonia* shows satisfactory level of sensitivity to Cefepime (12% resistance), Piperacillin + Tazobactam (8% resistance) and Imipenem (4% resistance). This is similar with study by Mohammad Akram.⁷ Satisfactory level of sensitivity to Carbapenems was also shown by other studies.^{10,36}

Other organisms like *Proteus* species, *Pseudomonas* species and *Enterobacter* Species showed higher degree of resistance to commonly used antibiotics. This is similar with other studies.^{51,52}

Variations are seen in data from different locations. This could be due to drug prescribing trends present in those particular areas in same countries. Our country data may vary from data from other countries may be due to easy availability of antimicrobial drugs over the counter.⁴

Now from above discussion, it is clear that antibiotic resistance is becoming a big problem for public health, which threatens the lives of hospitalised individuals as well as those with chronic conditions.⁷

Thus, in our study, resistance to third generation cephalosporins was found to coexist with resistance to two or more antibiotics like Ampicillin, Norfloxacin and Cotrimoxazole, etc. As also reported by Anbumani Narayanswamy,¹⁰ Subha et al⁵¹ and Duttatroy et al.⁵³

CONCLUSION

Increasing rate of resistance to commonly used antibiotics is an alarming sign for future of healthcare sector. Injudicious use of antibiotics could be the most important reason for such high rate of increasing resistance. Our study reveals that commonly used antibiotics are almost of no use for treatment of UTI patients. But special drugs like Cefepime, Piperacillin + Tazobactam and Imipenem could be kept as reserved drugs to treat infections. But care should be taken that these drugs should not be used irrationally. These wonder drugs should be used judiciously for which the clinicians need to be made aware about the current scenario of resistance pattern regarding the multidrug resistant organisms. Otherwise, resistance to these reserved drugs will emerge which will ultimately take us to situation like "NO ANTIBIOTIC ERA"..... and which is a bitter truth about the Superbugs.....!!!

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