Multidrug Resistant *Escherichia coli* in Nosocomial Urinary Tract Infections at a Tertiary Care Hospital in Kerala, India

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Abstract: *Background*: Nosocomial UTI (NUTI) is a major cause of morbidity in hospitalized patients and presence of urinary catheter is its single most important predisposing factor. Antibiotic resistance is a problem in these infections.

Objectives: To estimate the prevalence of NUTI in our setting, identify the predisposing factors and etiological agents of NUTI; to determine the antibiotic susceptibility patterns of the pathogens; to identify the risk factors for the acquisition of antibiotic resistance in these patients and to study the effect of duration of catheterization on the nature of infection.

Materials and Methods: After identifying suspected cases of NUTI based on signs and symptoms, relevant clinical details were noted and the urine samples were collected aseptically and cultured on blood agar and MacConkey's agar using the calibrated-loop-semi-quantitative method. Isolates from samples with significant bacteriuria were identified. Antibiotic susceptibility test was performed using modified Kirby-Bauer disk diffusion method. Resistance of the *E. coli* isolates to fluoroquinolones and third generation cephalosporins was confirmed by minimum inhibitory concentration (MIC) using agar dilution method for ciprofloxacin and cefotaxime respectively.

Results: Catheterization was the most common (88.1%) predisposing factor for NUTI. *E. coli* was the most common single isolate, accounting for 96 (41.2%) of the total 233 isolates. Multidrug resistance was observed in 81 (84.37%) of *E. coli* isolates. High rates of resistance were found with ampicillin (91.66%), ciprofloxacin (75%), cotrimoxazole (71.87%), cefuroxime (82.29%), cefotaxime (79.16%), cefepime (67.7%) and amoxicillin/clavulanate (63.54%) among the *E. coli* isolates. However, imipenem (0% resistance), meropenem(0%), piperacillin-tazobactam (15.62%), amikacin (5.2%) and nitrofurantoin (26.04%) appeared to have retained greater activity.

Conclusion: Considering the high rates of resistance found with fluoroquinolones, ampicillin, cotrimoxazole and cephalosporins, these can no longer be recommended for empiric therapy of NUTIs. Instead, imipenem, pipericillin-tazobactam, amikacin and nitrofurantoin should be considered for initial therapy with prompt de-escalation following receipt of the culture and sensitivity result.

Keywords: Catheterization, *E. coli*, multidrug resistance, nosocomial urinary tract infection.

INTRODUCTION

Nosocomial urinary tract infection (NUTI) accounts for over 1 million cases annually and approximately 40% of all nosocomial infections [1, 2]. Catheter associated UTIs (CAUTI) constitute 80% of all nosocomial UTIs [3]. "Almost 100 million catheters are sold annually worldwide" [4]. "NUTIs are significant not only due their high incidence and subsequent economic cost (an estimated $424 million to $451 million spent annually in the United States to manage these infections)" [5] but also because they may lead to serious complications, prolong the hospital stay, cause often unnecessary antibiotic usage and encourage the development of antibiotic resistance among hospital bacterial strains. The etiology, risk factors, pathogenesis, methods of diagnosis and surveillance, clinical outcome and effectiveness of preventive and therapeutic measures are now largely known [6]. Further, in recent years, resistance of these uropathogens to antibiotics has increased [7-13] Besides, the etiological agents and drug resistance vary among hospitals and through time [14] Therefore, it is imperative to know the bacterial pathogens responsible for NUTI in a particular setting and monitor their antibiotic susceptibility patterns periodically as it would be helpful in improving the efficacy of empirical treatment.

MATERIALS AND METHODS

Patients

This study was conducted between April 2013 and March 2014 at Kannur Medical College Hospital, Anjarakandy, Kerala, India. A UTI was considered as nosocomial if it was neither present nor incubating when the patient was admitted to the hospital and developed two days after hospitalization [15,16] or up to 48 hours after discharge from the hospital. It also applied to those patients who were transferred from another hospital and those who were readmitted to the

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hospital because of a UTI. Diagnosis was made as per the CDC definition of NUTI [17] i.e., symptomatic infection with the presence of one of the following signs or symptoms - fever, urgency, increased frequency of urination, dysuria, and a urine culture of $>10^5$ CFU/ml [18] with no more than two species of organisms [19]. Patients were included from all the wards in the hospital. Of the 9544 patients admitted to the hospital during the study, we collected clinical data with respect to the presence of signs and symptoms of a urinary tract infection. Those with signs and symptoms were requested to provide a urinary specimen, or a specimen was collected directly from the urinary catheter. Patients with a positive urine culture were included in the analysis if the diagnosed UTI could be considered to be nosocomial. Clinical data obtained with regard to finding the associated factors for NUTI in each patient included age, gender, duration of hospital stay, underlying disease, other co-morbid conditions; whether catheterized and if so, the duration of catheterization; details of antibiotic therapy, other instrumentation of the genitourinary tract and presence of signs and symptoms of UTI. Clinical data also included past history (one year) of UTI, previous (six months) exposure to antibiotics, previous (one year) history of urinary catheterization and older age (>65 years).

Specimen Collection

To obtain urine sample from a catheterized patient, the soft rubber connector between the catheter and the collecting tube was clamped by means of a sterile artery forceps for ten minutes for urine to collect. The rubber tube was disinfected with surgical spirit. It was then punctured by means of a sterile syringe and needle and urine aspirated. In patients who were not on catheter, clean-catch midstream sample was collected.

Processing and Culture

The urine samples were processed within one hour of collection. Wet mount and Gram's smear were used to detect cellular exudates and bacteria. Calibrated loop (4mm - internal diameter, carrying 0.04ml urine) method was used to inoculate blood agar and MacConkey's agar plates. The inoculated plates were incubated at 37oC for 24 to 48 hours. Bacteria grown in colony counts $\geq 10^5$ CFU/ml were considered positive [20]. The bacterial species were identified using standard methods [21].

Antibiotic Susceptibility Testing

E. coli isolates grown in significant colony counts were subjected to antibiotic susceptibility testing using modified Kirby-Bauer disk diffusion method as recommended by the CLSI [22]. *E. coli* ATCC 25922 was used as the reference strain. The antibiotic discs used were ampicillin, amikacin, gentamicin, ciprofloxacin, ofloxacin, cotrimoxazole, cefuroxime, cefotaxime, ceftazidime, cefixime, cefepime, nitrofurantoin, nalidixic acid, amoxicillin/clavulanate, piperacillin/tazobactam and imipenem for gram negative isolates. Results were interpreted as sensitive, intermediate or resistant according to CLSI criteria [22] "For calculations, all isolates showing zone of inhibition indicating intermediate susceptibility were considered resistant. Multi-drug resistance (MDR) isolates were defined as those isolates having co-resistance to at least three different antibiotic classes" [23]. Resistance of the MDR *E. coli* isolates to each of the antibiotic tested was confirmed by Minimum Inhibitory Concentration (MIC) using agar dilution method according to CLSI guidelines [22, 24]. Extended spectrum beta-lactamase (ESBL) production was detected phenotypically by the combined disc diffusion method using cefotaxime (30µg) and cefotaxime/clavulanic acid (30/10 µg); and ceftazidime (30µg) and ceftazidime/clavulanic acid (30/10 µg) according to CLSI guidelines [22].

RESULTS

For the study, we screened 9544 patients, of whom 221 reported to have signs and/or symptoms of UTI. Of these, 193 fulfilled the definition of NUTI and were included in the final analysis, proving 233 strains. Out of 193 cases, 170 cases (88.1%) were Catheter Associated UTIs (CAUTIs) and 23 (11.9%) were not associated with catheter use. More than two-thirds (68.38%) of the patients were older than 51 years. Out of the total 193 patients, 115 (59.58%) were males and 78 (40.42%), females.

Most patients had multiple co morbid conditions in addition to UTI. Prominent among them were diabetes mellitus in 50 patients (30.57%), nephropathies causing renal insufficiency in 38 patients (19.67%), neurological conditions including stroke and degenerative disorders in 28 patients (14.51%), prostatic hyperplasia in 33 patients (17.1%) and cancers in 12 patients (6.22%). A high number of patients - 66 patients (34.2%) gave past history of hospitalization(prior to 6 months).

The effect of duration of catheterization on bacteriuria was studied (Table **1**).

The majority of patients with a CAUTI were catheterized for more than 1 week up to 1 month (53.3%), while 36.5% of the patients had a catheter for up to 1 week only. Longer duration of catheterization was associated with a higher frequency of poly-microbial infections. (Table **1**)

A total of 233 strains were isolated out of 193 cases of NUTI (Table **2**).

One hundred and fifty three cases (64.56%) were mono-microbial infections and 40 cases (35.44%) were bi-microbial infections. *E. coli* was the commonest [96 isolates, (41.2%)] pathogen isolated. *Enterococcus faecalis* and *Proteus vulgaris* were isolated more often in poly-microbial infections than in mono-microbial infections (Table 2).

Antibiotic susceptibility testing was done on *E. coli* strains against 16 antibiotics. They were found to have high resistance rates. (Table **3**).

An attempt was made to identify the presence of known risk factors for acquisition of antibiotic resistance in the patients. Out of 193 patients, 149 (77.2%) gave history of prior (six months) antibiotic exposure, 61 (31.6%) had prior (one year) UTI, 64 (33.16%) had prior (one year) catheterization and 39 (20.2%) were aged > 65 years.

Table 1. Duration of catheterization and nature of infection.

Duration of Catheterisation	Mono-Microbial Infections (%)	Poly-Microbial Infections (%)	Total	%
Upto 7 days	56 (90.32)	6 (9.68)	62	36.47
> 7 days - 30 days	69 (85.83)	22 (24.17)	91	53.53
> 30 days	5 (29.41)	12 (70.59)	17	10.0
Total	130	40	170	100

Table 2. Bacterial Pathogens in NUTI.

Isolate	Monomicrobial	Polymicrobial	Total (%) n=233
Escherichia coli	66	30	96 (41.20)
Klebsiella pneumoniae	27	08	35 (15.02)
Enterococcus spp.	12	16	28 (12.02)
Pseudomonas aeruginosa	13	11	24 (10.30)
Candida albicans	17	3	20 (8.58)
Proteus vulgaris	5	6	11 (4.72)
Citrobacter freundii	6	1	7 (3.0)
Acinetobacter baumannii	3	1	4 (1.71)
Coagulase Negative Staphylococcal species	2	2	4 (1. 71)
Enterobacter cloacae	2	2	4 (1. 71)
Total	153	80	233(100)

Table 3. Antibiotic resistance in *E. coli* isolated from NUTI.

Antibiotics (Disk Potency)	Number (%) of Resistant Isolates		
	ESBL-ve n=19(19.8)	ESBL+ve n=77(80.2)	Total (n=96)
Ampicillin	11 (57.9)	77(100)	88(91.7)
Gentamicin	02 (10.5)	25(32.4)	27(28.1)
Amikacin	00 (00)	05(6.5)	05(5.2)
Cotrimoxazole	08 (42.1)	61(79.2)	69(71.8)
Ciprofloxacin	04 (21.1)	68(88.3)	72(75)
Ofloxacin	03(15.8)	65(84.4)	68(70.8)
Nalidixic acid	10 (52.6)	72(93.5)	82(85.4)
Nitrofurantoin	04 (21.1)	21(27.2)	25(26.0)
Cefuroxime	02 (10.5)	77(100)	79(82.2)
Cefixime	01(05.2)	77(100)	78(81.2)
Cefotaxime	00(00)	77(100)	77(80.2)
Ceftazidime	00(00)	77(100)	62(80.2)
Cefepime	00(00)	77(100)	65(80.2)
Amoxicillin/clavulanate	00(00)	61(79.2)	61(63.5)
Piperacillin/tazobactam	00(00)	15(19.4)	15(15.6)
Imipenem	00(00)	00(00)	00(00)

Out of the 96 isolates, 77(80.2%) were ESBL producers and 81(84.37%) were multidrug resistant.

DISCUSSION

"NUTI is the most common hospital acquired infection with the implications in terms of number of patients affected, duration of excess hospital stay and overall costs are huge [25, 26]. In our study, we reported a period prevalence of NUTI of 2.31% (193/9544). The most common causative specimen was *Escherichia coli*, which showed a high level of resistance for a wide variety of antibiotics.

Some of the risk factors for the development of NUTI as evaluated by previous studies are female sex, older age (>50 years), rapidly fatal underlying disease, orthopaedic or urologic patients, urethral catheterization, prolonged duration of catheterization, diabetes mellitus and renal insufficiency [26-29]. The most important determinant for bacteriuria is the duration of catheterization [25]. In the present study, 88% of NUTIs were found to be catheter associated and a high percentage (63.5%) of these patients were on catheter for more than one week.

There is a clear distinction between NUTI and community acquired UTI with respect to their microbial profiles [3, 30]. *E. coli* constituted 40.5% of the total isolates in the present study whereas it has been shown to cause 90% of the community acquired UTIs [30]. The preponderance of *E. coli* as a urinary pathogen can be explained in parts by the Prevalence Theory which postulates that the strains responsible for UTI are merely those present in feces and the Specific Pathogenicity Theory which states that those *E. coli* which possess specific virulence factors - which most commensals probably lack-are uropathogenic [32]. But *E. coli* causing NUTI lack many of the virulent factors possessed by those causing community acquired UTI [33].

We found that as the duration of catheterization increased, the incidence of poly-microbial infections also increased. We further observed that *E. coli* and enterococci occurred as the most common combination in poly-microbial infections. Moreover, enterococci were the second most common isolates from poly-microbial infections next only to *E. coli*. Enterococci may aggravate the pathogenicity of co-existing bacteria [34].

The high antibiotic resistance rates in *E. coli* causing UTI found by us was quite alarming although not entirely surprising. Out of the 96 isolates, 77 were ESBL producers and 81 were multidrug resistant. High rates of resistance were found with ampicillin (91.66%), ciprofloxacin (75%), cotrimoxazole (71.87%), cefuroxime (82.29%), cefotaxime (79.16%), cefepime (67.7%) and amoxicillin/clavulanate (63.54%). High rates of antibiotic resistance among uro-pathogens in hospital settings have also been shown by previous studies [7-13].

Among the factors that contribute to these high rates of antibiotic resistance are the selection of mutant strains from the patients' own flora receiving antibiotic treatment and the transfer of genetic determinants of resistance e.g., plasmids or transposons. The selection may be enhanced by antibiotic pressure, immunosuppression, presence of foreign body *viz.,* the catheter that impedes the local host defenses and favors colonization. Subsequently, these resistant strains spread among patients in the hospital - the transmission occurring mainly by cross-contamination *via* the hands of the health care staff. "Factors predisposing to this transmission include the length of hospital stay, intensity and duration of exposure to antibiotics, severity of underlying illness and use of catheter" [33].

To address the growing concerns of antibiotic resistance in hospitals, in addition to stringent infection control precautions, excessive and injudicious use of antibiotics particularly those having broad spectrum activity needs to be aggressively discouraged. Furthermore, CAUTI does not always warrant antibiotic treatment because it is usually asymptomatic [most cases (90%) of bacteriuria during catheterization are asymptomatic and usually resolves after removal of the catheter [35]. Patients catheterized for longer durations develop bacteriuria whether treated or not - the risk increasing by 5% per day [35]. Hence antibiotic prophylaxis just to prevent bacteriuria is not recommended unless the clinical situation demands it. "However, when an episode of CAUTI becomes symptomatic, the resulting sequelae can range from mild (fever, urethritis, and cystitis) to severe (acute pyelonephritis, renal scarring, calculus formation, and bacteremia). Left untreated, these infections can lead to urosepsis and death" [36, 37]. "These complicated infections commonly recur and result in long-term morbidity due to the presence of encrustation and blockage of the catheter by crystalline biofilms that increase resistance to the host immune response and to antibiotics" [38].

Based on the findings of our study, we observed high rates of drug resistance in our setting indicating the urgent need for antibiotic stewardship. Another notable finding was the susceptibility of a high percentage of ESBL producing *Escherichia coli* to Nitrofurantoin, which is less often used though it is cheap and freely available in our setting.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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