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Original Research Article

To Evaluate Different Phenotypic Diffusion Methods in the Identification of ESBL Producing Uropathogenic Escherichia Coli

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Abstract

Introduction: A urinary tract infection (UTI) is an infection in any part of urinary system namely kidneys, ureters, bladder and urethra. UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both complicated and uncomplicated UTIs is uropathogenic *Escherichia coli* (UPEC). ***Materials and Methods:*** This is a prospective study and observational study conducted in the Department of Microbiology, Surabhi Institute of Medical Sciences. A total of 200 consecutive urine samples were screened from patients with symptomatic UTI. Clean-catch midstream urine samples were collected in sterile disposable container and processed within one hour. Semi quantitative loop measuring 2.2 mm diameter with a holding capacity of 0.004ml was employed to culture urine on CLED agar and MacConkey's agar. The inoculated plates were incubated over night at 37^0C Isolates in significant number (colony count $\geq 10^5$ CFU/ml) were identified by standard procedures. ***Results:*** A sum of 200 patients who satisfied the inclusion principles during the investigation were enlisted. The present study shows the pathogens causing UTIs and their antibiotic susceptibility pattern. Escherichia.coli 48.5% was the predominant pathogen followed by Klebsiella pneumoniae 23%, Proteus spp. 13.5%, Staphylococcus aureus 7.5%, Pseudomonas aeruginosa 2.5%, Citrobacter spp. 3%, Staphylococcus saprophyticus 0.5%, Enterococcus faecalis 1% and Acinetobacter spp. 0.5%. In our study, high susceptibility of meropenem (76.2%) and imipenem (72.1%) was seen and least were Ciprofloxacin 13.5%. ***Conclusion:*** Infections caused by ESBL- producing bacteria often limits therapeutic options, leading to high disease burden. Therefore, diagnostic laboratories are in need of reliable, cost efficient and less labour-intensive methods to use in the detection of ESBL- producing bacteria. The public health implications of this are disturbing thus the need to rapidly detect these pathogens in the laboratory.

Keywords: Escherichia coli, Extended spectrum β lactamases, Uropathogenic.

INTRODUCTION

A urinary tract infection (UTI) is an infection in any part of urinary system namely kidneys, ureters, bladder and urethra. Most infections involve the lower urinary tract the bladder and the urethra. [1] Women are at greater risk of developing a UTI than are men. Infection limited to your bladder can be painful and annoying. However, serious consequences can occur if a UTI spreads to kidneys. [2]

Clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities; these infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis). Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility. [3] Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defence, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and presence of foreign bodies such as calculi, indwelling catheters. [4]

UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). [5] For the agents involved in uncomplicated UTIs, UPEC is followed in prevalence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcusfaecalis*,group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp. [6] For complicated UTIs, the

order of prevalence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *S. aureus*, *P. mirabilis*, *P. aeruginosa* and GBS. [7]

Extended spectrum β lactamases (ESBLs) producing pathogens exhibit resistance not only to newer β-lactams, including third generation cephalosporins and monobactams, but also to other classes of antibiotics. [8] ESBL resistance genes are located on plasmids which are transferrable to other strains, thus posing considerable infection control issues. UTIs caused by ESBL-producing *E. coli* and *K. pneumoniae* are the most common ESBL infections in childhood. [9]

The CLSI has proposed disk diffusion methods for screening for ESBL production by klebsiellae, *Escherichia coli*, and *Proteus mirabilis*. Laboratories using disk diffusion methods for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. [10] Cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone is used. However, the use of more than one of these agents for screening improves the sensitivity of detection. If any of the zone diameters indicate suspicion for ESBL production, phenotypic confirmatory tests should be used to ascertain the diagnosis. [11]

MATERIALS AND METHODS

This is a prospective study and observational study conducted in the Department of Microbiology, Surabhi Institute of Medical Sciences. A total of 200 consecutive urine samples were screened from patients with symptomatic UTI. Clean-catch midstream urine samples were collected in sterile disposable container and processed within one hour. Semi quantitative loop measuring 3.26 mm diameter with a holding capacity of 0.004 ml was employed to culture urine on CLED agar and MacConkey's agar. The inoculated plates were incubated over night at 370C. Isolates in significant number (colony count ≥ 105 CFU/ml) were identified by standard procedures.

Inclusion criteria: Specimens collected from all clinically suspected cases of UTI of all age groups, of both OPDs and IPDs. Conditions in which asymptomatic UTI occurs. (Diabetic Mellitus, Pregnancy) Single isolate of E. coli per patient was included in the study. Urine samples collected both via naturalis and catheters.

Exclusion criteria: Patients on antibiotics during last one month. Samples obtained from the collection bag in catheterized patients. Insignificant bacteriuria. Polymicrobial growth in culture.

Antibiotic Susceptibility Test

Antibiotic susceptibility testing was done by Kirby Bauer Disk Diffusion method on Mueller Hinton Agar as per Clinical and Laboratory Standard Institute guidelines (CLSI) to determine the resistance patterns of the isolates. *Escherichia coli* ATCC 25922 was used as the control. Samples showing an inhibition zone size of ≤ 22 mm with ceftazidime and ≤ 27 mm with cefotaxime were considered as potential ESBL producer and were further investigated for confirmation.

Detection of ESBL by Disk Approximation method

Isolates that showed intermediate/resistance to 3rd generation cephalosporin were screened to detect ESBL production. A modified double disk synergy (Disk Approximation Test) was carried out on resistant isolates. Amoxicillin/clavulanic acid (20µg/10µg) disk was placed in the centre of the MHA plate on which a lawn culture of the test organism (turbidity matched to 0.5 McFarland turbidity) had been made, ceftazidime (Ce) (30µg) and cefotaxime (Ca) (30µg) were placed on either side at a distance of 15 mm centre to centre from the amoxicillin/clavulanic acid (Ac) disc. Plates were incubated at 35oC for 18-24 hrs and the pattern of zone of inhibition was noted. Isolates that exhibited a distinct potentiation towards amoxicillin + clavulanic acid disc were considered potential ESBL producer. *Escherichia coli* ATCC 25922 was negative and positive controls respectively.

Combination Disc Diffusion Method

A cefotaxime 30µg and a cefotaxime + clavulanic acid (30µg+10µg) discs (Hi-media, Mumbai) were placed at a distance of 25 mm on a Mueller-Hinton Agar plate incubated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37oC. A ≥ 5mm increase in the diameter of inhibition zone for the combination disc versus ceftazidime disc confirmed ESBL production.

RESULTS

A sum of 200 patients who satisfied the inclusion principles during the investigation were enlisted. The information was examined, and the last perceptions were classified as beneath. In this examination, the most extreme number of patients were in the age gathering of 21-30 years age gathering which were 34% (n =68) of complete followed by age group 31–40 years having 26.5% (n = 200) in this gathering in table 1.

Table 1: Distribution of the Percentage of patients according to age group

Age group Years	Frequency	Percentage
<20	**21**	**9.5**
21-30	**68**	**34**
31-40	**53**	**26.5**
41-50	**32**	**16**
51-60	**22**	**11**
>60	**6**	**3**

Table 2: Gender wise distribution

Gender	Frequency	Percentage
Male	**71**	**35.5**
Female	**129**	**64.5**
Total	**200**	**100**

In table 2, of the 200 samples 129 were female and 71 males, which correspond to 64.5% of female and the 35.5% male.

Table 3: Distribution of various uropathogens in culture positive samples

Name of the organism	Frequency	Percentage
Escherichia coli	97	48.5
Klebsiella pneumonia	46	23
Pseudomonas aeruginosa	5	2.5
Acinetobacter spp.	1	0.5
Enterococcus faecalis	2	1
Proteus spp.	27	13.5
Staphylococcus aureus	15	7.5
Staphylococcus saprophyticus	1	0.5
Citrobacter spp.	6	3
Total	200	100

In table 3, the present study shows the pathogens causing UTIs and their antibiotic susceptibility pattern. Escherichia. coli 48.5% was the predominant pathogen followed by Klebsiella pneumoniae 23%, Proteus spp. 13.5%, Staphylococcus aureus 7.5%, Pseudomonas aeruginosa 2.5%, Citrobacter spp. 3%, Staphylococcus saprophyticus 0.5%, Enterococcus faecalis 1% and Acinetobacter spp. 0.5%.

Table 4: Susceptibility rates of isolated *Escherichia coli* to various tested antibiotics (n=97)

Antibiotic drugs	Frequency	Percentage
Amikacin	51	52.5
Ampicillin	20	20.6
Amoxy–clav	39	40.2
Aztreonam	55	56.7
Cefotaxime	21	21.6
Ceftrioxane	19	19.5
Cefuroxime	13	13.4
Cefatzidime	45	46.3
Ciprofloxacin	12	12.3
Cotrimoxazole	29	29.8
Gentamicin	37	38.1
Imipenem	70	72.1
Levofloxacin	17	17.5
Meropenem	74	76.2
Nitrofurantoin	54	55.6
Norfloxacin	28	28.8
Ofloxacin	47	48.4
Piperacillin + tazobactam	39	40.2

In table 4, in our study high susceptibility of meropenem (76.2%) and imipenem (72.1%) was seen and least were Ciprofloxacin 13.5%.

Table 5: Distribution of ESBL (n=97)

Isolates	ESBL		
	KB disc Diffusion Method	Disk Approximation Method	Combination Disc Method
Escherichia coli	75 (77.3%)	65 (67.0%)	63 (64.9%)

DISCUSSION

The prevalence of ESBL among members of Enterobacteriaceae constitutes a serious threat to current β lactam therapy leading to treatment failure and consequent escalation of costs of hospital stay. Among the wide array of antibiotics, β lactam are the most widely used for over 50% of all the systemic infections.

In the present investigation, the most frequent pathogen seen in the age group of 21 to 30 years (34.5%). According to Kalal et al observed that 31.7% were affected in the age group of 15- 59 years. [12] Savitha T and Bhowmick et al observations are dissimilar to our study and proved that UTI is more common in older age group (41- 50 years). [13,14] Irene Eriksson et al reported that UTI is common in older age due to associated risk factors such as urinary incontinence. [15]

In our study showed that the prevalence of UTI in females (64.5%) was higher than males (35.5%). It strongly correlates with other findings which revealed that the frequency of UTI is greater in females as compared to males. Manikandan C et al and Kasi Murugan et al observed a prevalence of 69.8% and 52.10% in females when compared to 30.2% and 47.9% in males respectively. [16,17] The reason behind this high prevalence of UTI in females is shorter urethra, due to its close proximity to anus, sexual intercourse, incontinence and other comorbid condition.

The present study shows the pathogens causing UTIs and their antibiotic susceptibility pattern. Escherichia. coli 48.5% was the predominant pathogen followed by Klebsiella pneumoniae 23%, Proteus spp. 13.5%, Staphylococcus aureus 7.5%, Pseudomonas aeruginosa 2.5%, Citrobacter spp. 3%, Staphylococcus saprophyticus 0.5%, Enterococcus faecalis 1% and Acinetobacter spp. 0.5%. It is strongly supported by the study done in Pattukkottai area in Tamilnadu by Manikandan C et al. in which the second most common uropathogen was *Klebsiella pneumoniae* (11.2%) followed by *Pseudomonas aeruginosa* (10.5%) and *Proteus* spp., (6.8%). [16] It is in contrast with the results of study done by Shanthi J et al in which Uropathogenic *Escherichia coli* (50%) was followed by *Citrobacter* spp., (14%) and *Pseudomonas aeruginosa* (20%). [18] Maripandi Arjunan et al showed that Uropathogenic *Escherichia coli* (31%) was followed by *Citrobacter* spp., (20%) and *Pseudomonas aeruginosa* (17.24%). [19]

In the present study, highest percentage susceptibility of meropenem (76.2%) and imipenem (72.1%) was seen and least were Ciprofloxacin 13.5%. The resistance pattern varies from place to place. It is similar to the study conducted by Tabasi M et al and the results suggested that *E. coli* was extremely resistant to Ampicillin (100%). He also observed a resistance of 68.3% to amoxicillin/clavulanic acid, 33% to Cotrimoxazole. [20]

In our study 46.15% ESBL producers belonged to Enterobacteriaceae family. There have been reports of ESBL's from major hospitals in India and some of them have recorded the incidence to be as high as 60-68%. [21] The high incidence of ESBL is a cause of concern to regulators of hospital antibiotic policy. Over reliance on third generation cephalosporins to treat gram negative bacterial infections is one of the prime causes for increased resistance to this class of antibiotics.

The difference observed in detection of ESBL positive isolates by two different methods may be justified by the lower sensitivity of phenotypic method and the influence of environmental factors on the incidence of resistance. [22] The lack of constant sensitivity of different phenotypic methods has been emphasized by some studies. [23] In contrast, the genotypic method using specific PCR amplification of resistance genes seems to have 100% specificity and sensitivity. The cost of molecular method is particularly reduced for the bacteria belonging to enterobactericeae family as their DNA is easily extractable by boiling method, a quick and cost-effective DNA extraction method. [24]

The disadvantage of these diffusion methods is that, they may not detect inhibitor-resistant beta lactamases. The ESBL confirmatory test is based on the demonstration of inhibition by clavulanate. But, other mechanisms of beta lactam resistance, like AmpC enzymes, change in the porin channel and variants ESBL enzymes may be present or coexist with ESBL, which interfere in the results of these diffusion tests.

CONCLUSION

Infections caused by ESBL- producing bacteria often limits therapeutic options, leading to high disease burden. Therefore, diagnostic laboratories are in need of reliable, cost efficient and less labour-intensive methods to use in the detection of ESBL- producing bacteria. The public health implications of this are disturbing thus the need to rapidly detect these

pathogens in the laboratory. Several techniques of ESBL detection and confirmation have been comprehensively explored and the choice of the testing method depends on the preferences and requirements of the laboratory professionals as well as the availability of the testing material. There is the need for reliable but simple phenotypic tests in the laboratory for the detection of these antibiotics resistant bacteria, which do not require highly skilled personnel; this is in order to ensure a swift response in the management and control of these pathogens.

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