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Antibiotic Susceptibility Pattern of ESBL producing Uropathogens

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A B S T R A C T

The increasing prevalence of infections caused by antibiotic-resistant bacteria makes empirical treatment of these infections difficult. We studied prevalence of ESBL and Amp C beta lactamases in 241 uropathogens and compare the antibiotic susceptibility pattern in them. In our study E coli was most common pathogen followed by pseudomonas, klebsiella. ESBL was produced by 38.58, Amp C by 12.03% and both type beta lactamases present in 3.37%. There is significant difference in antibiotic susceptibility of ESBL producing and non producing strains ESBL and Amp C producing isolates are less susceptible to commonly used antibiotics there is significant difference in their susceptibility except Nitrofurantoin difference is not significant (p value is <0.05) which is oldest drug, now it is commonly not uses to treat UTI. Resistance to Amikacin was found to be comparatively lower in both ESBL and non-ESBL producers. Gentamicin, Chloramphenicol and Cotrimoxazol were found to be more effective on non-ESBLs as compared to ESBL producing uropathogens.

Introduction

Urinary tract infection (UTI) is a common infection in both female and male. It is easily diagnosed and easily treatable in the young and healthy patients. Over last few years UTI caused by multidrug resistant uro-pathogens is increasing worldwide (Dalela *et al.*, 2012).

Common etiological agents are *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Proteus* species, *Staphylococcus aureus*, *Enterococcus* species, *Staphylococcus saprophyticus.* (Baby padmini *et al.*, 2004) *E. coli* is the most common organism causing both community as well as hospital acquired infections although the distribution of pathogens that cause UTIs is changing (MIMS Medical Microbiology *et al.*, 2013).

In India, the prevalence of ESBLs has been reported from 1990s. Prevalence of these

enzymes is ranging from 23 to 72% as reported in earlier studies. Antibiotic resistance pattern varies according to geographic locations and is directly proportional to the use and misuse of antibiotics (Buzayan *et al.*, 2014).

Wide spread use of third generation Cephalosporins, prolonged antibiotic exposure, sever chronic illness, prolonged hospital stay, prior infection with ESBL producing strains and indwelling catheters these are common risk factor for acquiring beta lactamases production by microorganism (Sasirekha *et al.*, 2013).

The increasing prevalence of infections caused by antibiotic-resistant bacteria makes empirical treatment of these infections difficult. The emergence of antibiotic resistance in the management of UTI infection is a serious public health issue, particularly in the developing countries where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation The resistance pattern of community acquired UTI pathogens has not been studied extensively. We studied ESBL and Amp C beta lactamases production in uropathogen.

The main aim of this study includes to study prevalence of ESBL and Amp C beta lactames in uropathogens. Difference in antibiotic susceptibility pattern between ESBL/AmpC producing strains and non ESBL producing strains

Materials and Methods

Study Design

Study was done prospectively on isolates obtained from consecutive, non repetitive urine samples with significant growth.

Site of study

Study was done at NIMS Medical College Jaipur and Research laboratory of Microbiology department of SHKM Govt Medical College Nalhar, Mewat.

Duration of study

From March 2015 to March 2016

Inclusion Criteria

The study included all clinical isolates obtained from significant growth of mid stream urine sample.

Exclusion criteria

1. Patients with urinary tract surgery
2. Patient took antibiotic in last 48 hour.
3. h/o trauma involving urinary tract
4. Patient of HIV or any other immunological disorder

Methodology

A clean voided early morning midstream urine specimens was collected in a sterile container after proper ano-genital toilet, before starting antibiotics. Urine samples was examined & processed in the laboratory as soon as possible after collection. In laboratory specimens were examined by wet mounts and Gram staining. Presence of any pus cells, micro-organisms, RBC's cast and crystals or any other finding has to be noted and cultured on Cysteine lactose electrolyte deficient agar (CLED), Mac-Conkey agar and 5% Blood agar using Semi quantitative standard loop method of culture using 0.01mm calibrated loop. These plates were incubated for 24 hrs at 37^0C and observed for growth.

Obtained growth consider as significant growth when colony count was as follows-

$>10^5$ CFU/ml of midstream urine sample in a female with no risk factors.

$>10^3$ CFU/ml of midstream urine sample in a symptomatic female or in a pregnant female

(CFU : colony forming unit) (Bailly and scott *et al.,* 2007)

Identification and antibiotic susceptibility test

Direct inoculation of urinary pathogens from CLED media was done for identification using VITEK® 2 compact system, an automated ID and susceptibility (AST) system (bioMe´rieux, USA). The system included an Advanced Expert System (AES) that analyzed MIC patterns and detected the phenotype of organisms. Pure subcultures of QC and clinical organisms were suspended in aqueous 0.45% (wt/vol) NaCl to achieve a turbidity equivalent to that of a McFarland 2.0 standard (range, 1.80 to 2.20). Strain characterization and antimicrobial susceptibility testing were performed with the VITEK 2 automated system using the ID-GNB and AST-037 cards, in accordance with the manufacturer's instructions. The VITEK 2 instrument automatically filled, sealed, and incubated the individual test cards with the prepared culture suspension. Cards were held at 35.5°C for 18 h, with optical readings taken automatically every 15 min, based on these readings, an identification profile was established and interpreted according to a specific algorithm. Final profile results were compared to the database, generating identification of the unknown organism.

ESBL screening

All the isolates showing resistance to 3rd generation cephalosporins, namely Ceftazidime, Ceftriaxone and Cefotaxime, were further tested for confirmation of β-lactamase production by phenotypic methods CLSI (2014).

Double disk synergy test: A disc of augmentin (20 μg amoxycillin and 10 μg clavulanic acid) and a 30 μg disc of ceftazidime was placed 15 mm out from edge of Augmentine disc at 90^0 angle so that its inner edge is 15 mm from it. Same was performed with cefotaxime 30 μg, ceftriaxone 30 μg, aztreonam 30 μg, cefpodoxim 10 μg so that they were spaced 90^0 apart lawn culture of the resistant isolate under test on Mueller-Hinton Agar The zone size around the test antibiotic disc increased towards the augmentin disc An "enhancement" or extension of the zone of inhibition is seen between any of the cephalosporin antibiotics and the clavulanate containing disks, This phenomenon is often referred to as the "KEYHOLE" effect, or "CLAVULANIC" effect.

Phenotypic confirmatory disk diffusion test (PCDDT) for ESBL detection: Ceftazidime (30 mcg) was used alone as well as in combination with Clavulanic acid (10mcg). Both the disks were placed on MH Agar plates pre-swabbed with the respective culture and incubated at 37 °C for 24 h. An increase in the zone diameter for Ceftazidime-Clavulanic acid by ≥ 5mm was considered positive for ESBL production.

AmpC disc test to detect Amp C beta lactamases production: A lawn culture of *Escherichia coli* 25922 was prepared on MHA plate. Sterile disk of 6 mm were moistened with sterile saline (20 µl) and inoculated with several colonies of test organism. The inoculated disk was then placed beside a cefoxitin disk almost touching on the inoculated plate. The plates were incubated at 37^oc overnight. A positive

test appeared as flattening or indentation of the cefoxitin inhibition zone (Black *et al.*, 2005).

Result and Discussion

In our study 241 were gram negative bacilli in which most common pathogen was *Escherichia coli* followed by *Pseudomonas*, *Klebsiella spp*, *Citrobacter spp*, *Enterobacter spp*, *Proteus spp* and *Acinetobacter*. 94 (38.58%) of strains produced ESBL, 29 (12.03%) produced Amp C and 9 (3.37%) produced both type of beta lactamases. 110 were not produce ESBL neither Amp C beta lactamases.

There is significant difference in antibiotic susceptibility of ESBL producing and non producing strains ESBL and Amp C producing isolates are less susceptible to commonly used antibiotics there is significant difference in their susceptibility except Nitrofurantoin difference is not significant (p value is <0.05) which is oldest drug, now it is commonly not uses to treat UTI. Resistance to Amikacin was found to be comparatively lower in both ESBL and non-ESBL producers. Gentamicin and Cotrimoxazol were found to be more effective on non-ESBLs as compared to ESBL producing uropathogens.

The co-existence of both AmpC β-lactamase and ESBL in some gram negative bacilli may give false negative tests in the detection of ESBL. The antibiogram pattern for these isolates possess a higher degree of resistance towards antibiotics that are routinely prescribed against urinary tract infections as compared to non beta lactamases producing isolates. (Dalela *et al.*, 2012, Chatterjee *et al.*, 2010)

Continuous monitoring systems and effective infection control measures are absolutely required to prevent the rapid and worldwide spread of ESBL and AmpC β-lactamase producing strains. The antibiotic sensitivity pattern of non ESBL producing strains revealed that they were highly susceptible to Imipenem and Piperacillin/ Tazobactam followed by Amoxycillin/ clavunate and amikacin. The maximum resistance was seen against cotrimoxazole and nitrofurantoin. The present study findings were similar to other studies in country and abroad (Taneja N *et al.*, 2008, Dalela *et al.*, 2012, Sinha *et al.*, 2011, Ruby Naz *et al.*, 2016).

Now- a- days the threat of the ESBL and AmpC β-lactamase producing strains not limited to the ICUs or the tertiary care hospitals only, but they are also found in OPD patients. ESBL producing gram-negative bacteria are emerging worldwide, challenging the clinicians, public health professionals and hospital infection-control teams.(Behroozi *et al.*, 2010) We compared antibiotic susceptibility pattern of ESBL producing strain and Amp C producing strain with other strain which was negative for both ESBL and Amp C beta lactamases and calculate P value.

Antimicrobial resistance is now recognised as widespread problem especially in gram negative bacilli. Increasing resistance to broad spectrum cephalosporins predominantly due to production of ESBL and AMP C bata lactamases reported in different studies ranging from 21% to 64% (Singhal *et al.*, 2005, Taneja *et al.*, 2006, Sinha *et al.*, 2011 Buzayan *et al.*, 2014, Khater *et al.*, 2014, Ruby Naz *et al.*, 2016).

Table.1 Comparison of antibiotic susceptibility pattern of ESBL and non ESBL/Amp C isolates

Antibiotic	ESBL n=94	Non ESBL/Amp C n=110	Chi square value	P value
Ampicillin	0	56(50.9%)	65.96	<0.05
Cotrimoxazol	42(44.6%)	80(72.72%)	16.58	<0.05
Nitrofurantoin	48(51%)	71(64.6%)	3.79	>0.05
Ciprofloxacin	56(59.6%)	89(80.9%)	11.22	<0.05
Gentamycin	57(60.6%)	87(79.09%)	8.31	<0.05
Imepenem	80(85.1%)	107(97.27%)	9.82	<0.05
Piperacillin+Tazobactam	70(74.4%)	98(89.1%)	7.45	<0.05
Amoxycillin clavulenate	30(31.9%)	86(78.2%)	44.23	<0.05

Table.2 Comparison of antibiotic susceptibility pattern of Amp C and non ESBL/Amp C isolates

Antibiotic	Amp c n=29	Non ESBL/Amp C n=110	Chi square value	P value
Ampicillin	0	56(50.9%)	24.72	<0.05
Cotrimoxazol	10(34.48%)	80(72.72%)	14.7	<0.05
Nitrofurantoin	15(51.72%)	71(64.6%)	1.59	>0.05
Ciprofloxacin	11(37.93%)	89(80.9%)	21.00	<0.05
Gentamycin	7(24.13%)	87(79.09%)	31.65	<0.05
Imepenem	23(79.31%)	107(97.27%)	12.22	<0.05
Piperacillin+Tazobactam	10(34.48%)	98(89.1%)	39.49	<0.05
Amoxycillin clavulenate	7(24.13%)	86(78.2%)	30.27	<0.05

This variability could be due to difference in the study design, population and geographical distribution and the variation is probably due to differential clonal expansion and drug pressure in community. This may be due to wide use of these drugs empirically because they are relatively cheap and also by being oral antibiotics they are easy to administer. Preventive measure against spread of beta lactamases production includes proper education and training, quick identification and isolation, judicial use of antibiotics specially third generation cephalosporins, intensified hand washing in wards and ICU, single use equipment and use of gloves for patients care. Continuous analysis of the antibiotic resistance pattern acts as a guide in initiating the empirical treatment of UTI and the therapy must be started only after the urine culture and the sensitivity testing have been done (Seigle *et al.*, 2008).

In conclusion, the fraction of ESBL producing isolates is increasing with time that shows higher resistance to a wide variety of commonly used antibiotics as compared to the non-ESBL-producers. It is strongly recommended to follow the 'Good Clinical Practices' and not to prescribe drugs without appropriate lab tests. Proper counselling of patients should be mandatory for proper and complete courses of medications to avoid the evolution of resistant strains. In the ESBL produce are high prevalence of ESBL and Amp C producing bacteria among MDR e coli were shown in this study this rate is alarming and need special consideration, routine screening for ESBL and AmpC production need to be

done for all uro-pathogens causing complicated urinary tract infection.

References

Babypadmini, S., Appalaraju, B. 2004. extanded spectrum Beta lactamases in urinary isolates of E coli and klebsiella pneumoniae prevalence and susceptibility pattern in a tertiary care hospital. *Indian J. Med. Microbiol.*, 22: 172-4.

Bailey and Scott's Diagnostic microbiology. 12th edition.

Betty, A., Forbes, Daniel, F., Sahm, Alice, S. 2007. *Weissfeld Blood stream Infect.*, pp 842-55.

Behrooozi, A., Rahbar, M., Yousefi, J.V. 2010. Frequency of extended spectrum beta-lactamase (ESBLs) producing *Escherichia coli* and *klebseilla pneumonia* isolated from urine in an Iranian 1000-bed tertiary care hospital *African J. Microbiol. Res.*, 4(9): 881-884.

Black, J.A., Thomson, K.S., Buynak, J.D. 2005. Evaluation of beta lactamases inhibitor in disc test for detection of plasmid mediated Amp C beta lactamases in well characterized clinical strains of Klebsiella spp. *J. Clin. Microbiol.*, 3: 4168-71.

Buzayan, M.M., Fauzia Rajab El-Garbulli. 2014. Detection of ESBL and AmpC - lactamases producing in uropathogen *Escherichia coli* isolates at Benghazi Center of Infectious Diseases and Immunity, *Int. J. Curr. Microbiol. App. Sci.*, 3(2): 145-53.

Chatterjee, S.S., Karmacharya, K. 2010. High prevalence of co- expression of newer Beta lactamases (ESBL, Amp C beta lactamases and metallo beta lactamases) in gram negative bacilli *Indian J. Med. Microbiol.*, 28: 267-68.

Clinical and Laboratory Standards Institute (CLSI). 2014. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement, CLSI document M100-S24. Wayne and Pennsylvania

Dalela, G., Gupta, S., Jain, D. 2012. Antibiotic resistance pattern in Uropathogens at a Tertiary Care Hospital at Jhalawar with Special Reference To Esbl, AmpC b-Lactamase and MRSA Production *J. Clin. Diag. Res.*, *6*(4): 645-651.

Khater, Hammouda. W. 2014. Sherif Rapid Detection of Extended Spectrum B-lactamase (ESBL) Producing Strain of *Escherichia coli* in Urinary Tract Infections Patients in Benha University Hospital, Egypt Enas Sh. *British Microbiol. Res. J.*, 4(4): 443-453.

MIMS. Medical Microbiology fifth edition 2013. Richard v Goerig, Hazel M Dockrell, Mark Zuckerman, Iran M Roitt, *Urinary tract infections*, pp 237-43.

Ruby Naz, Mohammad Khalid Farooqui, Ruchi Girotra. 2016. Bacteriological Profile of Septicaemia and Extended Spectrum Beta Lactamases Production in Multi Drug Resistant strains. *Int. J. Curr. Microbiol. Appl. Sci.*, 5(2): 598-607.

Sasirekha, B. 2013. prevalence of ESBL, AMPC B- LACTAMASES and MRSA among uropathogen and its antibiogram *EXCLI J.*, 12: 81-88.

Sharma, A., Shrestha, S., Upadhyay, S. 2011. Clinical and Bacteriological profile of urinary tract infection in children at Nepal Medical College *Teaching Hosp. Nepal Med. College J.*, 13(1): 24-26.

Siegel, R.E. 2008. Emerging gram negative antibiotics resistance: dounting challenges declining sensitivity and dire consequences, *J. Respiratory Care*, 53(4): 471-79.

Singhal, S., Thakur, T., Khan, S., Upadhyay, D.J., Chugh, S., Gaind, R. *et al. 2005.* Evaluation of methods for Amp C beta lactamases in gram negative clinical isolates from tertiary care hospitals. *Indian J. Med. Microbiol.*, 23(2): 120-24.

Sinha, P., Sharma, R., Rishi, S. 2008. prevalence of extended spectrum beta lactamases and Amp C beta lactamases producers among E coli isolates in a tertiary care hospital in Jaipur. *Indian J. Pathol. Microbiol.*, *5*1: 367-69.

Swetha, V., Sreenivasa, U., Prakash, H. Subbarayudu, S. 2014. Aerobic bacteriological profile of urinary tract infections in a tertiary care hospital *Int. J. Curr. Microbiol. Appl. Sci.*, 3(3): 120-125.

Taneja, N., Rao, P., Arora, J. 2008. Occurrence of ESBL & Amp-C b-lactamases & susceptibility to newer antimicrobial agents in complicated UTI. *Indian J. Med. Res.*, 2: 85-88.

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