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Short Communication

Isolation and Characterization of Microflora from Human Urine

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A B S T R A C T

The research work was conducted to isolate & characterized the bacteria isolated from mid-stream urine of human. A total of 30 samples were collected from patients with urinary tract infection (n=10samples); diabetic patients (n=10samples) & apparently healthy individuals (n=10samples). *Escherichia coli, Staphylococcus* spp. *and Bacillus* spp. was the predominant bacterial flora of human urine. Among the isolates, the prevalence of *Escherichia coli* was highest (80%) compared to *Staphylococcus* spp.(14%) and *Bacillus* spp.(6%). *E. coli* isolated from 6 different sources were found to be highly virulent, moderately virulent, less virulent and avirulent categories as observed in day-old suckling mice. Antibiotic sensitivity profiles suggest that nalidixic acid will be the first drugs of choice to treat the UTI caused by *E. coli* and arythromycin, ampicillin and azithromycin will be the second drugs of choice to treat the UTI caused by *Staphylococcus* spp and *Bacillus* spp. respectively.

Keywords: Bacterial flora, urinary tract infection (UTI); isolation and antibiotic sensitivity profile

Urinary tract infection (UTI) caused by bacteria includes cystitis (most common type of UTI), pyelonephritis, asymptomatic bacteriuria, and acute urethral syndrome constitute one of the most frequent causes of illness in humans (Oliveira *et al.*, 1992). In most cases, the infection is confined to the bladder (Bamberger *et al.*, 2001). Symptomatic UTI is identified if the person exhibits three of these four criteria: (1) fever 100.4ºF (38ºC) or higher; (2) new or increased burning sensation with urination, frequency, or urgency; (3) new flank or suprapubic pain or tenderness; and (4) worsening of mental or functional status (JoAnn, 2009). Urinary tract infection most commonly results from exposure to stool organisms. The most frequently isolated causative agents of UTIs are members of the family *Enterobacteriaceae*, in particular *E. coli* is the causative organism in 80% to 85% of cases of acute cystitist; other causative organisms are *Staphylococcus saprophyticus*, *Klebsiella pneumoniae,* and *Proteus mirabilis* (Hooton *et al.*, 2004). UTIs are most common in sexually active women and increase in diabetics and people with anatomical malformations of the urinary tract. Acute lower urinary tract infections are common in adult women, and as many as 6% of members of the adult female population experience 3 or more episodes during a given year (Terje *et al.*, 2002). These infections affect as many as 50% of women at least once during their lifetime (Morgan *et al.*, 1993), and 25% of those who acquire a UTI will have another infection within the following 6 months (Foxman, 1990). In children, infection is more common in young girls, except in the neonatal age group, where boys predominate. The infections are a serious health problem affecting millions of people each year. It is responsible for much illness and contributes significantly to the cost of providing health care in economically developed countries which account for 7 million office visits, more than 1 million emergency room visits, and 100,000 hospitalizations each year (Schappert,1999). The estimated annual cost to the U.S. health care system is approximately $1.6 billion (Foxman, 2000). In 1995, an estimated 11.3 million women in the United States received antibiotic treatment for at least 1 presumed UTI, resulting in associated costs of $1.6 billion during that year (Terje *et al.*, 2002). It accounts for about 8.3 million doctor visits each year (Vital and Health Statistics. Series 13, No. 157; U.S. 2004). For the better control of human urinary tract pathogens (uropathogens) the first priority is to enumerate bacteria in the urine and urine culture to isolate the agents and their antibiotic sensitivity for effective treatment. Evidence based practice for the reporting of urine culture results is still unfortunately lacking. However, no detailed study has so far been performed in our country in this regard.

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A total of 30 samples (mid-stream urine–10ml per sample) were collected from man & woman affected with Urinary Tract Infection (with high fever, frequent urination and loss of health); diabetic patients and apparently healthy man & woman (10 samples from each source) at National Healthcare Network of Diabetic association, Bangladesh Agricultural University (BAU), Mymensingh. The collected samples were inoculated onto Nutrient agar (NA), MacConkey agar (MC), Triple Sugar Iron agar (TSI), Salmonella-Shigella agar (SS), Eosin methylene blue agar (EMB) and Blood agar (BA) incubated at 37ºC for 24-48 hours and colony morphology of grown bacteria were examined with grams staining method (Merchant and Packer, 1967) and motility test with hanging drop slide (Cowan, 1974). Biochemical characterization of the isolates were performed with Sugar fermentation test, Catalase test, Coagulase test, Indole test, Methyl Red test(MR) and Voges-Proskaucer test (V-P) (Cheesbrough, 1985). Susceptibility of the isolated bacteria to different antibacterial agents was performed through disc diffusion method. Commercially available antibiotic discs (Mast Group Ltd, Merseyside, UK and BENEX Limited, Clare, Ireland; USA) were used for the test to determine the drug sensitivity pattern. This method allows the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that result from different rates of diffusion of the agent into the medium surrounding the disc. Sensitivity to antibiotic was mostly determined on Nutrient agar with ampicillin (10µg/disc), amoxycillin (10 µg/disc), azithromycin (15µg/disc),

erythromycin (15µg/disc), nalidixic acid (30µg/disc), penicillin (10 UI/disc). The result of the antibiotic sensitivity test was interpretated according to the guidelines of NCLS, 2007. The virulence of 6 representative *Escherichia coli* isolates, one from each source was determined by performing the lethality assay in day-old suckling mice by oral inoculation of bacteria with crude toxin in separate groups. The test *Escherichia coli* isolates were cultured in 5 ml nutrient broth at 37^0C for 18 hours. After 18 hours the broth culture was shaked well and 1 ml was taken with a sterile syringe from the test tube containing both bacteria and crude toxin. The CFU was determined by inoculating the overnight broth culture of the test organisms on the MacConkey agar plates after serial 10 fold dilution. After incubation of the plates at 37^0C for 18 hours, the plates having single colonies within 30-300 were counted and the CFU per ml inoculum was calculated. Fifty microlitre of bacteria with crude toxin (approx. 3×10^7 CFU) of each representative isolate of *Escherichia coli* were inoculated orally in separate groups of day-old suckling mice and observed for mortality at every hour for 5 days. A known virulent and an avirulent control *Escherichia coli* isolates were also inoculated in the mice. Uninoculated control mice were also included. Isolates that killed more than 50% of mice were considered under killer group, and isolates that killed 0-50% of mice were considered as non-killers (Johnson *et al*, 2006).

The cultural and biochemical characteristics of the isolated bacteria are summarized in Table 1. Bacteria recovered from patients with urinary tract infection and diabetes as well as apparently healthy man & woman showed characteristics colony morphology of *E. coli, Staphylococcus* spp. and *Bacillus* spp. on different culture media. *E. coli* isolates were Gram negative, arranged in Single, paired or in short chain and produced characteristics metallic sheen on EMB agar. The *Staphylococcus* spp. were Gram positive cocci, motile arranged in grape like cluster produced golden yellow color colony on blood agar. *Bacillus spp* produced thick grayish white cream colored colony and haemolysis on blood agar. The cultural characterization of all isolated bacteria were on agreement with Cheesbrough, 1985. *E. coli* and *Staphylococcus* spp fermented five basic sugars with the production of acid and gas. On the other hand, *Bacillus* spp fermented five sugars and produced both acid and gas from maltose. *E. coli* were catalase, MR and indole positive; but V-P negative. *Staphylococcus* spp and *Bacillus* spp catalase, indole, MR positive but V-P negative. The biochemical properties of all isolated bacteria were in agreement with Cheesbrough, 1985.

Antibiotic sensitivity pattern of *Escherichia coli*, *Staphylococcus* spp. *and Bacillus* spp. are presented in Table 2. *E. coli* isolates recovered from human urine were sensitive to nalidixic (80%) and resistant to erythromycin(60%); *Staphylococcus* spp. isolates were sensitive to erythromycin (60%) and resistant to ampicillin (40%); *Bacillus* spp. isolates were sensititive to erythromycin (40%) and azithromycin (40%)and resistant to penicillin (60%). The graphical representation of mean death time in hours of day-old suckling mice after experimental inoculation with different *E. coli* isolates are shown in Fig. 1. All of the mice used for this experiment were dead. Cent percent mice were died following inoculation of crude toxin of *E. coli* indicating these were virulent strains (death of mice occurred between 11 to 19 hrs). Isolates recovered from apparently healthy woman urine were moderately virulent (66.7%) and from apparently healthy man urine were less virulent (33.3%). For these two isolates death of the mice occurred within 34 to 44 hrs.

Bacteria isolated from human mid-stream urine were *Escherichia coli, Staphylococcus* spp. *and Bacillus* spp. Among the isolates, *Escherichia coli* were found in all samples. In this study prevalence of *E. coli* was the highest (80%), followed by *Staphylococcus spp*.(14%) and *Bacillus spp.*(6%) in all samples. These findings were were in agreement with the findings of Hooton *et al.* (2004). Colony morphology and gram staining characteristic s of *E. coli* recorded in this study were similar to the findings of Buxton and Fraser (1977), Ali *et al.* (1998), Sharada *et al.* (1999). *Staphylococcus* spp recovered from urine samples were similar cultural and staining characteristics reported by Marchant and Packer (1967), Freeman (1985), Carter (1979) and Brooks *et al*. (2002). Isolated *Bacillus* spp showed similar cultural and staining characteristics observed by Colins and Lyne, 1976 and Toply and Wilson, (1995). The biochemical properties of all isolated bacteria were in agreement with Cheesbrough 1985. The mice lethality assay showed similar pathogenic nature of the isolated E. *coli* strains reported by Dang *et al.* (2001), Johnson *et al.* (2006) and Zinnah *et al.* (2007). *E. coli* isolates from human urine were sensitive to nalidixic acid (80%).These findings were in close agreement with Gulsun *et al.* (2005). *Staphylococcus* spp isolates from human urine were sensitive to erythromycin (60%). The present findings were contradictory with Singla *et al.* (1993). The isolated *Bacillus* spp. were sensitive to ampicillin (20%). These findings were in close agreement with Guven (2005).

Table 1. Cultural and biochemical characteristics of the isolated bacteria

Total samples	Sources of samples	Number of samples examined	Name of bacterial isolates	Number of isolates	Prevalence (%)		
					Escherichia coli	*Staphylococcus* spp.	*Bacillus* spp
30	Adult woman	5	*Escherichia coli*	5	80	14	6
			Staphylococcus spp.	2			
			Bacillus spp.	1			
	Adult man	5	*Escherichia coli*	5			
			Staphylococcus spp.	1			
			Bacillus spp.	0			
	Diabetic woman	5	*Escherichia coli*	5			
			Staphylococcus spp.	1			
			Bacillus spp.	0			
	Diabetic man	5	*Escherichia coli*	5			
			Staphylococcus spp.	0			
			Bacillus spp.	1			
	Healthy woman	5	*Escherichia coli*	5			
			Staphylococcus spp.	1			
			Bacillus spp.	0			
	Healthy man	5	*Escherichia coli*	5			
			Staphylococcus spp.	0			
			Bacillus spp.	0			

Table 2. Antibiotic sensitivity pattern of *Escherichia coli*, *Staphylococcus* spp. *and Bacillus* spp.

Name of Bacterial isolates		Antibiotics and their percentage of sensitivity																							
		AMP				A				AZM				E				NA				P			
		H	M	L	R	H	M	L	R	H	M	L	R	H	M	L	R	H	M	L	R	H	M	L	R
Escherichia coli	No	2	1	2	0	1	1	2	1	1	1	2	1	0	0	2	3	4	1	0	0	1	0	1	3
	%	40	20	40	0	20	20	40	20	20	20	40	20	0	0	40	60	80	20	0	0	20	0	40	60
Staphylococcus spp.	No	2	3	0	0	2	2	1	0	0	1	2	2	3	2	0	0	0	0	3	2	1	1	2	1
	%	40	60	0	0	40	40	20	0	0	20	40	40	60	40	0	0	0	0	60	40	20	20	40	20
Bacillus spp.	No	1	0	2	2	0	2	2	1	2	1	2	0	2	3	0	0	1	0	2	2	0	1	1	3
	%	20	0	40	40	0	40	40	20	40	20	40	0	40	60	0	0	20	0	40	40	0	20	20	60

AMP = Ampicillin, A = Amoxycillin, AZM= Azithromycin, E = Erythromycin, NA= Nalidixic Acid,
P= Penicillin, H= Sensitive, M = Intermediate, L = Less sensitive, R= Resistant, %= Percentage

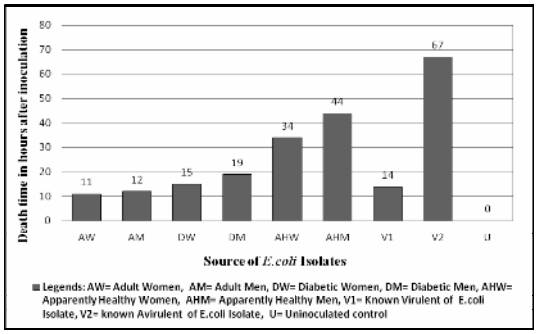

Fig. 1. Graphical representation of mean death time in hours of day-old suckling mice after experimental inoculation with different *E. coli* isolates.

From this study it was concluded that *Escherichia coli, Staphylococcus* spp. *and Bacillus* spp. were the predominant bacterial flora of human urine sample. Among the isolates, the prevalence of *E. coli* was highest (80%) compared to *Staphylococcus* spp. (14%) and *Bacillus* spp. (6%) in all samples. Among these isolated bacteria *E. coli* was alarming for human urinary tract particularly for aged woman because of their potential pathogenic nature. Pathogenicity of 6 representative *E. coli* isolates, one from each source was determined by performing lethality assay in day-old suckling mice models. Study revealed variable results indicating that the isolates were of highly virulent, moderately virulent and less virulent as well as avirulent catagories. Nalidixic Acid will be the first drugs of choice to treat the UTI caused by *E. coli* in human; erythromycin, ampicillin and azithromycin will be the second drugs of choice to treat the UTI caused by *Staphylococcus* spp and *Bacillus* spp.

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