

Antibiotic Sensitivity of Bacteria Causing Urinary Tract Infection

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[Received: 1 January 2015; Accepted: 15 March 2015; Published: 1 June 2015]

Abstract

Background: Urinary tract infection is very common in both male and female. **Objectives:** The purpose of the present study was to see the antibiotic sensitivity pattern of isolated from urinary tract infected patients. **Methodology:** This cross sectional study was carried out in Dhaka Medical College Hospital, Dhaka for a period of 12 months. Clinically diagnosed cases of urinary tract infection irrespective of age and sex having pus cells ≥ 5 /HPF in the deposits of centrifuged urine were selected as study population. Data regarding organisms causing UTI and their antibiotic sensitivity patterns were collected. For urine culture the urine samples were inoculated on HiCrome UTI agar, CLED agar, 5% sheep blood agar and MacConkey's agar media with a calibrated loop having diameter of 1.45 mm which contains 0.001 ml of urine. The inoculation at 37° C for 24 hours and CFU count of 10^5 /ml of urine were considered positive for UTI. Identification of bacteria was done by standard biochemical techniques and their distinct colony characteristics. All the isolated organisms were tested for antimicrobial sensitivity against different antimicrobial agents by disc diffusion method on Mueller-Hinton agar plates. **Result:** Diagnosis of bacteria causing UTI with their sensitivity to different antibiotics was performed with a total of 300 samples from both male (38.66%) and female (61.33%) of different age groups. Among 300 samples 107 strains were isolated. Out of 107 identified strains, 95(31.67%) samples showed single growth and 6(2%) samples showed mixed growth. *Escherichia coli* (64.49%) was found to be the predominant organism. Regarding antimicrobial sensitivity pattern *Esch. coli* showed 98.55 to 63.77% sensitivity to imipenem, amikacin, ceftazidime and nitrofurantoin. Other isolated organisms showed 50 to 100% sensitivity to ceftazidime, amikacin, imipenem except *Klebsiella*, *Pseudomonas* and *enterococci spp.* which showed 40% and less sensitivity. **Conclusion:** In conclusion *Escherichia coli* is the most commonly isolated bacteria which is highly sensitive to imipenem. [Bangladesh J Infect Dis 2015;2(1):13-18]

Keywords: Antimicrobial sensitivity; urinary tract infection; bacterial pathogens

[How to Cite this article Parveen R, Yusuf MA, Sharmin I, Islam MS, Rahim I. Antibiotic Sensitivity of bacteria Causing Urinary Tract Infection. Bangladesh J Infect Dis 2015;2(1):13-18]

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Conflict of Interest: Authors have declared no conflict of interest.

Contributions to authors: RP has contributed from protocol preparation to manuscript writing as well as in data collection. MAY, IS, MSI & IR have prepared and have revised the manuscript.

Introduction

Urinary tract infection (UTI) is associated with multiplication of organisms in the urinary tract and is defined by the presence of more than 10^5 organisms per ml in a midstream sample of urine¹. It is estimated that about 35.0% of healthy women suffer symptoms of UTI at some time in their life². Urinary tract infection is caused mainly by normal bowel flora-principally *Escherichia coli*, responsible for $\geq 75\%$ of cases³. Other Gram negative *Enterobacteriaceae*, Gram positive *Enterococcus faecalis* and *Staphylococcus saprophyticus* are responsible for remainder of most commonly acquired UTI⁴. Nosocomial infections are frequently caused by *Enterococcus faecalis*, *Klebsiella* species, *Enterobacter* species, *Citobacter* species and *Pseudomonas aeruginosa*⁵. Urinary tract infection is the leading cause of Gram-negative sepsis in hospitalized patients⁶. Urinary tract infections are more common in women than in men though male over 60 years with prostatic hypertrophy are the exceptions⁷. Women are more prone to UTIs than men because in females, the urethra is much shorter & closer to the anus than in males⁸. Urine samples are among the most common specimen sent for microbiology studies. A large laboratory may examine 200-300 urine samples each day⁹. This heavy workload reflects the frequency of UTI both in general practice and in hospital settings and demands a cost effective method for the diagnosis of UTIs.

One of the most important and readily available laboratory tests in patients with suspected UTI is detection of pyuria. Pyuria is present in almost all symptomatic UTIs and its absence should strongly suggest another diagnosis. Thus, the quantification of pyuria is usually made on the basis of direct microscopic examination of urinary sediment from a centrifuged specimen¹⁰. White blood cells >5 /HPF is considered to be significant¹¹. The aim of the microbiology laboratory in the management of UTI is accurate and timely diagnosis with appropriate antimicrobial sensitivity testing thus reducing morbidity⁷. The increase in resistance of microorganisms to antimicrobial agents, especially in hospitalized patients needs identification of pathogens¹¹. Therapeutic decision should be based on accurate, up-to-date antimicrobial susceptibility pattern. Rapid and accurate diagnosis, along with early initiation of appropriate antibiotic therapy has great potential to minimize the risk of a poor outcome. It also reduces chronicity & drug resistance, decreasing patient's sufferings and financial expenditure⁸. For this reason, knowledge

of the etiological agents of UTIs and their antimicrobial resistance patterns in specific geographical locations may aid clinicians in choosing the appropriate antimicrobial empirical treatment. Thereby the study was undertaken to find out the most frequent causative organisms of UTI and to determine the antibiotic susceptibility patterns of microbial agents isolated from urine culture in order to facilitate better treatment and management of UTIs.

Methodology

This cross sectional study was carried out among out-patient department and in-patient department of Dhaka Medical College Hospital, Dhaka for a period of 12 months. Clinically diagnosed cases of urinary tract infection irrespective of age and sex having pus cells ≥ 5 /HPF in the deposits of centrifuged urine were selected for the purpose of the study during the study period. Patients having pus cells <5 /HPF in a centrifuged urine sample were excluded from this study. The necessary information was collected using a structured questionnaire to assess the study subject. Data regarding organisms causing UTI and their antibiotic sensitivity patterns were collected. All study subjects were advised to collect the mid-stream urine sample in wide-mouthed sterile containers. In case of female, they were instructed to clean the area around the urethral opening with clean water and collect the urine with labia held apart. Samples were processed within 1 hour of collection. For direct microscopy, 5 ml of urine was centrifuged at 1500-2500 rpm for 5 minutes. One drop of sediment was taken on a clean glass slide, covered with a cover slip and was examined under light microscope using 10 x and 40 x magnifications. The presence of pus cells ≥ 5 /HPF was considered to be significant pyuria⁸. For urine culture the urine samples were inoculated on HiCrome UTI agar, CLED agar, 5% sheep blood agar and MacConkey's agar media with a calibrated loop having diameter of 1.45 mm which contains 0.001 ml of urine. The inoculation at 37° C for 24 hours and CFU count of 10^5 /ml of urine were considered positive for UTI. Identification of organism was done by standard biochemical techniques¹¹ and their distinct colony characteristics. All the isolated organisms were tested for antimicrobial sensitivity against different antimicrobial agents by disc diffusion method on Mueller-Hinton agar plates¹². Antibiotic discs were purchased from commercial source (Oxoid Ltd, UK). Plate was dried in an incubator at 37° C for 30 minutes. With a sterile inoculating wire loop five

colonies of the test organisms were taken and were emulsified in 5 ml of sterile normal saline¹³. The turbidity of the inoculum in the test tube was adjusted by adding more bacteria or more sterile saline to turbidity equivalent to that of 0.5 McFarland’s nephelometric standard¹¹ which approximately corresponds to 1.5×10^8 organisms/ml. A sterile cotton swab was immersed in the bacterial suspension and the excess broth was removed by rotating the swab with firm pressure against the side of the tube. The swab was then streaked evenly on the dried surface of plate in 3 different plains by rotating the plate approximately 60° angle each time to get uniform distribution of the inoculums. A final circular motion was made around the rim with the swab. The inoculum was allowed to dry for 5 minutes at room temperature with the lid closed. The discs were then placed on the inoculated surface by a sterile fine tipped forceps 15 mm away from the edge of the petri dishes and having 25 mm gap in between two discs. Six discs were placed (90 mm petri dish). The plates were then inverted and were incubated aerobically at 37° C for 18-24 hours. Interpretation of results was done using the zone sizes. Zone of inhibition of growth produced by each drug was considered into the three susceptibility categories, namely sensitive (S), intermediate (I) and resistant (R). All bacteria were assayed against the following antimicrobial agents: amoxicillin (30 µg), Cephadrine (30 µg), cotrimoxazole (25 µg), cefotaxime (30 µg), ceftazidime (30µg), ceftriaxone (30µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), gentamicin (10 µg), doxycycline (30 µg), imipenem (10 µg), aztronam (30 µg), amikacin (30 µg).

Results

A total 300 clinically diagnosed UTI cases having pus cells ≥5/HPF were included in this study. Most of them (31.33%) were in the age group of 21-30 years (Table 1).

Table 1: Distribution of Positive Cases of UTI Causing In Different Age Groups (n=300)

Age group (years)	Frequency	Percentage
≤ 10	20	6.66
11-20	42	14.00
21-30	94	31.33
31-40	72	24.00
41-50	29	9.67
51-60	23	7.67
>60	20	6.67
Total	300	100.00

Among 300 samples, 95(31.67%) samples showed growth of single organism, 6(2%) samples showed mixed growth and 199(66.33%) samples yielded no growth (Figure I). Among 116 urine samples collected from males, 35(34.65%) samples showed growth of organism. 184 urine samples collected from females, 66(65.35%) samples yielded growth of organism. The difference was not statistically significant (p>0.05) (Table 2).

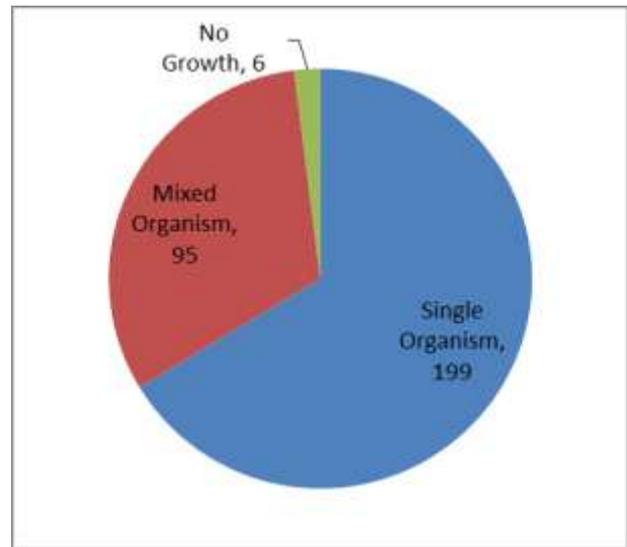


Figure I: Pie diagram shows the results of urine culture

Total 107 strains were isolated from 101 culture positive samples. Among these *Esch. coli* (64.49%) was the most common aetiologic agents followed by *Klebsiella* species (11.21%), *Pseudomonas* spp. (9.35%), *Enterococcus faecalis* (5.61%), *Proteus* species (3.74%), *Staphylococcus saprophyticus* (2.80%) and *Enterobacter* species (2.80%) in descending order (Table 3).

Table 2: Sex distribution of culture positive and culture negative cases among the study population (n=300)

Culture	Male	Female	Total
Positive	35(34.65%)	66(65.35%)	101(100%)
Negative	81(40.70%)	118(59.30%)	199(100%)
Total	116(38.67%)	184(61.33%)	300(100%)

$\chi^2 = 1.68; p > 0.05$

Regarding the sensitivity towards antibiotics, *Esch. coli* was found to be most sensitive to imipenem (98.55%) followed by amikacin (82.61%) and ceftazidime (82.61%) followed by cefotaxim (65.22%), nitrofurantoin (63.77%). *Esch.coli* was found to be least sensitive to doxycycline (11.59%), nalidixic acid (17.39%), amoxicillin(23.19%), cotrimoxazole (24.64%) and cephradine (24.63%).

High efficacy of imipenem (91.67%), amikacin (75%) followed by nitrofurantoin (66.67%) was observed against the *Klebsiella spp.* It was found that *Pseudomonas spp.* was found to be sensitive only to imipenem which was 90%. Amikacin and imipenem were highly effective (100%) against *Staphylococcus saprophyticus* followed by cefotaxim (75%), ceftazidime (75%), ceftriaxone (75%) and ciprofloxacin (75%). The antimicrobial sensitivity pattern was shown in Table 4.

Table 3: Isolation of Different Organisms among Culture Positive Cases (n=107)

Isolated Bacteria	Single	Mixed	Total
<i>Escherichia coli</i>	64	05	69(64.49)
<i>Klebsiella spp.</i>	11	01	12(11.21)
<i>Pseudomonas spp.</i>	08	02	10(9.35)
<i>Enterococcus spp.</i>	03	03	06(5.61)
<i>Proteus spp.</i>	04	00	04(3.74)
<i>Sta. saprophyticus</i>	03	00	03(2.80)
<i>Enterobacter spp.</i>	02	01	03(2.80)
Total	95	12	107(100)

Figures in parentheses represent percentage

Discussion

A total 300 samples of urine from clinically diagnosed UTI cases having pus cell ≥ 5 /HPF were examined. Of them, 95(31.67%) samples showed single growth, 6(2%) showed mixed growth and 199(66.33%) samples yielded no growth. From

101(33.67%) culture positive samples, 107 strains were isolated, of which 95 strains were from 95 samples of single growth and 12 (6x2) strains were from 6 samples of mixed growth.

In the present study, from 101 culture positive cases a total 107 strains were isolated. Of which, 69(64.49%) were *Esch. coli* followed by 12(11.21%) *Klebsiella spp.*, 10(9.35%) *Pseudomonas spp.*, 6(5.61%) *Enterococci spp.*, 4(3.74%) *Proteus spp.*, 3(2.80%) *Staph saprophyticus* and 3(2.80%) *Enterobacter spp.* Sharmin²² from Bangladesh reported *Esch. coli* as the predominant (53.2%) organism. Chowdhury et al¹⁵ reported 64%, Khaleque et al¹⁶ showed 63.4%, Talukder²⁹ showed 64% and Hossain et al¹⁷ showed 60% detected organism as *Esch. coli*. These findings are comparable with the findings of the present study. However, this study differs from study done by Bhuiyan and Abdullah¹⁸, Islam et al⁴ from Bangladesh and Hames and Rice¹⁹ from University of Oklahoma. They reported 92%, 73.8% and 90% *Esch. coli* from urine respectively. In the present study, the second most common organism was *Klebsiella spp.* (9.35%). Kawser²⁰ from Bangladesh worked on ICU patients of different hospital and found that the second most common organism was *Klebsiella* species (10.7%). This finding is in accordance with the finding of the present study but differ from Shahnaz et al²¹ who reported next common organism was *Pseudomonas spp.* (18%).

Table 4: Antibiotic Susceptibility Observed Among the Bacterial Species Causing UTI

Antibiotic against which susceptibility was observed	Bacterial Species Identified						
	<i>E. coli</i> (n=69)	<i>Klebsiella spp.</i> (n=12)	<i>Pseudomonas spp.</i> (n=10)	<i>Enterococci spp.</i> (n=6)	<i>Staph. saprophyticus</i> (n=3)	<i>Proteus spp.</i> (n=4)	<i>Enterobacter spp.</i> (n=3)
Amoxicillin	16(23.19)	0(0.0)	---	1(16.67)	1(33.33)	0(0.0)	0(0.0)
Cephadrin	17(24.63)	2(16.67)	---	0(0.0)	2(75.0)	1(25.0)	0(0.0)
Cotrimoxazole	17(24.64)	1(8.33)	--	1(16.67)	1(25.0)	0(0.0)	0(0.0)
Cefotaxim	45(65.22)	4(33.33)	4(40.0)	2(33.33)	2(75.0)	2(50.0)	2(75.0)
Ceftazidime	57(82.61)	5(41.67)	4(40.0)	2(33.33)	2(75.0)	2(50.0)	2(75.0)
Ceftriaxone	38(55.07)	4(33.33)	3(30.0)	2(33.33)	2(75.0)	3(75.0)	2(75.0)
Ciprofloxacin	28(40.58)	2(16.67)	3(30.0)	1(16.67)	2(75.0)	1(25.0)	1(25.0)
Nalidixic acid	12(17.39)	1(8.33)	---	1(16.67)	1(25.0)	1(25.0)	0(0.0)
Nitrofurantoin	44(63.77)	8(66.67)	---	4(66.67)	1(25.0)	2(50.0)	1(25.0)
Gentamicin	35(50.72)	3(25.0)	2(20.0)	2(33.33)	1(33.33)	0(0.0)	1(25.0)
Doxycycline	8(11.59)	0(0.0)	----	0(0.0)	1(25.0)	0(0.0)	0(0.0)
Imipenem	68(98.55)	11(91.67)	9(90.0)	6(100.0)	3(100.0)	4(100.0)	3(100.0)
Aztreonam	41(59.42)	5(41.67)	2(20.0)	3(50.0)	--	3(75.0)	2(75.0)
Amikacin	57(82.61)	9(75.0)	6(60.0)	3(50.0)	3(100.0)	2(50.0)	2(75.0)

Note: Figures in parentheses indicate percentage

Antibiogram of organisms in the present study showed varying susceptibility pattern. *Escherichia coli* and *Klebsiella spp.* showed higher sensitivity to

imipenem (91.67-98.55%) and amikacin (75 to 82.61%). It was observed that *Esch. coli* showed higher sensitivity against ceftazidime (82.61%) and

moderate sensitivity against cefotaxim (65.22%), nitrofurantoin (63.77%), ceftriaxone 95.07%) and gentamicin 95.72%), whereas *Klebsiella* species showed lower sensitivity to ceftazidime (94.67%), cefotaxim (93.33%) and ceftriaxone (93.33%). About 82.61% of *Esch. coli* was resistant to nalidixic acid followed by cotrimoxazole (75.36%) and ciprofloxacin (57.97%). Sharmin²² reported similar sensitivity pattern of *Esch. coli* and *Klebsiella spp.* against imipenem (99.9-100%), amikacin (70-75.5%), ceftazidime (89.7-45%), cefotaxim (66.6-40%), ceftriaxone (20-54%) and least sensitivity against other drugs. Ling et al²³ from China reported that 40.8% of *Esch. coli* was resistant to ciprofloxacin which was lower than the present study. Easin²⁴ reported similar findings. She observed that *Klebsiella* was highly resistant (70-100%) to all drugs except ciprofloxacin (100%) and imipenem (80%). Hossain et al¹⁷ reported, 43% of *Esch. coli* was resistant to ciprofloxacin and 29% to ceftriaxone. Study done in Holy Family Red Crescent Hospital²⁴ reported that 26% of *Esch. coli* were resistant against ciprofloxacin and 25% against ceftriaxone. Islam⁴ reported minimum resistance against ciprofloxacin (18.2%) and ceftriaxone (20.0%). Sharif²⁶ showed only 15% of *Esch. coli* were resistant to ceftriaxone and ciprofloxacin. Resistance pattern against ciprofloxacin and ceftriaxone studied by above mentioned authors were not consistent with the present study. Reason of these variations might be due to the fact that as they year's passing ciprofloxacin and ceftriaxone are becoming more resistant against *Esch. coli* probably due to over and irrational use and easy availability of the drug in our country. Mazzulli²⁷ from Canada, reported that only 1.8-2.3% of *Esch. coli* were resistant to ciprofloxacin. Reason might be due to rational use of drugs in their country and these drugs are not easily available.

In the present study *Pseudomonas spp.* showed higher sensitivity to imipenem (91.67%) and amikacin (75.0%). Least sensitivity was showed against ceftazidime (40%) and other antibiotics (20 to 30%). Sharmin²² and Chowdhury¹⁵ from Bangladesh reported similar sensitivity to above antibiotics. Contrary to the present study higher sensitivity to ceftazidime (62.5%) was reported by Kawsar²⁰. Similarly Wadud et al²⁸, Islam et al⁴ and Shahnaz et al²¹ from Bangladesh reported higher sensitivity pattern to ceftazidime, ciprofloxacin, ceftriaxone and gentamicin. *Pseudomonas* species can rapidly develop resistance especially when single drug is employed due to frequent mutations and its own innate mechanisms of antibiotic

resistance²⁴. In this study, *Enterococci*, *Enterobacter* and *Proteus* showed 100% sensitivity to imipenem. *Enterobacter* species showed 75.0% sensitivity to ceftriaxone and ceftazidime. *Proteus* species and *Enterococci* species showed 50% and 33.33% sensitivity to ceftriaxone and ceftazidime respectively. Contrary to our findings, moderate sensitivity against ceftriaxone (75%) and ciprofloxacin (50.0%) against *Enterococcus* were reported by Kawsar et al²⁰ and Shahnaz et al²⁵. The sensitivity pattern to various antimicrobial agents varies in different studies, in different parts of the same country at different times in the same hospital. This might be due to emergence of resistant bacteria caused by the indiscriminate use of antimicrobial agents²⁹.

In this study it was observed that 100% of *S. saprophyticus* were sensitive to imipenem and amikacin. Better sensitivity was observed to ciprofloxacin, ceftriaxone and ceftazidime. Similar findings were observed by Shahnaz et al²¹ from Bangladesh.

Conclusion

The prevalence of antimicrobial resistance among microorganisms that cause UTI is increasing worldwide and is a major factor in selecting antibiotics for treatment. Regular monitoring is required to establish reliable information about susceptibility pattern of urinary pathogens for optimal empirical therapy of patients with urinary tract infection. The emergence and spread of resistance can be reduced through appropriate and careful use of antimicrobial agents and increasing awareness among the population to the hazards of inappropriate antimicrobial use through public health education campaign.

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