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Original Article

Antimicrobial Susceptibility Pattern of Community Isolates of Urinary Tract Infection in Bangladesh

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Abstract

Background: *Overuses and injudicious uses of maximum antibiotics that have been prescribed empirically in community acquired UTI are being the prime cause of increasing resistance pattern among uropathogens in Bangladesh. There are minimum reports on antimicrobial resistance pattern among uropathogens in different regions of Bangladesh. No reports have been carried out to determine the response of uropathogens to fosfomycin yet in our country. So, the study aimed to determine the prevalence of uropathogens causing UTI in community and to determine the increasing resistance pattern among them with special emphasis on their ciprofloxacin and fosfomycin resistance status.* ***Methodology:*** *Identification of isolated bacteria was performed from collected 187 culture positive cases. Their antimicrobial resistance pattern was determined by Kriby- Bauer modified disc diffusion test. Minimum inhibitory concentration test of ciprofloxacin was performed among the ciprofloxacin resistant bacteria by agar dilution method.* ***Result:*** *The study demonstrated a higher prevalence of UTI in female (70.1%) than in male (29.9%). The most common isolated bacteria was E. coli (80.2%) followed by Klebsiellaspp.. Higher resistance rate of 171 Enterobacteriaceae to ampicillin and 3rd generation cephalosporins was observed 71.9% and 49.1%-59.7% respectively in all 6 districts of Bangladesh. Ciprofloxacin resistance has been found higher 43.9% and 50% among Enterobacteriaceae and Staphylococcus spp. More than 50% of ciprofloxacin resistant bacteria had MIC 64ug/ml. All the strains of Enterobacteriaceae were sensitive to fosfomycin except 20% Klebsiella.*

Conclusion: *Rising antimicrobial resistance pattern among uropathogens in different districts provide physicians an important information to make a discreet choice of antibiotics in empiric therapy of community acquired UTI in Bangladesh to reduce further evolution of multidrug resistant organisms.*

Key words: *Urinary Tract Infection; Clinical and Laboratory Standards Institute; American Type Culture Collection; Escherichia coli;Minimum Inhibitory Concentration.*

Introduction: Urinary tract infection is the second most common infection in community and the most common infection in hospital setting (Valiquette, 2001). Community acquired UTI is one of the most common problems for which patients seek medical attention to the physicians (Deshmuckh et al., 2017). It is estimated that about 150 million of UTI occur worldwide as many as 50% of women and 12% of men experienced at least one symptomatic UTI during their lives and about 25% of affected women have recurrent UTI (Begum, 2016).

The predominant etiology of UTI remains E. coli, Klebsiella, Staphylococci, Proteus, Enterococi, Enterobacter, Pseudomonas and Candida spp. (Erdem et al., 2018). Antibiotic therapy for uncomplicated community acquired UTI is usually treated empirically due to unavailability of culture facility and delay in obtaining laboratory results (ViKet al., 2018).

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Resistance patterns of antimicrobials have been increased in community acquired UTI in different regions of Bangladesh. Antibiotic susceptibility patterns of microorganisms can vary in different regions, might be due to prevailing resistant bacteria in different regions resulted from overuses and inappropriate uses of antibiotics. Moreover, High resistance rate among multidrug -resistant uropathogens to ciprofloxacin have been observed in developing countries (Fasugba et al., 2015, Gupta et al., 2001). So, the choice of drugs in the treatment of UTI is now becoming quite narrow. Multidrug resistant gram-negative bacilli causing community acquired uncomplicated UTIs still retain susceptibility to nitrofurantoin and fosfomycin (Gardiner, 2019). Several studies in Rajshahi and Dhaka of Bangladesh demonstrated that rate of resistance to nitrofurantoin is increasing (Haque et al., 2015; Nahar et al., 2017). As fosfomycin is not started in treatment of UTI, its susceptibility appears to be better than nitrofurantoin.

Due to an increasing therapeutic failure observed in empirical treatment, it has become important to identify the pattern of antibacterial resistance that can guide the therapeutic approach (Zhanel et al., 2006). So, the study was designed to identify the causative bacteria of community acquired UTI and to determine their resistance pattern against antimicrobial agents with special emphasis on fosfomycin and most used ciprofloxacin.

Materials and method:
This cross-sectional study was conducted in BIRDEM General Hospital, Dhaka, Bangladesh from September, 2018- August, 2019 and approved by ethics committee of BIRDEM Academy.

Samples: A total of 971 urine samples were collected from urinary tract infected patients of different age and sex attending at the outpatient departments of Vital Research Laboratory (Feni), Diabetic Association Medical College (Faridpur), Jahurul Islam Medical College (Kishorganj), Khaja Yunus Ali Medical College (Shirajganj), Satkhira Diagnostic center (Shatkhira) and Brahmanbaria Medical College (Brahmanbaria). History was collected and informed written consent was taken from patients by trained medical technologist from each institution respectively.

Laboratory procedures at district level:
The isolated bacteria from samples having both microscopy positive (> 5 pus cells/HPF) and culture positive (colony count: ≥ 1 X 105 cfu per ml) were preserved for this study. A few colonies of identified organisms from culture plate were inoculated on Mueller Hinton Slant in screw cap tubes. Inoculated tubes were incubated at 37°C aerobically for 24 hours and then stored in deep chamber (-10°c) of refrigerator. The inoculated bacterial strains in screw cap tubes were transported to BIRDEM General Hospital in ice pack box by courier within 2 weeks of isolation and preservation.

Laboratory procedures at BIRDEM microbiology laboratory:
Subculture was done in Blood agar and MacConkey agar media from inoculated strains of Mueller Hinton slant. These plates were aerobically incubated at 37°C for 24 hours and were checked for bacterial growth and forre-identification. The uropathogens were further identified by colony morphology, hemolytic criteria, staining character, pigment production and biochemical test as per standard techniques (Cheesbrough, 2006).

Antimicrobial susceptibility test:
The identified bacterial species were tested for antimicrobial susceptibility by Kriby-Bauer modified disc diffusion technique on Mueller Hinton agar media according to CLSI guideline 2017. Antibiotic tested for gram positive cocci were penicillin, amoxyclave, cefoxitin, ciprofloxacin, cotrimoxazole, linezolid, nitrofurantoin, vancomycin, teicoplanin. And for gram negative bacteria, ampicillin, cefuroxime, ceftazidime, ceftriaxone, cefotaxime, cefixime, cefepime, amoxiclave, amikacin, gentamicin, imipenem, ciprofloxacin, cotrimoxazole, nitrofurantoin, fosfomycin were tested. For pseudomonas spp., ceftazidime, aztreonam, gentamicin, amikacin, netilmicin, piperacillin-tazobactam, ciprofloxacin, cotrimoxazole, imipenem, fosfomycin were tested.

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ATCC strains E. coli 25922 and Pseudomonas aeruginosa 23853 and Staphylococcus aureus 25923 were used to assess the potency of the discs. The zone sizes were interpreted as sensitive, intermediate and resistant according to CLSI (2017). For vancomycin and teicoplanin disc Barry et al (1987) and for Fosfomycin disc Lu et al. (2011) were followed.

MIC of ciprofloxacin:

The MIC for 79 ciprofloxacin resistant isolates in Kriby-Bauer method was determined in Mueller-Hinton agar media containing ciprofloxacin in final concentrations of 1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5ug/ml. From each inoculum of ciprofloxacin resistant isolates containing 1 107cfu/ml, 1ul was placed on different antibiotic concentration impregnated Mueller-Hinton agar plate respectively. The MIC of ciprofloxacin for these strains was defined as the lowest concentration of antibiotic impregnated Mueller Hinton agar plates inhibiting no visible growth after 24 hours incubation at 37°C. Enterobacteriaceae, Pseudomonas aeruginosa and Staphylococcus spp. were regarded resistant to ciprofloxacin if MIC ≥4ug/ml (CLSI, 2017).

Results:

Table 1 shows that out of 971 urine samples, 187 (19.3%) urine cultures were positive. Isolation rate was lowest (14.4%) in Feni and highest (39.1%) in Brahmanbaria. Frequency of UTI in case of female (70.0%) was higher than in male (29.9%). The highest frequency was in the age group of >60 years (Table 2).

Table 1: Rate of culture positive UTI cases in selected laboratories

District	Total urine samplesN	N (%) of culture positive cases
Feni	298	43 (14.4)
Faridpur	158	32 (20.3)
Satkhira	75	18 (24.3)
Kishoreganj	280	46 (16.4)
Sirajganj	92	21 (22.8)
Brahmanbaria	69	27 (39.1)
Total	971	187 (19.3)

Out of 187 isolated bacteria, gram negative bacteria were 181 and gram-positive bacteria were 6. E. coli (80.2%) was the most predominant isolate followed by Klebsiella spp. (5.4%), Enterobacter spp. (5.4%) and Pseudomonas spp. (5.4%) (Table 3).

Table 2: Distribution of culture positive UTI cases according to age and gender (N=187)

Age group (in years)	Male N (%)	Female N (%)	Total N (%)
2m-10	15 (8.0)	17 (9.1)	32 (17.1)
11-20	2 (1.1)	10 (5.4)	12 (6.4)
21-30	6 (3.2)	34 (18.2)	40 (21.4)
31-40	5 (2.7)	20 (10.7)	25(13.4)
41-50	4 (2.1)	14 (7.5)	18 (9.6)
51-60	8 (4.3)	17 (9.1)	25(13.4)
>60	16 (8.6)	19 (10.2)	35 (18.7)
Total	56 (29.9)	131 (70.0)	187 (100)

m= months

Table 3: Pattern of organisms isolated from urine culture.

Patterns of Organisms	N (%)
E. coli	150 (80.2)
Klebsiella spp.	10 (5.4)
Enterobacter spp.	10 (5.4)
Pseudomonas spp.	10 (5.4)
Proteus mirabilis	1 (0.5)
Staph. aureus	1 (0.5)
Coagulase negative *Staphylococcus*	5 (2.7)

Table 4 shows all E. coli with high resistance pattern to cephalosporins, amoxiclave, ciprofloxacin and cotrimoxazole ranging from 29.3%-70.6% and low resistance pattrens to

Table 4: Antibiotic resistance pattern of Enterobacteriaceae

Isolates	% of Resistance AMPCFMCAZCROCTXCXMCPMAMCIPMAKCNCIPCOT												
E.coli (n=150)	70.6	48.0	48.0	50.0	59.3	53.3	29.3	53.3	1.3	7.3	10.0	**46.0**	46.0
*Klebsiella*spp. (n=10)	90.0	70.0	80.0	80.0	80.0	80.0	60.0	80.0	10	30.0	40.0	40.0	30.0
*Enterobacter*spp. (n=10)	70.0	50.0	50.0	50.0	50.0	60.0	10.0	50.0	0.0	20.0	30.0	10.0	20.0
Proteusmirabilis(n=1)	100	0.0	0.0	0.0	0.0	0.0	100	0.0	0.0	0.0	0.0	100	100

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Areas	% of All Resistant Enterobacteriaceae												
Urban areas (n=85)	72.9	62.3	63.5	61.1	62.3	62.3	25.8	62.3	3.5	11.7	17.6	47.0	37.6
Rural areas (n=86)	70.9	36.1	46.5	41.8	58.1	47.6	34.8	46.5	0.0	6.9	8.1	40.6	50.0
P value	0.77	0.00*	0.00*	0.01*	0.57	0.05*	0.21	0.03*	0.08	0.28	0.06	0.42	0.1

Abbreviations: Ampicillin (AMP), Cefuroxime (CFM), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefixime (CXM), Cefepime (CPM), Amoxiclave (AMC), Imipenem (IPM), Amikacin (AK), Gentamicin (CN), Ciprofloxacin (CIP), Cotrimoxazole (COT). $P < 0.05$ is statistically significant by chi-square test.

imipenem (1.3%) and amikacin (7.3%). Klebsiella spp. and Enterobacter spp. showed similarly high resistance pattern to cephalosporins, amoxiclave, ciprofloxacin and cotrimoxazole and low resistance pattrens to imipenem.Among the 6 peripheral laboratories, Feni, Faridpur and Satkhira were considered as suburban areas as the laboratories were situated in the district headquarters. The laboratories in Kishoreganj, Sirajganj and Brahmanbaria were considered as rural areas due to their situations in the rural areas. The antimicrobial resistance rate among enterobacteriaceae in suburban and rural areas is also summarized in table 4.The resistance patterns were observed comparatively higher against cefuroxime, ceftazidime, ceftriaxone, cefixime, and amoxiclave in suburban areas than rural areas ($p < 0.05$). However, the resistance rate to ampicillin, cefotaxime, cefepime, amikacin, ciprofloxacin, cotrimoxazole showed no significant difference ($p > 0.05$) between urban and rural areas.

Figure 1 showed low resistance of E. coli and Klebsiella spp. respectively 3.3% and 10% to nitro furantoin commonly used as empirical treatment. And all Enterobacteriaceae were also sensitive to fosfomycin except Klebsiella spp. (20%).

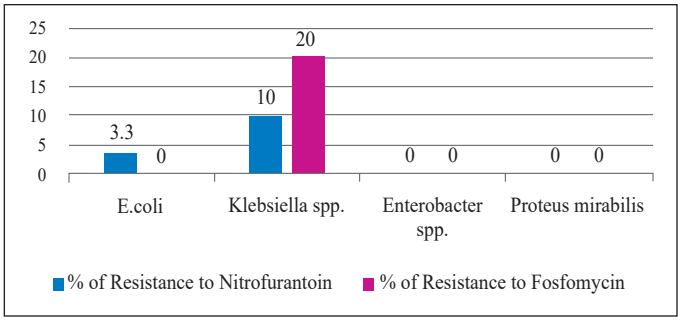

Figure 1: Nitrofurantoin and Fosfomycin Resistance Pattern of Enterobacteriaceae

Pseudomonas spp. showed resistanceto cotrimoxazole (60%), respectively to ceftazidime, gentamicin, and netilmicin 30%, respectively to piperacillin/tazobactam, ciprofloxacin and amikacin (20%), respectively to imipenem and fosfomycin 10%. And no sensitivity to aztreonam and colistin (Figure 2).

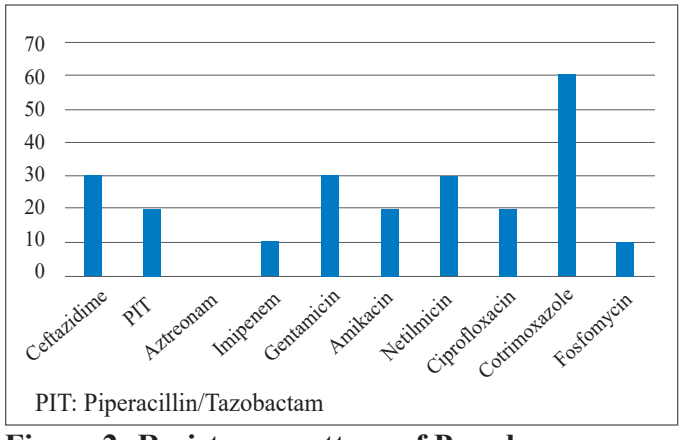

Figure 2: Resistance pattern of Pseudomonas spp. (n=10)

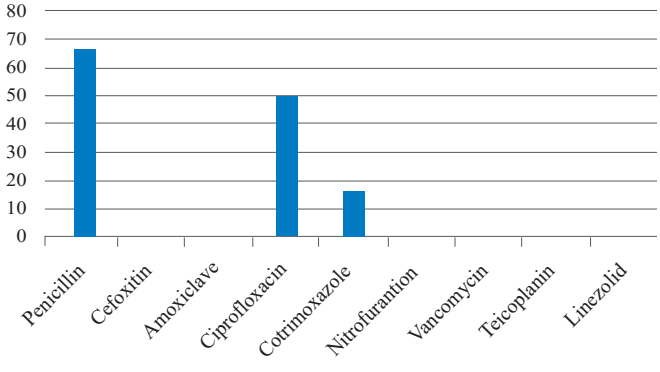

Figure3: Resistance pattern of Staphylococcus spp. (n= 6).

Staphylococcus spp. showed resistance to penicillin (66.7%), ciprofloxacin (50%), cotrimoxazole (16.7%) and showed sensitivity to amoxiclave, cefoxitin, nitrofurantoin, vancomycin, teicoplanin and linezolid (Figure 3).

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Table 5 represents MIC of ciprofloxacin was 0.5 µg/ml for one isolate, 1 µg/ml for 3 isolates, 2 µg/ml for 2 isolates, between 4-1024 µg/ml for 64 isolates among 70 ciprofloxacin resistant E. coli. Among 4 Klebsiella spp., MIC was 8 µg/ml for one isolate, between 256-1024 µg/ml for other isolates. MIC of ciprofloxacin was 64 µg/ml for one Enterobacter spp. and for two Pseudumonas spp., it was 32 ug/ml and 512 µg/ml respectively and for three CoNS it varied between 8 µg/ml to 16 ug/ml.

Table 5: MIC distribution among ciprofloxacin resistant urinary isolates (n= 80)

Ciprofloxacin resistant is olates	N of isolates with following MIC(µg/ml											
	0.5	1	2	4	8	16	32	64	128	256	512	1024
E.coli (n=70)	1	3	2	2	1	4	14	17	13	6	6	1
Klebsieblla (n= 4)	00	0	0	1	0	0	0	0	1	1	1	
Enterobacter spp. (n=1)	0	0	0	0	0	0	1	0	0	0	0	0
Pseudomonas spp. (n=2)	0	0	0	0	0	1	0	0	0	0	1	0
CoNS (n=3)	0	0	0	01	2	0	0	0	0	0	0	

CoNS= Coagulase negative Staphylococcus. Breakpoint used in interpretation of ciprofloxacin resistance (CLSI, 2017) for Enterobacteriaceae, Pseudomonas spp. and Staphylococcus spp.: Sensitive ≤ 1 µg/ml, Intermediate 2 µg/ml, Resistant ≥ 4 µg/ml

Among 79 ciprofloxacin resistant isolates in Kriby-Bauer disc diffusion method, 2 isolates have shown intermediately resistant, and 4 isolates have shown sensitive in MIC method.

Discussion:

UTI is the second most common diagnosis for which empirical antibiotics are prescribed in both primary and secondary care. More than 50% of the antibiotics prescribed for a suspected UTI in older adults being considered unnecessary (Crnich et al., 2017). As the antibiotic uses and changes relate the pattern of antimicrobial resistance, it is now more important to assess the management and outcome of UTIs (Gharbi et al., 2019). Antibiotic resistance in uropathogens is increasing worldwide. To decline the frequency of antibiotic resistance among bacteria, report about distribution pattern of microorganisms and their regional antimicrobial susceptibility rate is essential to guide the treatment choice.

In the present study, culture positive cases were 19.3% which is similar to data of 20.7% and 16.8% in Yasmeen et al. (2015) and Rahman et al. (2014). The occurrence of UTI was found to be more in female than in male and this is accordance with other findings (Odoki et al., 2019; Pardeshi, 2018; Abejew et al., 2014; Maliagno et al., 2012). This high prevalence of UTI in women is due to the proximity of the female urethra to the anus (Stamm and Norrby, 2001).

Rate of UTI in female was found highest (18.2%) in age group of 21-30 years followed by 10.7% in age group of 31-40 years and lowest (5.4%) in age group of 11-20 years. Similar pattern has been also reported in other studies (Odoki et al., 2019 and Dash et al.2013). The rate of UTI in male was found highest (8.6%) in age group of > 60 years that corresponds with the findings of Thattil and Santhosh (2018). Obstruction of outlet of urinary bladder due to benign prostatic hyperplasia is implicative for higher rate of UTI in male after 60 years of age (Griebling, 2005).

The most common uropathogens isolated were E. coli (80.2%) followed by Klebsiella spp. (5.4%), Enterobacter spp. (5.4%), Pseudomonas spp. (5.4%), Proteus mirabilis (0.5%) and coagulase negative Staphylococcus spp. (2.67%) that corresponds with the study of Nahar et al. (2017) in Bangladesh and Bajpai et al. (2014) in India.

E. colishowed high resistance pattern to cephalosporin (29.3%-59.3%), amoxiclave (53.3%), ciprofloxacin (46%) and cotrimoxazole (46%) and showed low resistance pattern to aminoglycosides (7.3%-10%), nitrofurantoin (3.3%) and imipenem (1.3%). Klebsiella spp. also showed high resistance to cephalosporins (60%-80%), aminoglycosides (30%-40%), ciprofloxacin (40%) and cotrimoxazole (30%) and low resistance to nitrofurantoin (10%) and imipenem (10%). Similar resistance pattern of E. coli and Klebsiella spp. has been also reported in other studies (Siddiqua et al., 2017; Prakash and Saxena, 2013; Iyamba and Wambale, 2014). High resistance rate among Enterobacteriaceae to commonly used antibiotics may have resulted from selective pressure as empiric treatment with ß-lactum drugs, ciprofloxacin and cotrimoxazole in community acquired UTI. The low resistance pattern of E. coli and Klebsiella spp. to nitrofurantoin and imipenem corresponds with the findings of other studies from Dhaka and Iran (Nahar et al., 2017; Yasmeen et al., 2015; Kashef et al., 2010).

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We observed higher resistance patterns against cefuroxime, ceftazidime, ceftriaxone, cefixime, and amoxiclave in suburban areas than that of rural areas ($p < 0.05$). Much more uses of these antibiotics results more evolution of resistant organisms in suburban areas. As the resistance rate to ampicillin, ciprofloxacin and cotrimoxazole showed no significant difference between suburban and rural areas($p > 0.05$). It determines frequent uses of these antibiotics both in suburban and rural regions.

Pseudomonas spp. were found to be highly resistant to cotrimoxazole (60%) but were less resistant to pipercillin/tazobactam (20%), ciprofloxacin (20%) and aminoglycosides (20-30%), imipenem (10%) which correlate with other study (Shah et al., 2015). Gram positive cocci were highly resistant to penicillin and ciprofloxacin with great sensitive to cefoxitin, amoxyclave, nitrofurantoin, vancomycin and linezolid. Lowest resistance to vancomycin, linezolid and nitrofurantoin also was reported in other studies (Erfani, 2015). So, vancomycin and linezolid followed by nitrofurantoin can be the potential drugs for treatment against gram positive cocci in community acquired UTI.

Ciprofloxacin is preferred as the initial agent for empiric therapy in UTI, because of its excellence activity against uropathogens (Schaeffer, 2003). Before 2000 a study reported lower resistance rate to ciprofloxacin (18%-30%) among uropathogens. More than 12 million urine specimens from US outpatient centers found that fluoroquinolone resistance rate of E. coli increased from 3% in 2000 to to 17.1% in 2010. Our study also demonstrated high resistance rate (40%-50%) of E. coli, Klebsiella, Enterobacter, Pseudomonas and Staphylococcus to ciprofloxacin. MIC of this ciprofloxacin resistant isolates to ciprofloxacin was evaluated and more than fifty percent ciprofloxacin resistant isolates had MIC $\geq$ 64ug/ml.

Knowledge of MIC will provide a physician valuable information for making a prescription with accurate and precise use of antibiotics. The MIC value is regarded to have the greatest importance in the optimization of targeted antibiotic therapy (Magréault et al., 2022). In this study the remarkably high MIC values for ciprofloxacin reflects the extent of high resistance pattern of organisms causing UTI in community. So, empirical therapy of ciprofloxacin may result treatment failure. Our study found low resistance pattern of isolated bacterial spp. to nitrofurantoin may be due to less uses in the treatment of UTI in the community.

Fosfomycin is available in oral preparation in Bangladesh since 2019 and may be a better choice of antibiotic for the empiric treatment of community acquired UTI. In our study all the strains of E. coli, Enterobacter spp. and Proteus mirabilis were sensitive to fosfomycin whereas a smaller number of Pseudomonas spp. (10%) and Klebsiella spp. (20%) were resistant to fosfomycin. Excellent susceptibility profile of bacteria causing UTI to fosfomycin also reported from other studies in Turkey, Korea, Canada, Greece and Italy (Gopichand et al. in 2019; Erdem et al, 2018; Kumar et al. in 2017; Karlowsky et al, 2014; Maraki et al. 2009; Marchese et al, 2003).

Conclusion:

Findings of our study including regional variability of antimicrobial resistance rate help the clinician of Bangladesh as a guidance in their treatment decision. As culture facility is not available in every district in Bangladesh, clinician should prescribe drug on basis of surveillance of antimicrobial drug resistance to minimize treatment failure. In conclusion, high MIC value of ciprofloxacin among maximum urinary isolates gives a fair idea about restricted uses of ciprofloxacin in future. Nitrofurantoin given orally was found to be effective for both gram-negative and gram-positive organisms. Fosfomycin was found to be highly potential antibiotic against gram negative organisms causing UTI in community on the other hand vancomycin and linezolid was found potential antibiotic against gram positive organisms. Amikacin can be a choice of injectable antibiotic as most of the common uropathogens are sensitive to it.

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