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Research Article

PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING UROPATHOGENS IN PREGNANT WOMEN

REKHA THAPA[1]*, PRAMILA LAMICHHANE[2]*, MEGHA RAJ BANJARA[1], GANESH PRASAD ACHARYA[3]

[1]Department of Microbiology, Tribhuvan University, Kathmandu, Nepal. [2]Department of Microbiology, Universal College of Medical Sciences, Bhairahawa, Nepal. [3]Department of Pathology, Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu, Nepal. Email: thaparekha101@yahoo.com

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ABSTRACT

Objective: This study aims to determine the prevalence of urinary tract infection (UTI) among pregnant women and multidrug-resistant (MDR) uropathogens with reference to extended spectrum beta-lactamase (ESBL) producers.

Methods: Three hundred urine specimens collected from pregnant women were studied. A semi-quantitative method was used for diagnosis of UTI. Isolation, identification, and antimicrobial susceptibility of an organism was done by standard microbiological procedure. ESBLs production was detected by double-disc synergy test method.

Results: UTI was found among 30.5% of pregnant women. Among 137 Gram-negative bacterial isolates, 72.0% were found to be MDR while only 7.30% were ESBL producers. Among total of *Escherichia coli* and *Klebsiella pneumoniae* isolates, 7.69% and 15.38%, respectively, were found to be ESBL producers. Parity (odds ratio [OR]: 1.58, $p<0.05$), education status (OR: 4.07, $p<0.01$), occupation of pregnant women (OR: 1.86, $p<0.05$), times of bathing (OR: 3.45, $p<0.01$), history of UTI (OR: 20.79, $p<0.01$) were found to be significantly associated with UTI from both univariate and multivariate analysis. Gentamicin, nitrofurantoin, ceftazidime, and amikacin were found to be the most effective antibiotic against uropathogens.

Conclusion: Frequent and consistent evaluation of the prevalence, etiologic agents, and predisposing factors of UTI during pregnancy is necessary in developing countries like Nepal in order to reduce its devastation effects during pregnancy on both maternal and fetal health. It is essential to have a regular and routine monitoring of ESBL producing clinical isolates in laboratory practice.

Keywords: Pregnant women, Urinary tract infection, Multidrug resistance, Extended spectrum beta-lactamase.

INTRODUCTION

Urinary tract infection (UTI) is the second most common infectious presentation in the community affecting all age group across the life span. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of six billion US dollars [1]. UTI is common during pregnancy due to a number of factors including ureteral dilatation, increased bladder volume and decreased bladder tone, along with decreased ureteral tone which contributes to increased urinary stasis and ureterovesical reflux [2]. Up to 70% of pregnant women develop glycosuria, which encourages bacterial growth in the urine [3]. Although UTI may be caused by any pathogen that colonizes the urinary tract (e.g., fungi, parasites, and viruses), most causative agents are bacteria of enteric origin [4]. The bacteria causing UTI in pregnancy essentially mirror those in non-pregnant patients [5]. UTI during pregnancy has been associated with complications such as pyelonephritis, hypertensive disease of pregnancy, anemia, chronic renal failure, premature delivery, and fetal mortality [6,7].

Uropathogens have shown a slow but steady increase in resistance to several antibiotics over the last decades. Extended-spectrum beta-lactamases (ESBL) producing enterobacteriaceae are among the most problematic multidrug resistance (MDR) bacteria worldwide [8] and are increasingly causing UTI both in hospitalized patients and outpatients making infections difficult to treat [9]. ESBLs are the beta-lactamases capable of hydrolyzing penicillin, broad-spectrum cephalosporins, and monobactams, and are generally derived from TEM and SHV-type enzymes but do not effect cefamycins and carbapenems. ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species [10].

Delay in the detection and reporting of ESBL production by Gram-negative bacilli (GNB) is associated with prolonged hospital stay, increased morbidity, mortality, and health-care costs [11]. Frequent and consistent evaluation of the prevalence, etiologic agents, and predisposing factors of UTI during pregnancy is necessary in developing countries like Nepal in order to reduce its devastation effects during pregnancy on both maternal and fetal health. It is essential to have a regular and routine monitoring of ESBL producing clinical isolates in laboratory practice where there is excessive use of antibiotics and lack of adequate antimicrobial resistance surveillance.

MATERIALS AND METHODS

Across sectional descriptive study was done from June to December 2010 among 300 pregnant women attending their antenatal checkup at Paropakar Maternity and Women's Hospital, Kathmandu, Nepal. Structured questionnaires were used to collect demographic data, behavioral characteristics, and history of the patient. Midstream urine was collected and processed in the microbiology laboratory. Semi-quantitative culture technique was used to detect the presence of significant bacteriuria. Culture was done on blood Agar (Hi-Media, India) and McConkey Agar (Hi-Media, India). Diagnosis of UTI was made when there were at least 10^5 organisms/ml of urine. A single isolated colony was considered for further studies, and identification was done using standard conventional, morphological, cultural, and biochemical tests [12]. Antibiotics susceptibility testing of GNB was performed by Kirby-Bauer disc diffusion method following CLSI recommendation. The antibiotic discs (Hi-Media, India) used were gentamicin (10 µg), amikacin (30 µg), cephalexin (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), norfloxacin (10 µg), ampicillin (10 µg), cotrimoxazole (25 µg), and nitrofurantoin (300 µg).

The control strains used were *Escherichia coli* ATCC 29522. Phenotypic confirmatory was done by double-disc synergy test (DDST). Amoxy-clavulanic acid (20/10 µg) disc (Hi Media) was placed in the center of ceftazidime (30 µg) and cefotaxime (30 µg) discs with 25-30 mm apart. After overnight incubation at 37°C in air, confirmation of ESBL producing organism was assessed when the zone of inhibition around ceftazidime and cefotaxime was expanded by at least 5 mm by the presence of clavulanic acid [13]. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive controls for ESBL production, respectively.

RESULTS

The prevalence of UTI among the pregnant women was found to be 30.5% (92/300). Among total 150 isolates, 13 were Gram-positive cocci of which 11 were *Staphylococcus aureus* and 2 were coagulase-negative staphylococci. Among 137 GNB, *E. coli* (52.0%) was the predominant one followed by *K. pneumonia* (17.3%). Of these 137 isolates, only 7.30% (10/137) were ESBL producers. Among total of *E. coli* and *K. pneumoniae* isolates, 7.69% (6/78) and 15.38% (4/26), respectively, were found to be ESBL producers (Table 1).

Majority of the sample 65.7% (197/300) were from the outpatient department of the hospital. In multivariate analysis of UTI with independent variables parity, educational status, occupation of pregnant women, times of bathing in a week, and history of UTI was found to be significantly associated with UTI (Table 2).

Among the total GNB isolates, 72.0% (100/137) were MDR. Gentamicin, nitrofurantoin, ceftazidime, and amikacin were found to be the most effective antibiotic against GNB while majority of the isolates were resistance to cephalexin (Table 3 and Fig. 1).

The antibiotic susceptibility pattern of *E. coli* showed that gentamicin, nitrofurantoin were the most effective drug followed by amikacin (Table 3).

DISCUSSION

The prevalence of UTI in pregnant was found 30.5% which is comparative with figures in Yemen i.e. 30% [14]. Different authors from Nepal have reported different prevalence rate of 8.7% [15], 45% [16] and 51.4% [17]. However, lower rate of 26.0% in Bangladesh [18], and 10.4% in Ethiopia [19] while higher rate of 49.4% in India [20] has been reported from different countries.

E. coli being the most predominant bacteria accounts for more than half of the isolated bacteria. The predominance of *E. coli* as the common causative agent of UTI among pregnant women has been reported by different authors [21,22]. *E. coli* being a commensal of the bowel is more likely to cause infection by fecal contamination due to poor hygiene, the anatomy proximity to the genito-urinary area in females and the urinary stasis during pregnancy [6,23]. Among total GNB isolates, 7.30% were ESBL producers. However, another study

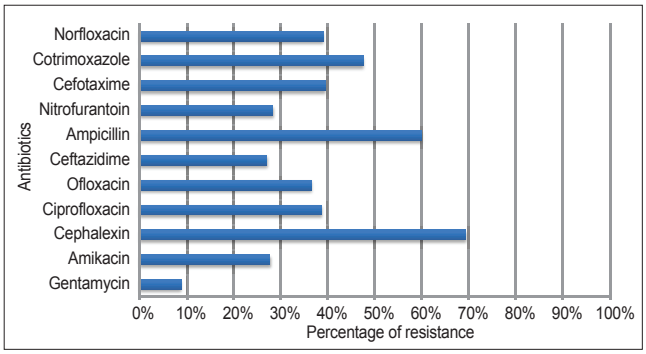

Fig. 1: Resistance pattern of Gram-negative uropathogens toward antibiotics

by Manandhar *et al.* in Nepal reported the prevalence of 27.5% [24]. Different similar studies in India and Pakistan have reported prevalence of ESBL producing GNB to be 25.2% and 2.45%, respectively [22,25]. ESBL prevalence varies in different countries. ESBL production by the bacterial isolates might be due to excessive use of broad–spectrum antibiotics in hospital and to a higher level in community setting, together with a lack of attention to laboratory screening of ESBL production by clinical isolates. Our study showed 7.69% of *E. coli* and 15.38% of *K. pneumonia* were, ESBL producers. Another study in Nepal

Table 1: Prevalence of ESBL production among Gram-negative uropathogens

Bacterial isolates (N=137)	Number (%)	ESBL producers (%)
E. coli	78 (52.0)	6 (7.69)
K. pneumoniae	26 (17.3)	4 (15.38)
P. mirabilis	24 (16.0)	-
E. aerogenes	6 (4.0)	-
C. freundii	2 (1.3)	-
P. aeruginosa	1 (0.7)	-
Total	137	10 (7.30)

E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, P. mirabilis: Proteus mirabilis, E. aerogenes: Enterobacter aerogenes, C. freundii: Citrobacter freundii, P. aeruginosa: Pseudomonas aeruginosa

Table 2: Multivariate analysis of UTI with independent variables

Variables	Number (%)	OR	95% CI	p value
Demographic characteristics				
Origin				>0.05
Outpatient	197 (65.7)	1.25	0.77-2.03	
Inpatient	103 (34.3)	1	-	
Address				>0.05
Rural	143 (47.7)	1.51	0.95-2.38	
Urban	157 (52.3)	1	-	
Age group				>0.05
≤20	46 (15.3)	1	-	
21-30	223 (74.3)	1.16	0.61-2.20	
31-40	31 (10.3)	1.56	0.61-4.0	
Parity				*p
Primiparous	163 (54.3)	1	-	
Multiparous	137 (45.7)	1.58	1.00-2.5	
Trimester				>0.05
First	69 (23.0)	1	-	
Second	145 (48.3)	6.90	0.92-51.78	
Third	86 (28.7)	9.83	0.65-147.34	
Education				**p
Illiterate	36 (12.0)	4.07	1.79-9.26	
Literate	264 (88.0)	1	-	
Maternal occupation				*P
Housewife	220 (73.3)	1.86	1.10-3.13	
Employed	80 (26.7)	1	-	
Behavioral characteristics				
Times of bathing/ week				**p
<2	75 (25.0)	3.45	1.95-6.10	
≥2	225 (75.0)	1	-	
Smoking habit				>0.05
No	281 (93.7)	1	-	
Yes	19 (6.3)	7.00	0.35-138.33	
History of pregnant women				
History of UTI				**p
No	258 (86.0)	1	-	
Yes	42 (14.0)	20.79	2.86-150.87	
Yes	9 (6.5)	8.56	0.22-322.71	

*p<0.05, **p<0.01, UTI: Urinary tract infection , OR: Odds ratio, CI: Confidence interval

Table 3: Antibiotic resistant pattern of Gram-negative bacilli

Antibiotics used	Bacterial isolates					
	***E. coli* (n=78)**	***K. pneumonia* (n=26)**	***P. mirabilis* (n=24)**	***E. aerogenes* (n=6)**	***C. freundii* (n=2)**	***P. aeruginosa* (n=1)**
Gentamicin	6 (7.7)	3 (11.5)	3 (12.5)	0 (0)	0 (0)	0 (0)
Amikacin	16 (20.5)	13 (50.0)	7 (29.2)	1 (16.7)	1 (50.0)	0 (0)
Cephalexin	49 (62.8)	21 (80.8)	17 (70.8)	5 (83.3)	2 (100)	1 (100)
Ceftazidime	21 (26.9)	10 (38.5)	3 (12.5)	2 (33.3)	1 (50.0)	0 (0)
Cefotaxime	34 (43.6)	8 (30.8)	10 (41.7)	1 (16.7)	0 (0)	1 (100)
Ciprofloxacin	32 (41.0)	10 (38.5)	7 (29.2)	3 (50.0)	1 (50.0)	0 (0)
Ofloxacin	27 (34.6)	11 (42.3)	7 (29.2)	5 (83.3)	0 (0)	0 (0)
Norfloxacin	46 (58.97)	2 (7.69)	1 (4.2)	3 (50.0)	1 (50.0)	1 (100)
Ampicillin	44 (56.4)	20 (76.9)	13 (54.2)	4 (66.7)	1 (50.0)	0 (0)
Cotrimoxazole	32 (41.0)	10 (38.5)	12 (50.0)	4 (66.7)	2 (100)	1 (100)
Nitrofurantoin	8 (10.3)	7 (26.9)	4 (16.7)	0 (0)	1 (50.0)	0 (0)

The numbers in parenthesis indicates percentage. *E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, P. mirabilis: Proteus mirabilis, E. aerogenes: Enterobacter aerogenes, C. freundii: Citrobacter freundii, P. aeruginosa: Pseudomonas aeruginosa*

reported 13.5% of *E. coli*, and 16.55% *K. pneumoniae* isolates to be ESBL producers [26].

Multiparity and education status were found to be the risk factors of UTI. Profound physiologic changes affecting the entire urinary tract during pregnancy has a significant impact on the natural history of UTI during gestation. These changes vary from patient to patient and more likely to occur in women who have pregnancies in rapid succession [27]. The high prevalence of UTI in illiterate women might be due to poor genital hygienic practices especially in the case of cleaning their anus after defecating or cleaning their genital after passing urine. Though maternal occupation was significantly associated with infection in our study, Masinde *et al*. [28] reported no association between maternal occupation and the infection status. Times of bathing were found to be high-risk factor for developing UTI. Amiri *et al*. [29] focused on the protective role of the good hygienic practices against most of the morbidities. History of past UTI and past urological problems was found to be the risk factors for UTI in pregnant women. Amiri *et al*. [29] also confirmed that a previous UTI might predispose to subsequent UTI through behavioral, microbiological or genetic factors. Alemu *et al*. [19] also reported UTI in pregnant women to be significantly higher among those with the previous history of UTI.

Gentamicin, nitrofurantoin, ceftazidime, and amikacin were the effective drug against uropathogens. Kattel *et al*. [30] reported gentamicin and ceftazidime; Bhatt *et al*. [31] reported nitrofurantoin while Chaudhary *et al*. [32] reported amikacin and nitrofurantoin to be the most effective drugs against uropathogens. Low level of resistance against nitrofurantoin might be due to its narrow range of clinical indications, which results in less usage. Considering the invitro activity of nitrofurantoin and availability as an oral drug, nitrofurantoin can be considered as one of the drugs of choice against UTI in pregnant women where multi-resistant uropathogens are prevalent [21]. Gentamicin was found to be the most effective drug against *E. coli* followed by nitrofurantoin and amikacin. In accordance to our study Alemu *et al*. [19] showed 94.7% of *E coli* susceptible to gentamicin while Bhatt *et al*. [31] reported nitrofurantoin to be the most effective drug against *E. coli.* A large percentage (72%) of the bacterial isolates was MDR. Higher MDR might be due to many factors including misuse of antibiotics by the health care professionals, non-skilled practitioners as well as general public and also inadequate surveillance due to lack of information arising from routine antimicrobial susceptibility testing.

Regular monitoring is required to establish reliable information about resistance pattern of uropathogens for optimal empirical therapy of patients with UTI. One should avoid unnecessary antibiotics and optimize their use to minimize resistance pressure. The study emphasizes the need for microbiology laboratories to adequately screen for ESBL-producing strains of the family *Enterobacteriaceae* as the infections caused by these organisms are not efficiently treated with these antibiotics. This study is limited by the fact it included highly selected pregnant women with symptoms of UTI only, and the duration of the study was only 6 months. Further studies are recommended to include both symptomatic and asymptomatic pregnant women with UTI over a longer duration to establish a trend in antibiotic resistance pattern.

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