Microbiological profile of symptomatic and asymptomatic bacteriuria recovered from female patients with urinary tract infection

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With the previous knowledge on the production of *β*-lactamase by the bacterial pathogens causing urinary tract infection, present study further investigated the presence of symptomatic and asymptomatic bacteriuria in female patients admitted into the Delta Medical College with suspected urinary tract infections (UTIs). The manifestation of uropathogens and their responses against locally available antibiotics (amoxicillin, 10 μg; cephradin, 30 μg; ciprofloxacin, 5 μg; cotrimoxazole, 23.8 μg; gentamicin, 10 μg; nalidixic acid, 30 μg) were inquired by means of conventional cultural techniques and double-disc diffusion methods, respectively. Among 110 urine samples collected from the patients with suspected UTI, 34 were found to be culture positive. Symptomatic (64.7%) and asymptomatic (36.3%) bacteriuria were noticed inside the puss cell of the UTI positive cases. *Escherichia coli* (73.5%) was the dominant bacteria while *Klebsiella* spp. (26.5%) was also exultant. Around 96% uropathogens were found to be sensitive against imipenem, and 75% against amikacin. *E. coli* was found to be sensitive against all of the antibiotics used, whereas *Klebsiella* spp. was found to be 100% resistant against nalidixic acid and cotrimoxazole.

Key words: Urinary tract infection; Pathogens; Antibiotic resistance; Symptomatic; Asymptomatic

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Urinary tract infections (UTIs) are already known to be the most frequently encountered bacterial illness with a relatively higher frequency of onset among women (1-6). On an average of 50% of females manifest symptoms of UTI at certain stage during their lives because of their physiological pattern of anus and urethral meatus (6-10). The prevalence of infection also varies especially with the age and sex of the individual (6, 11, 12).

The most frequent uropathogen is known to be the Gram negative bacterium *Escherichia coli* which have been reported to cause 80% of the community-acquired UTI and 40% of the healthcare-associated UTI (6, 13). Other common uropathogens include *Klebsiella pneumoniae*, *Enterococcus* spp., *Pseudomonas aeruginosa*, *Candida* spp., *Proteus* spp. and *Enterobacter* spp. (5, 6, 14, 15). The medication strategies of UTI have also been linked to the identification of the drug-resistance traits of the uropathogens against the commonly used antibiotics (5, 6, 10).

Recent studies conducted in Bangladesh also revealed that 12% of the rural women were suffering from asymptomatic bacteriuria (10). Our earlier investigation revealed the prevalence of uropathogens and their antibiotic resistance patterns as well as their extended-spectrum *β*-lactamase activity (5, 6). The current investigation further improvised the study of UTI in Bangladesh through (i) the manifestation of UTI among female patients admitted into the Delta Medical College based on their age and (ii) the responses of isolated uropathogens against locally available antibiotics commonly prescribed by the physicians.

MATERIALS AND METHODS

Study population. The study was conducted at Department of Microbiology, Delta medical college and Hospital. Patients who were prescribed by doctors for undergoing tests for UTIs were included in the study. A total of 110 randomly chosen female patients of different sex groups were included in this study (Table 1).

Ethical consideration. Ethical permission was not required in the study as the patients were prescribed by the doctors to undertake the UTIs test.

Isolation and identification of uropathogens. After collecting the specimens aseptically (16), physical and chemical examinations were carried out (data not shown), then samples were centrifuged and examined microscopically (Optima Biological Microscope G206, manufactured in Taiwan) (6).

For bacteriological analysis, Nutrient agar (NA), MacConkey agar (MAC) and Erosin Methylene Blue (EMB) agar plates were used. By means of a calibrated loop of 3 mm diameter, the appropriately labeled media plates were streaked aseptically with an aliquot of 0.01 ml urine sample. The plates were then incubated aerobically at 37 °C for 12-24 hours. Bacteriological identification was done by the examination of the overnight culture and Gram-staining. Standard biochemical tests were performed also to identify the bacteria of interest (5, 6, 17).

Antibiotic susceptibility test. Agar disc diffusion assay was used to determine the bacterial susceptibility towards different antibiotics (amoxicillin, 10 μg; cephradin, 30 μg; ciprofloxacin, 5 μg; cotrimoxazole, 23.8 μg; gentamicin, 10 μg; nalidixic acid, 30 μg) *in vitro* as described previously (5, 6, 18, 19). The zone diameters for individual antimicrobial agents were translated into susceptible and resistant categories (20).

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RESULTS AND DISCUSSION

Prevalence of UTI according to age. Out of 110 patients, 34 (30.9%) patients were found to bear UTI. Women in the age group 21-30 years were most likely to suffer from UTI (72.7%) above than the age group of 10–20 years (Table 1). Noor et al. (6) reported most of the occurrence of UTI among 16-45 aged females. Such infections might occur through their shorter and closer urethra, which is indeed corroborating with the previous suggestive reports (6-10).

Microbiological profile of UTI. All the UTI positive samples were shaded with substantial number of total viable bacteria of 10^5 cfu/ml or more (Table 2). Among 34, 22 (64.7%) UTI positive cases experienced symptomatic bacteriuria with recovery of 5-9 pus cells/high power field. Other 12 (35.3%) UTI cases were found to have asymptomatic bacteriuria (Table 2). Notably, the presence of symptomatic and asymptomatic bacteriuria inside the pus cells (Table 3) accord the possibility of pyonephrosis (21).

TABLE 1. UTI positive cases among different patients' age group

Age group	Patients	
	Tested (n)	UTI positive (%)
10-20 years	22	4 (11.8%)
21-30 years	22	16 (72.7%)
31-40 years	22	8 (36.4%)
41-50 years	22	6 (27.3%)
51-60 years	22	0 (0%)
Total	110	34 (30.9%)

isolated in this study belonged to the *Enterobacteriaceae* family (Table 3). *E. coli* was identified as the most common causative agent of UTIs (73.5%) over *Klebsiella* spp. (26.5%), which was in consistence with the previous reports (5, 6).

Drugs resistance profile of the bacterial isolates causing UTI. The drug resistance and sensitivity profile of the microorganisms showed that *E. coli* isolates were sensitive to amoxicillin 10 μg (80%); nalidixic acid 30 μg (64%), ciprofloxacin 5 μg (24%); gentamicin 10 μg

TABLE 2. Number of viable bacteria and pus cells, and determination of symptomatic and asymptomatic bacteriuria in UTI positive samples

No. of UTI positive sample	Total viable bacteria (cfu/ml)	Number of pus cells (cell/hpf)	Symptomatic bacteriuria (64.7%)	Asymptomatic bacteriuria (35.3%)
01	1.3×10^5	6-9	+	-
02	1.8×10^6	5-7	+	-
03	2.3×10^5	6-8	+	-
04	3.1×10^5	5-8	+	-
05	2.8×10^6	6-9	+	-
06	1.2×10^5	6-9	+	-
07	1.7×10^5	2-3	-	+
08	1.9×10^6	5-8	+	-
09	4.1×10^5	2-3	-	+
10	3.2×10^5	2-3	-	+
11	1.3×10^6	2-3	-	+
12	2.2×10^5	6-9	+	-
13	1.5×10^6	5-7	+	-
14	5.1×10^5	6-8	+	-
15	4.8×10^6	2-3	-	+
16	7.8×10^5	2-3	-	+
17	5.3×10^5	2-3	-	+
18	1.6×10^6	6-9	+	-
19	1.8×10^5	5-7	+	-
20	2.9×10^6	6-8	+	-
21	1.3×10^5	6-8	+	-
22	5.2×10^5	5-8	+	-
23	1.9×10^6	6-9	+	-
24	1.6×10^6	6-9	+	-
25	1.3×10^5	6-8	+	-
26	3.4×10^4	5-8	+	-
27	2.9×10^5	6-9	+	-
28	2.1×10^6	6-9	+	-
29	7.1×10^5	6-8	+	-
30	3.1×10^5	2-3	-	+
31	1.9×10^5	2-3	-	+
32	1.3×10^5	2-3	-	+
33	1.6×10^5	2-3	-	+
34	1.4×10^5	2-3	-	+

hpf = High power field; (+) = Positive; (-) = Negative

TABLE 2. Identification and confirmation of the causative agents of urinary tract infections

Isolate No.	TSI			Citrate	Gram staining	Colony Characteristics on EMB agar	Suspected microorganisms
	Slant	Butt	Gas				
01	Y	Y	+	+	Gram negative rods	Green Metallic sheen	*E.coli*
02	Y	Y	+	-	Gram negative rods	Green Metallic sheen	*E.coli*
03	Y	Y	+	+	Gram negative rods	Green Metallic sheen	*E.coli*
04	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E.coli*
05	Y	Y	+	+	Gram negative rods	Glossy pink	*Klebsiella* spp.
06	Y	Y	+	-	Gram negative rods	Green Metallic sheen	*E.coli*
07	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E.coli*
08	Y	Y	+	+	Gram negative rods	Glossy pink	*Klebsiella* spp.
09	Y	Y	+	+	Gram negative rods	Glossy pink	*Klebsiella* spp.
10	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
11	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
12	Y	Y	+	+	Gram negative rods	Glossy pink	*Klebsiella* spp.
13	Y	Y	+	+	Gram negative rods	Glossy pink	*Klebsiella* spp.
14	Y	Y	+	+	Gram negative rods	Glossy pink	*Klebsiella* spp.
15	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
16	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
17	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
18	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
19	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
20	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
21	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
22	Y	Y	+	+	Gram negative rods	Glossy pink	*Klebsiella* spp.
23	Y	Y	+	+	Gram negative rods	Glossy pink	*Klebsiella* spp.
24	Y	Y	+	+	Gram negative rods	Glossy pink	*Klebsiella* spp.
25	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
26	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
27	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
28	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
29	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
30	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
31	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
32	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
33	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*
34	Y	Y	+	+	Gram negative rods	Green Metallic Sheen	*E. coli*

TSI = triple sugar iron test; Y = yellow (acidic); R = red (alkaline); EMB = Erosin Methylene blue
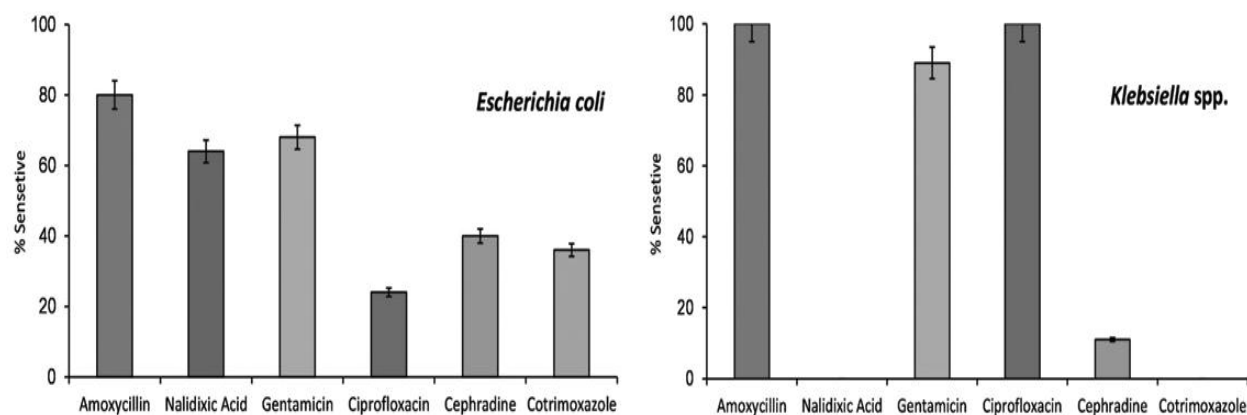

FIG. 1. Antimicrobial resistance pattern of *E. coli*, *Klebsiella* spp. against amoxycillin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), cephradin (30 µg), cotrimoxazole (23.8 µg). *E. coli* was found to be sensitive to some extent against all of the antibiotics used. Notably, *Klebsiella* spp was found to be 100% resistant against nalidixic acid and cotrimoxazole. The presented data were statistically analyzed by showing standard errors considered as 5%. All experiments were carried out three times and 95% accuracy was found.

cephradin 30 μg (40%); cotrimoxazole 23.8 μg (36%) (Figure 1), whereas *Klebsiella* spp. were found to be 100% resistant against nalidixic acid 30 μg; and cotrimoxazole 23.75 μg (Figure 1). Considerably, several studies in the developed and developing countries have revealed higher levels of resistance against the most commonly used antibiotic agents, possibly due to the dissemination of the drug-resistance genes within the infectious microorganisms (6, 18, 22-25).

CONCLUSION

Overall, the current investigation clearly showed the incidences of urinary tract infections are most frequent in female patients within the age group of 21-30 years admitted into the Delta Medical College. Moreover, encountering asymptomatic bacteriuria in pus cells of the tested population pictured severe health risk due to its unpredictable nature and appeal more attention from the public health sectors.

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