International Journal of Life science & Pharma Research

SCREENING OF URINARY ISOLATES FOR THE PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROBACTERIA OTHER THAN ESCHERICHIA COLI.

***MANJULA MEHTA, SONIA BHARDWAJ AND JYOTI SHARMA.**

***Department of Microbiology, Dr Harvansh Singh Judge Institute of Dental Sciences and Hospital, Panjab University, Chandigarh.India**

ABSTRACT

A total of 447 urine samples were processed in the Department of Microbiology . In this retrospective study 164 isolates 70.72% were *E.coli* and 16.46% were Gram negative bacteria other than *E.coli*, 12.80 % were Gram positive cocci. Antimicrobial susceptibility of uropathogens was tested by disc diffusion method. The Enterobacteria other than *E.coli* (n=27) were predominantly of Klebsiella over other isolates i.e Pseudomonas, Acinetobacter, Proteus, Citrobacter. On the other hand, among Gram positive cocci (n=21) there was predominance of Enterococcus (61.93%) over Staphylococci and Streptococci species. Antimicrobial sensitivity patterns of Enterobacteria other than *E.coli* revealed resistance to Penicillins followed by Quinolones like Nalidixic acid (33.3%), Nitrofurantoin (52.6%) and Co-trimoxazole (26.6%). On the other hand, Gram positive cocci showed maximum resistance towards Cephalexin (100%) followed by Nalidixic acid (85.7%), Co-trimoxazole (71.42%) and Norfloxacin (69.27%). However the drug of choice remains as Augmentin and other combination drugs for Gram negative bacteria whereas for Gram positive bacteria Nitrofurantoin, Augmentin and Linezolid remains as drug of choice, none was found to be resistant to Vancomycin.

Key Words -Urinary tract Infection, Enterobacteria other than *E.coli*, antimicrobial susceptibility.

INTRODUCTION

Urinary tract infection (UTI) is a serious health problem and frequently encountered serious morbidity afflicting all segments of human population (Azra *et al*,2007). The incidence of UTI is greater in women (20%) as compared to men that may be either due to anatomical predisposition or uroepithelial mucosa adherence to the mucopolysaccharide lining or other host factor(Mary *et al*,2000). UTI in men is less common and primarily occurs after 50 years of age. The knowledge of etiology and antibiotic resistance pattern of the organism causing urinary tract infection is essential because of increasing resistance due to persistence and mismanagement of the ailment. The World Health Organization (WHO) has called antibiotic resistance an emerging disease. Bacteria may be innately resistant or may acquire resistance to antibiotics. It has been found that fluoroquinolones are extremely effective in treatment of UTIs. Ampicillin and Nitrofurantoin have also been recommended to treat UTIs in routine. The present study was aimed at isolation and diagnosis of bacterial infection that causes UTI and antibiotic susceptibility pattern of bacterial isolates from these patients.

MATERIALS AND METHODS

A total of 447 urine samples from patients with suspected UTI were processed in the Department of Microbiology ,Dr Harvansh Singh Judge Institute of Dental Sciences & Hospital, Chandigarh from December 2009 – August 2012.

Collection of Sample

The patients were properly instructed on how to collect the sample under aseptic conditions. Clean Catch mid Stream specimen of urine was collected from each patient. The name, age and sex were clearly mentioned on the universal container containing specimen.

Processing of Specimen

A modified semi-quantitative technique using a standard calibrated bacteriological loop of urine was performed to transfer the 0.01 ml of urine sample on Blood agar and MacConkey agar media. After allowing the urine to be absorbed into the agar, the plates were then inverted and incubated at 37°C for 18-24 hrs. The colony count was done using semi quantitative method. A significant bacterial count was taken as count equal to or in excess of 10^5 bacteria per milliliter.

Identification of isolates

Pure isolates were identified as described by Collee *et al*, 1989 using morphological, cultural and biochemical characters.

Antibiotic susceptibility testing

An antibiogram was done by agar disc diffusion technique as described by Bauer *et al*, 1996. Appropriate antibiotic discs were tested depending upon whether the organism was gram positive or gram negative. Interpretation of results was done based on the diameter of the zone. The antibiotics tested were Ciprofloxacin, Norfloxacin, Amikacin, Gentamicin, Nitrofurantoin, Nalidixic acid, Ceftazidime+Clavulinic acid, Piperacillin + Tazobactam and Co-trimoxazole for GNB. For GPC antibiotics tested were Ciprofloxacin, Co-trimoxazole, Cefixime, Cephalexin , Norfloxacin, Augmentin, Linezolid, Gentamicin, Amoxycillin, Erythromycin, Nitrofurantoin, Nalidixic acid, Cefotaxime, Vancomycin (Hi Media, India).

RESULTS

A total of 447 urine samples were received, positivity of urinary tract infection was found to be 36.68 % (164/447). Out of these isolates *E.coli* comprised 70.73% (116/164) of the isolates. Enterobacteria other than *E.coli* were 16.46 % (27/164) and Gram-positive cocci 12.80 %(21/164) of the isolates as depicted in Table- 1. Among the Enterobacteria other than *E.coli* there was pre-dominance of Klebsiella (44.44%) followed by other isolates i.e Pseudomonas, Acinetobacter, Proteus and Citrobacter. On the other hand, among GPCs there was a predominance of Enterococci (61.90%) over Staphylococcus and Streptococcus species as in Table 2. In the antimicrobial sensitivity pattern (AST) as shown in Table 3,4; there was a varying sensitivity pattern by each organism for the same antimicrobial. AST patterns of Enterobacteria other than *E.coli* revealed resistance to Amoxicillin (91%) followed by Nalidixic acid (33.3%), Nitrofurantoin (52.6%) and Co- trimoxazole (26.6 %). On the other hand, Gram-positive cocci showed maximum resistance towards Cephalexin (100%) followed by Nalidixic acid (85.7%), Co-trimoxazole (71.42%) and Norfloxacin (69.27%). Sex-wise distribution of positive cultures is given in Table 5. Female subjects predominated over males in terms of urine culture positivity in both Enterobacteria other than *E.coli* as well as GPC.

Table –1
Distribution of the urinary isolates.

Micro-organism	Percentage
E.coli	70.73
Enterobacteria other than *E.coli*	16.46
Gram positive cocci	12.80

Table-2
Distribution of bacteria other than Escherichia coli and yeast.

Micro-organism		Percentage
Enterobacteria other than *E.coli*.	Klebsiella,	44.44
	Pseudomonas,	22.22
	Acinetobacter,	11.11
	Proteus,	7.40
	Citrobacter,	3.70
Yeast	Candida.	11.11
Gram positive Cocci	*Staphylococcus aureus*	14.28
	Streptococcus	23.80
	Enterococcus	61.90

Table 3
Gram Negative Bacteria other than E.coli resistance pattern to antibiotics.

Antibiotic	Percentage
Amoxycillin (Am)	91
Norfloxacin (Nx)	23.52
Nalidixic acid (Na)	33.3
Nitrofurantoin (Nf)	52.6
Gentamicin(G)	0
Co-trimoxazole (Co)	26.6
Ceftazidime+ Clavulinic acid (CaC)	18.75
Piperacillin+Tazobactum (Pt)	0

Table- 4
Gram positive Cocci resistance patterns to antibiotics.

Antibiotic	Percentage
Ciprofloxacin (cf)	52.94
Co-trimoxazole (co)	71.42
Cephalexin (Cp)	100. 00
Norflox (Nx)	69.23
Augmentin (Ac)	10.52
Linezolid (Lz)	0
Gentamicin (G)	46.15
Amoxycillin(Am)	63.63
Erythromycin (E)	44.44
Nitrofurantoin(Nf)	0
Nalidixic acid (Na)	85.7
Cefotaxime (Cf)	0
Vancomycin (Vc)	0

Table 5
Distribution of male and female subjects among positive cultures.

Micro-organism	Males	Percentage	Females	Percentage
GNB (n=27) Enterobacteria Other than *E.coli*	10	37.03	17	62.96
GPC(n=21)	7	33.33	14	66.66

DISCUSSION

Micro-organisms causing UTI vary in their susceptibility to antimicrobials from place to place and from time to time (Jameison *et al*, 2006). The percent positivity for urinary cultures was found to be 36.68% (164/447). One possible explanation behind such low positive isolation rate may be that most of these patients present with pyrexia. AST patterns of Enterobacteria other than *E.coli* revealed resistance to Amoxycillin (91%) followed by Quinolones ie Nalidixic acid (33.3%), Nitrofurantoin (52.6%) and Co-trimoxazole (26.6%). Similar findings have been observed by many workers around the world (Jenson *et al,* 2006;Barnell *et al*, 1997; Nassar,2000; Moges *et al*,2000; Rafay,2000).The possible explanation behind the resistance shown to these antibiotics, may be because these antibiotics have been in use for a long period and must have been abused and as a result the organisms must have developed mechanisms of circumventing their mode of action. Gram positive cocci showed maximum resistance towards Cephalexin (100%) followed by Nalidixic acid (85.7%), Co-trimoxazole (71.42%) and Norfloxacin (69.2%). On observing the susceptibility pattern of Gram positive bacterial pathogens, it was noted that Staphylococcus showed highest sensitivity to Augmentin (Ojumba, 2005).The low sensitivity to other antibiotics like Ampicillin and Nalidixic acid is in similarity to other studies (Ferri *et al*,2005;Reid,1987). The present study showed the higher incidence of disease in females as compared to males. The higher incidence of UTI in women could probably be due to predisposition of uroepithelial mucosa adherence to mucopolysaccharide (Talan *et al*, 2000;Tankhiwale *et al*,2004)). It has been found that in children approximately 5% of girls and 1% of boys have a UTI by 11 years of age (Foxman,2003;Guidori *et al* 2008; Howes,2005). The drug of choice remains as Augmentin and other combination drugs for GNB, whereas for GPCs Nitrofurantoin, Augmentin and Linezolid remains as drug of choice though none was found to be resistant to Vancomycin. The knowledge of antimicrobial pattern of routinely isolated uropathogens in a particular area may provide guidance to clinicians regarding the empirical treatment of UTI when therapy must be started before laboratory tests are available.

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