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Screening And Antibiotic Profile of Uropathogens with Reference To Extended-spectrum Beta-lactamases (ESBL)

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Abstract-The most common human bacterial infections are urinary tract infections with global infection rates nearing 180 million per year. These infections occur due to bacterial colonization of any tissue along the urinary tract, from the urethral opening to the kidneys. The emergence of antibiotic resistance in the management of urinary tract infections possesses a serious public health issue. In the present study 22 bacterial isolates were obtained from 15 urine sample collected from patients suspected with urinary tract infection. 9/22 (41%) were found to be gram positive cocci and 13/22 (59%) were found to be gram negative bacteria. Among the gram positive bacteria isolated the most predominant organism was found to be *Staphylococcus aureus* (56%) followed by *Staphylococcus epidermidis* (33%) and *Enterococcus faecalis* (11%). The most predominant gram negative bacteria were found to be *E.coli* (38%) followed by *Klebsiella pneumoniae* and *Acinetobacter baumanii* (23%), *Pseudomonas aeruginosa* and *Proteus vulgaris* (8%). 69% of gram negative bacteria were found to be ESBL positive. 54% of gram negative bacterial isolates were found to be positive for inducible *AmpC* β-lactamase.

Keyword: ESBL, UTI, AmpC, MRSA.

1. INTRODUCTION

The most common bacterial infections are urinary tract infections (UTIs). UTIs accounts for 35% of nosocomial infections making them the most common hospital-acquired infection, and they are the second most common cause of bacteraemia in hospitalized patients (Stamm, 2002). UTI causes bacterial infections of one or more parts of urinary system after bacteria overcome the natural host defence mechanism (Al-Dujiaily, 2000). Bladder infection or cystitis is the infection of lower urinary tract and infections in the upper urinary tract is known as kidney infection or pyelonephritis. Urgency of urination, dysuria, pyuria, irritation of urinary tract, discomfortable pressure, bloody urine which may have a strong smell and tiredness are associated with cystitis. Fever and flank pain as well as the symptoms of cystitis are significant in kidney infections (Lane and Takhar, 2011).

These infections are caused by both Gram-negative which includes a large number of aerobic bacilli such as *Escherichia sp, Klebsiella sp, Enterobacter sp, Citrobacter sp, Proteus sp, Serratia sp, Salmonella sp and Pseudomonas sp.* and Gram-positive bacteria which includes *Staphylococcus sp, Streptococcus sp and Enterococcus sp.* Among this 80-90% of UTI are caused by *E. coli* (Rushton, 1997) and in ambulatory patients and of nosocomial infections, *Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Enterococcus faecalis* are the most frequently isolated (Andreu *et al.,* 2008).

One of the greatest discoveries of modern medicine has been antibiotics, but the development of microbial resistance towards antibiotics increased due to availability and increased use of antibiotics (Gottlieb and Nimmo, 2011). Developing countries show increasing amounts of antimicrobial resistance (Sadeghabadi *et al.,* 2014). Antimicrobial resistance is increasingly a global threat for public health and a serious threat to modern medicine according to the World Health Organization in 2014.

2. MATERIALS AND METHODS

a) Collection of urine sample:

Clean catch midstream urine samples were collected in a sterile wide mouth container from

patients suspected with urinary tract infections. The specimens were labelled, transported to laboratory in ice packs and were processed within one hour for aerobic bacterial culture. Urine was observed by naked eyes for the altered colour, presence of turbidity and deposits.

b) Isolation and identification of bacterial isolates:

10 ml of the urine sample was transferred into sterile centrifuge tubes and centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded and the sediment was inoculated on various media which included- Blood agar, MacConkey's agar, cetrimide agar and nutrient agar. The plates were incubated at 37^{o}C for 24 hrs. Colony morphology of the isolates was observed on following growth of the organisms. For pure culture single colony was further streaked on nutrient agar and incubated for 24 hours at 37°C. Bacterial isolates were identified based on the standard biochemical methods.

Antibiotic sensitivity testing

Once the bacterial isolate was identified, the antibiotic sensitivity testing was carried out by Kirby Bauer disc diffusion method (Bauer *et al*., 1996) for the following antibiotics- (in µg/disc)- ceftazidime (30mcg), ceftazidime/clavulanic acid (30 mcg/ 10 mcg), imipenem (30mcg), cefoxitin (30 mcg), ceftriaxone (30mcg), cefotaxime (30mcg), gentamicin, erythromycin, clindamycin, ofloxacin and teicoplanin.

Cefoxitin Disc diffusion Method

Muller Hinton Agar was prepared and sterilized by autoclaving at 121° C/15 lbs for 15 minutes. About 15ml melted MHA was poured into sterile petri plate and allowed to solidify. Two well isolated colonies from overnight growth of the test organism were inoculated into Muller Hinton broth and incubated at 37°C for 3hrs. The turbidity of the growth was compared with 0.5 McFarland and lawn culture was made on to the plates. Cefoxitin disc (30µg) was placed after drying the plates for 1 minute and incubated at 37°C for 24hrs and was observed for the zone of inhibition around the disc. The zone of inhibition was measured and was interpreted as per CLSI standards.

ESBL screening by Disc diffusion test:

This test requires the use of ceftazidime (30mcg) alone and in combination with clavulanic acid (10mcg). Ceftazidime and with ceftazidime/clavulanic acid (30 mcg/10 mcg) discs were placed with distance between the two discs 10mm edge to edge on MHA plate inoculated with standard inoculum (0.5 McFarland) of the test organism to form a lawn culture and was incubated overnight at 37^{o}C. An increase in the zone diameter by > 5 mm of ceftazidime versus its zone when tested in combination with clavulanic acid was considered as an ESBL producer. (Jayakumar, 2007).

Disc Antagonism Test (*AmpC* β-lactamase inducibility)

In this test, lawn culture of test isolate (0.5 Mcfarland) was made over Muller-Hinton agar plate (MHA) and ceftazidime (30µg) and cefoxitin (30µg) disc were placed 20 mm apart from centre to centre. Plates were incubated for 18-20 hours at 37^{o}C. *AmpC* β-lactamase inducibility was recognized by isolates showing blunting of ceftazidime zone of inhibition adjacent to cefoxitin disc and were considered screen positive. (Sanders *et al.,* 1982)

3. RESULTS

Prevalence of Bacterial Isolates from Urine Sample:

22 bacterial isolates were obtained from 15 urine sample collected from patients suspected with urinary tract infection. 9/22 (41%) were found to be gram positive cocci and 13/22 (59%) were found to be gram negative organisms. (Table 1 & 2)

Table 1: Prevalence of gram positive bacteria, n=9

S. No.	Name of the bacterial isolates	Total number obtained	Percentage of bacterial isolates
1	*S.aureus*	5	56%
2	*S.epidermidis*	3	33%
3	*Enterococcus faecalis*	1	11%

Table 2: Prevalence of gram negative bacteria, n=13

S. No.	Name of the bacterial isolates	Total number obtained	Percentage of bacterial isolates
1	*E.coli*	5	38%
2	*Klebsiella pneumoniae*	3	23%
3	*Pseudomonas aeruginosa*	1	8%
4	*Acinetobacter baumanii*	3	23%
5	*Proteus vulgaris*	1	8%

Antibiotic sensitivity testing:

Gram positive bacteria exhibited 100% sensitivity towards teicoplanin, while they showed 63% resistance towards erythromycin followed by gentamicin (47%), clindamycin (36%) and ofloxacin (23%). Gram negative bacterial isolates exhibited 100% sensitivity towards imipenem. The isolates were found to be highly resistant to ceftazidime (78%) followed by cefotaxime (68%), cefoxitin (65%) and ceftriaxone (61%).

MRSA screening by cefoxitin disc diffusion method:

A total of 8 Staphylococcus sps were isolated in the present study. Out of the 8 isolates, 5 (63%) were found to be *Staphylococcus aureus* and 3 (37%) were *Staphylococcus epidermidis*. All the Staphylococcal isolates were subjected to MRSA screening by cefoxitin disc diffusion method. Among 5 *Staphylococcus aureus*, 2(40%) were found to be MRSA and 3 (60%) were found to be MSSA, while all the *Staphylococcus epidermidis* were found to be sensitive to methicillin.

Screening for ESBL production among gram negative organisms:

A total of 13 gram negative isolates were obtained in the present study. Out of the 13 isolates, 9 (69%) were found to be positive for the production of ESBL by disc diffusion method using ceftazidime and with ceftazidime/clavulanic acid. (Table 3)

Table 3: Ceftazidime antibiotic sensitivity pattern of gram negative bacteria

Total number of gram negative bacteria	Ceftazidime sensitive Isolates	Ceftazidime resistant isolates
13	4 (31%)	9 (69%)

Screening for *AmpC* β-lactamase production:

The disc antagonism test was done to detect inducible *AmpC* β-lactamase for all the isolates. 7/13 (54%) isolates were found to be positive for inducible *AmpC* β-lactamase. The isolates showed blunting of the ceftazidime zone of inhibition adjacent to the cefoxitin disc.

***AmpC* β-lactamase screening of gram negative bacteria**

Total number of gram negative bacteria	Cefoxitin sensitive isolates	Cefoxitin resistant isolates
13	6 (46%)	7 (54%)

4. DISCUSSIONS

One of the most important causes of morbidity in the general population and the second most common cause of morbidity among hospital visitors is the urinary tract infection. UTI is also the most common causes of nosocomial infection among hospitalized patients (Ronald and Puttulo, 1991). UTI increases in men due to prostate enlargement and neurogenic bladder with advancing age (Liperky, 1989). Recurrent UTI are common and can lead to irreversible damage to the kidneys, resulting in renal hypertension and renal failure in server cases (New, 1992). Women are more prone to develop UTI in the community. About 20% of the women experience a single episode of UTI during their lifetime, and 3% of women had more than one episode of UTI per year (Gebre-Selassie, 1998). Pregnant women are more susceptible to the infection (Van Nostrand *et al*., 2000). UTI associated with catheter is a severe problem with about 10% of the patients developing bacteriuria (Srinivassa *et al*., 1999).

In the present study 22 bacterial isolates were obtained from 15 urine sample collected from patients suspected with urinary tract infection. 9/22 (41%) were found to be gram positive cocci which included *Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus faecalis* and 13/22 (59%) were found to be gram negative organisms which included *E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumanii* and *Proteus vulgaris.* Among the gram negative bacteria, *E.coli* was the most common pathogen isolated predominantly. The isolation rate of urinary pathogens of the present study is found to be consistent with reports of the studies Chandra *et al*., 2013 and Savitha, 2011.

The origin of urinary tract infections and the antibiotic resistance of the uropathogens have been changing over the years, both in the community and nosocomial infections (Manges *et al*., 2006; Kahan *et al*., 2006). Therefore, a wide range of antibiotic resistance has been recorded among the uropathogens across the world. In the present study, gram positive bacteria exhibited 100% sensitivity towards teicoplanin, while they showed 63% resistance towards erythromycin followed by gentamicin (47%), clindamycin (36%) and ofloxacin (23%). Gram negative bacterial isolates exhibited 100% sensitivity towards imipenem. The isolates were found to be highly resistant to ceftazidime (78%) followed by

cefotaxime (68%), cefoxitin (65%) and ceftriaxone (61%). Amoxycillin with clavulanic acid which are commonly used antibiotics showed poor in-vitro sensitivity against majority of the organisms isolated in this study.

In the present study, a total of 5 *Staphylococcus aureus* isolates were obtained. Of the 5 isolates, 2 (40%) were found to be MRSA and 3 (60%) were found to be MSSA. The incidence of MRSA varies according to the region, 25% in Western part of India (Patel *et al*., 2010) to 50% in South India (Gopalakrishnan *et al.,* 2010). The prevalence of MRSA in a study from Chennai was reported as 40-50 per cent. The prevalence of MRSA in the present study was found to be 40% which was in agreement with Patel and Gopalakrishnan.

ESBL producing *E. coli* isolates are frequently found to be resistant to other antibiotics, in particular fluoroquinolones (Lautenbach *et al*., 2001). Studies in some places like in Nagpur showed 50% of ESBL producers and in another study done in 2005, from New Delhi, showed 68.78 % of the strains of gram negative bacteria to be ESBL producers and this was in agreement with our study. Studies in other places like in Varanasi by Upadhyay *et al.,(2010)* showed the prevalence of ESBL producers to be 3.3% and Rodrigues *et al.*,(2004) in a study showed 5.9% of bacterial isolates harbouring ESBLs in Mumbai, which was less in comparison with our study. In the present study, a total of 13 gram negative isolates were obtained. Out of the 13 isolates, 9 (69%) were found to be positive for the production of ESBL by disc diffusion method using ceftazidime and with ceftazidime/clavulanic acid.

It is necessary to identify *AmpC* β-lactamase producing bacteria as they can cause major therapeutic failure if they remain undetected. *AmpC* β-lactamase producing organisms are increasing and they poses a major therapeutic challenge due to treatment failure (Arora and Bal, 2005), and have been responsible for several nosocomial outbreak. The disc antagonism test was done to detect inducible *AmpC* β-lactamase in all the isolates. In the present study, 7/13 (54%) isolates were found to be positive for inducible *AmpC* β-lactamase. The isolates showed blunting of the ceftazidime zone of inhibition adjacent to the cefoxitin disc. Our study exhibited more positive when compared with reports from Aligarh by Shahid *et al*.,(2004) in 2004 who reported 20% AmpC positivity, from Kolkata by Arora *et al*. in 2005 to be 17.3% (Arora and Bal,2005) and from Varanasi to be 22% (Bhattacharjee *et al.*,2008).

5. CONCLUSION

Our study shows high prevalence of ESBL producers among *E.coli* and *Klebsiella pneumoniae*. Gram positive bacteria showed high sensitivity towards teicoplanin, while gram negative bacteria towards imipenem. *AmpC* β-lactamase detection using disc antagonism method should be done for an effective UTI treatment. As different centres show variations among antibiotic resistance rates, it will be helpful that every region perform surveillance studies to determine local antibiotic resistance rates for the development of treatment protocols.

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