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Incidence, distribution and antibiogram of uropathogens isolated from patients with urinary tract infections

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ABSTRACT

The purpose of this study was to investigate the bacterial etiology of urinary tract infections in one of the busiest hospitals of Tamil nadu. Bacterial identification was based on standard culture and biochemical characteristics of isolates. Antimicrobial was performed for all the isolates by disk diffusion method on the Mueller-Hinton agar plates with and without 10 µg of amoxy clav. Susceptibility test results were interpreted according to the criteria established by the Clinical & Laboratory Standard Institute (CLSI). E. coli was the most frequent isolate throughout the period (36.1 % of the total isolates). It was followed by Klebsiella pneumoniae and Proteus sp., Pseudomonas aeruginosa, Enterococcus sp., and Streptococcus agalactiae. E. coli occurred more frequently in women (69.8%) than in men (61.4%). The lowest percentage of susceptibility of E. coli was manifested against piperacillin and ampicillin. An increase in the production of ESBL was observed. The pathogenicity of urinary tract infections and their susceptibility profiles are important to be evaluated in countries like India where a severe misuse of antibiotics at all levels in some places are observed.

Key words: UTI infections, ESBL-producers, antibiotic susceptibility, drug resistance.

INTRODUCTION

One of the most prevalent problems faced by healthcare services is the increasing prevalence of antimicrobial resistance. Urinary tract infections (UTI) are the most common bacterial infections affecting humans throughout their lifetime. They are the frequent cause of morbidity in outpatients as well as most frequently involved in the cause of nosocomial infection in many hospitals (Sussman M 1998) [1]. The commonest urinary pathogen accounting for over 80% of community-acquired infection is due to *Escherichia coli*. However, other organisms gain a greater foothold in patients with complicated UTI [2]. Compounded by a diminishing number of new agents entering clinical practice, resistance is widely recognized as a major threat to public health sectors. UTI is a serious ailment in human due to increasing frequency, recurrence and difficulty in eradication; it poses stiff challenge to the medical professionals. It is much more common in women than in men, due to anatomical and physiological reasons; by virtue of its position urinogenital tract is more vulnerable to bacterial infections caused by both internal and external flora [3]. UTIs are often treated with different broad-spectrum antibiotics, one with a narrow spectrum of activity may be appropriate because of emerging concerns about infection with resistant organisms, and antimicrobial susceptibility testing of the urinary pathogens constitutes the basis for antibiotic therapy. However, in view of the increasing bacterial resistance, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy [4].

Enterobacteriaceae and *Escherichia coli* in particular are the notorious pathogens [5] causing infections by adhering to, invading, and replicating the umbrella cells of the bladder epithelium [6]. *E. coli* replication is facilitated by inflammation, leading to increased bacterial survival and invasion to the deeper layers of the urothelium. Consequently, these urothelial cells become reservoirs in which pathogens persist in a quiescent state becomes

reservoirs and may be the source of recurrent UTIs. In general practice, there are concerns that some common infections are becoming increasingly difficult to treat and that complications due to antibiotic resistant bacteria may take longer to resolve.The distribution of urinary pathogens in hospitalized patients is differs with *Escherichia coli* accounting for about 50% of infections. *Enterococcus, Klebsiella, Enterobacter, Citrobacter, Serratia, Pseudomonas aeruginosa, Providencia,* and *Staphylococcus epidermidis* account for most of the rest [7]. Aerobic nonfermenting gram-negative bacilli (nonfermenters) are a heterogeneous group of organisms that are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative rather than fermentative pathway [8]. Risk factors include immunosuppression, trauma, foreign body, broad-spectrum antibiotic use, infused body fluids like saline irrigations and also urinary catheterization when infections are caused by these pathogens [9]. We present data on antimicrobial susceptibility and resistance in UTIs patients attending to a tertiary care hospital in Salem Tamilnadu.

MATERIALS AND METHODS

Isolation and Identification of Organisms

Mid stream urine (MSU) specimens collected from both inpatients and outpatients attending the hospital (1100 bed capacity) for routine culture and sensitivity test, for six months period were included in this study. All samples were inoculated on blood agar as well as Mac Conckey agar and incubated at 37˚C for 24 hours, and for 48 hours in negative cases [10]. A specimen was considered positive for UTI in view of the number of yielded colonies (≥105 cfu/mL) and the cytology of the urine through microscopic detection of bacteriuria and PMNs (≥8 leukocytes/mm3). These MSU specimens were studied for significant bactereuria by grams staining (Hall GS et al 1995) (3). They were further processed for species identification by standard biochemical tests [11].

Susceptibility Testing

In-vitro antibiotic sensitivity test was performed by Kirby Bauer's disc diffusion method using Muller Hinton Agar as per Clinical Laboratory Standards Institute (CLSI) guidelines and susceptibility pattern was noted. Antimicrobial agents tested were Norfloxacin (10µg), Gentamycin(10µg), Amoxy clav(30µg), Nalidixic acid(30µg), Ciprofloxacin(30µg), Carbenicillin(100µg), Ceftazidime(30µg), Chloramphenicol(30µg), Amikacin(10µg), Meropenem(30µg), Methicillin(30µg). The CLSI-ESBL phenotypic confirmatory test with ceftazidime, cefotaxime, ceftriaxone, and cefixime was performed for all the isolates by disk diffusion method on the Mueller-Hinton agar plates with and without 10 μg of amoxy clav. Susceptibility test results were interpreted according to the criteria established by the Clinical & Laboratory Standard Institute (CLSI). A minimum of 5mm increase in the zone of diameter of third-generation cephalosporins, tested in combination with amoxy clav versus its zone when tested alone, was considered indicative of ESBL production. *E. coli* ATCC 25922 was used as ESBL-negative and *K. pneumonia* 700603 was used as ESBL-positive reference strain.

RESULTS

Majority of patients belonged to the age group of 20 to 29 years and most of them were females (Table-1). Out of the 18 *Pseudomonas aeruginosa* isolates which were resistant to commonly used antibiotics, 15 were from inpatients indicating the possibility of nosocomial infection. Patient surveillance was evaluated for UTI from 3 to 150 days it was present as early as day 3 and as late as day 75, with a mean of 19.5 days. Reinfection was observed in 7/19 (36.8%) patients with previous UTI, from day 17 to 65. The first infections developed were caused mainly by *E. coli*, other Enterobacteriaceae and *Pseudomonas aeruginosa*. The secondary infections were caused mainly by *E. coli* (42%) and *Enterococcus* spp. (28.1%). Table-2 represents the symptomatic correlation of UTI infection since few patients with positive symptoms were negative for pathological screening may be relating to other clinical correlations. Therapeutic alternatives for these cases were aztreonam, trimethoprim-sulfamethoxazole, netilmicin and fosfomycin.

A multiresistant *Enterococcus* was recovered, which was sensitive only to vancomycin. As expected, *E. coli* was the most frequent (average of 60.64% of the total isolates) followed by *Citrobacter* spp. and *Pseudomonas aeruginosa* (Fig-1). If *Candida* and minor bacterial isolates are not included, Gram-negative bacteria accounted for 92% of the UTI, while Gram-positive infections were responsible only for 8%. Analysis of the results according to patient gender (not shown) indicated that although *E. coli* is the predominant isolated pathogen from both sexes, it occurred more frequently in women (69.8% in women compared to 61.4% in men). The percentage sensitivity pattern of the commonly used antibiotics are represented (Fig-2) Over the past few years, the susceptibility to cephalosporins, including generations 3 and 4, tends to decrease; this is coupled by an increase in the production of ESBL the susceptibility profiles of ESBL producing *E. coli* to families of antibiotics other than *beta*-lactams. Tigecycline and piperacillin-tazobactam (data not shown) seem to have the highest antibacterial activity on these organisms.

DISCUSSION

Infection of the urinary tract is one of the most common infectious diseases and it would affect all age groups peoples including men, women and children in worldwide [12]. The increasing prevalence of infections caused by antibiotic-resistant bacteria makes the empirical treatment of UTIs difficult and outcome unpredictable [13]. In poor-resource settings where the availability of alternative effective antibiotics is limited, serious problems are anticipated in the treatment of multidrug resistant strains. Women are predisposed for UTI infections with 56% being infected in our study, the short urethra is considered to be a primary risk factor [14].This study is consistent with the findings of previous studies in which *E. coli* was the predominant pathogen isolated from patients with UTIs [15]. Many studies worldwide have also reported a sharp increase in ciprofloxacin resistant *E. coli* isolates from UTIs. The prevalence of ciprofloxacin resistance in Bangladesh was 26% [16].We also find increased resistance for norfloxacin and ciprofloxacin in *E. coli*.

In our study *Citrobacter* was the second most frequently isolated microorganism (14%) which is in accordance with [17]. *Citrobacter* types mostly cause urinary tract infections. *Pseudomonas aeruginosa is* an established pathogen of urinary tract. *Pseudomonas spp*. was the commonest non-fermenter isolate in the present study being significant in 20% of cases. This study revealed that for *Pseudomonas* spp. amikacin followed by ciprofloxacin in the group of first and second line antibiotics and also meropenem to be effective followed by cephalosporins in the group of third line reserved antibiotics. A previous study has reported that for *Pseudomonas aeruginosa*, amikacin, ceftazidime and piperacillin are the recommended antibiotics [18]. Many other studies reported multiple drug resistance in *Pseudomonas aeruginosa* isolates [19]. The rate of isolation of *Klebsiella pneumoniae* described is consistent to other studies. *Candida* spp. was commonly isolated; however, their clinical significance was not always evident.

Table-1: Bacterial load in patients of different sex and age groups and percentage of UTI infections

Age groups	**Male**		**Female**		**Total no. of patients infected**	**% TVC**	**%UTI**
	Total no.	No. Infected	Total no.	No. Infected			
1-10	11	3	7	1	4	22.2	12.4
20-29	27	5	34	23	28	45.9	25.9
30-39	19	12	28	13	25	53.1	29.6
40-49	17	7	22	16	23	58.9	32.9
50-59	5	2	9	6	8	57.1	31.8
Total	79	29	100	59	88	237.2	32.3

Table-2: Symptological correlation of bacterial infection in UTI

Symptoms	**% of 38 patients with positive growth and symptoms**		**% of 28 patients with negative growth and positive symptoms**		**% of 30 symptom less pregnant women patients**	
	Number	Percentage	Number	Percentage	Number	Percentage
Burning micturation	29	76.3	13	65	27	90
Increased frequency of urination	15	39.4	9	45	17	56.66
Fever	12	31.5	6	30.2	3	10
Abdominal pain	8	21.0	8	40.1	12	40.3
Heamaturia	1	2.6	-	-	-	-
Pyuria	3	7.8	1	5	3	10

Figure-1: Percentage of organisms involved in UTI

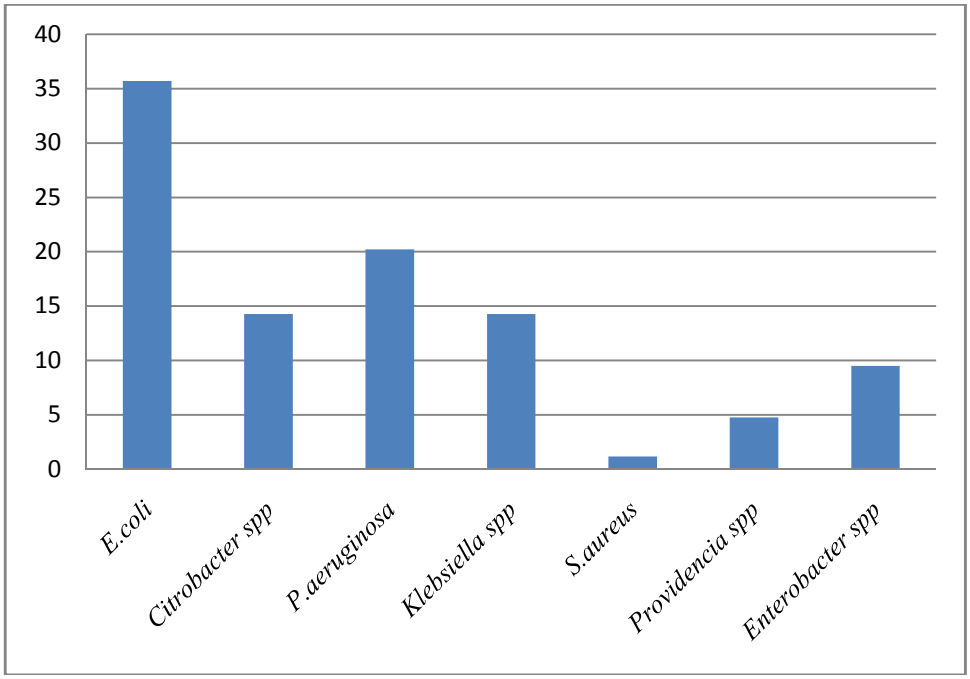
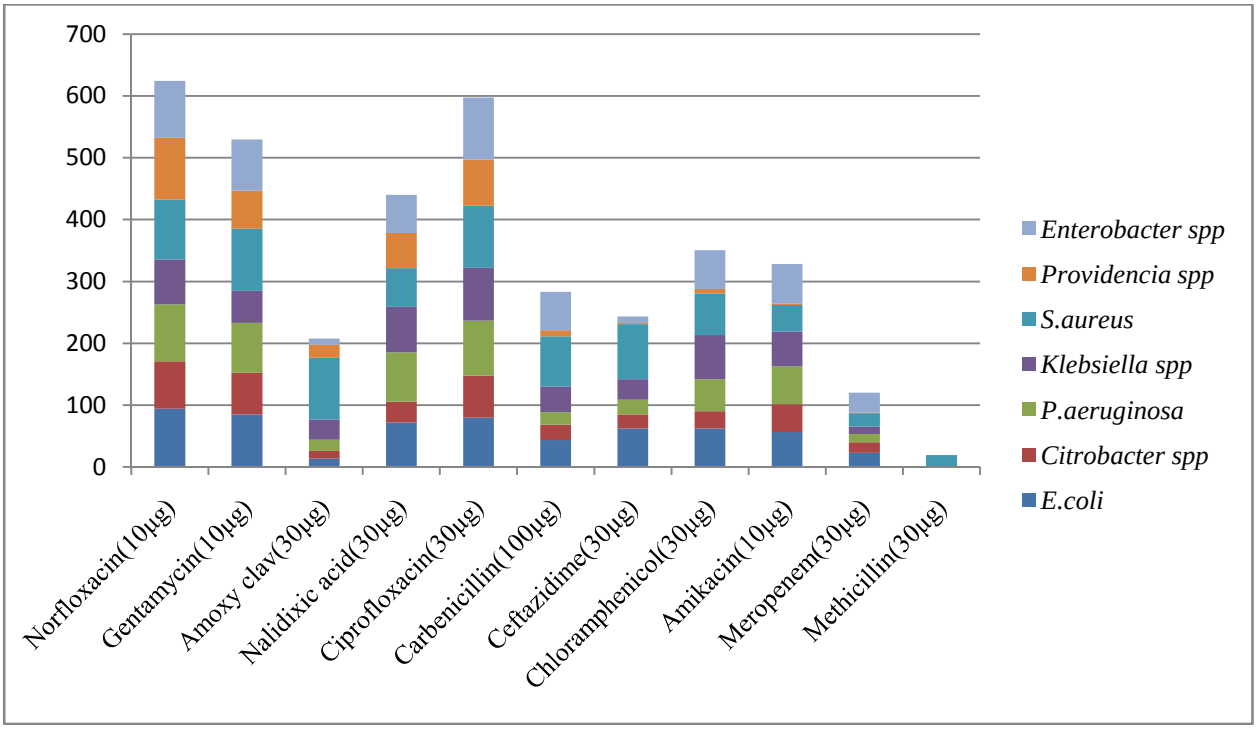


Figure-2: Antibiotic sensitivity pattern of isolated pathogens



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