Current antimicrobial susceptibility pattern of uropathogens in a maternal and child health care hospital in Bangladesh

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Abstract

Introduction: Urinary tract infection is still one of the most common infections among all age groups. The causative microorgnisms of UTI and their sensitivity to different antibiotics varies in different areas, and changes with time. This necessitates periodic studies of the causative uropathogens and their antibiotic sensitivity pattern.

Aim: To observe the profile of common uropathogens and their antibiotic sensitivity patterns to commonly used antimicrobial agents.

Materials and Methods: A contemplative study was done at the department of Microbiology of Ad-din Women's Medical College, Dhaka (AWMCH), Bangladesh, during January to December, 2017. Clean-catch midstream urine samples were collected from 7139 suspected urinary tract infection patients of different age and sex groups. Uropathogens were identified by standard microbiological techniques and antimicrobial susceptibility pattern was determined by Kirby Bauer Disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines.

Result: In this study, Out of 7139 patients, 1664 (23.3%) were growth positive for urine cultures. Majority of the patients (88.5%, 6315/7139) were female. The predominant isolate was *E. coli* 712 (42.8%), followed by Coagulase negative Staphylococcus (CONS) 589 (35.4%), *Acinetobacter* 126 (7.5%), *Enterobacter* spp. 72 (4.3%), *Klebsiella* spp. 62 (3.7%), *Enterococcus* spp. & *Proteus* spp. 16 (1.9%). Imipenem (92%), amikacin (83.8%), piperacillin- tazobactum (85.4%), gentamycin (69.4%), levofloxacin (65.6%) shows higher sensitivity to Gram negative bacteria, whereas high resistance to ampicillin (17%), cephradin (11.8%), cotrim (26%) and amoxiclav (28%) were observed. On the other hand, Gram positive bacteria showed high resistance to nalidixic acid (70-95%), erythromycin (68-90%), and high sensitivity to nitrofurantoin, meropenem, vancomycin and linezolid. Vancomycin, linezolid and nitrofurantoin for Gram positive bacteria and amikacin, meropenem, pipercillin tazobactam, and colistin for Gram negative bacteria are still useful. As an empirical antibiotic against Gram negative organisms, amoxyclav is less effective as only 20% pathogens were susceptible. Both Gram positive and negative bacteria are highly resistance to quinolones, nitrofurantoin and cephalosporins with few exceptions.

Conclusion: Empiric antimicrobial agents should be selected on the basis of current antibiotic sensitivity pattern of the uropathogens prevalent in that area.

Keywords: Uropthogens, Urinary tract infection, Current Antimicrobial susceptibility pattern.

Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections encountered in medical practice today in all age groups of population,[1] which can also be considered as a major public health problem because of morbidity and financial loss. It also accounts for up to 40% of all hospital acquired infections.[2] In spite of tremendous improvement in the diagnosis and treatment of urinary tract infections (UTIs), these infections still remain a major clinical problem.

Most infections are caused by retrograde ascent of fecal flora to urinary bladder and kidney via urethra, specially in females with their shorter and wider urethra.[3] It is estimated that about 35% of healthy women suffer from symptoms of urinary tract infection at some point in their life. UTIs in female are also preceded by Vaginal colonization with uropathogens.[4] Moreover, they are susceptible to trauma during sexual intercourse and bacterial passage through urethra up to the bladder during pregnancy and delivery due to their urethral and vaginal anatomy.[5,6]

Gram negative bacilli are mostly responsible for urinary tract infection. Of them E coli is most common causative agent of UTI.[7] Other important causative Gram negative agents are *Klebsiella, Enterobacter, Citrobacter, Proteus and Serratia species*. The commonly isolated Gram positive pathogenic bacteria include Coagulase negative staphylococcus (*Staphylococcus epidermidis, Staphylococcus saprophyticus)* and *Enterococcus species* which results in UTI from their subsequent colonization of vaginal and perianal skin.[8]

The gastrointestinal tract is the major reservoir of *S. saprophyticus*. Association between UTI by *S. saprophyticus* and their rectal, vaginal, and urethral colonization was observed by Latham et al.[9] in an early study. It is the second most common cause of UTI after E.coli in females of reproductive age.[10]

Identification of the uropathogens and their antibiotic susceptibility pattern is usually observed by doing Urine culture. Empirical antibiotic therapy is often given to the patient before the laboratory results of urine culture are available for reduction of the existing symptoms and prevention of renal complications. Antibiotic resistance is one of the major causes of treatment failure in case of UTIs.[11] Indiscriminate use of antibiotics are responsible for emergence of resistant microorganisms to one or more of drug and gradual narrowing of scope for effective drugs to fight with bacterial infections including UTIs.[12] Moreover, the prevalence and pattern of antimicrobial sensitivity of

uropathogens are constantly changing with increasing uses of antimicrobial agents. So area-specific monitoring studies aimed to understand the types of uropathogens and their susceptibility patterns to various antibiotics may help clinicians to select proper empirical treatment.[13]

The aims of this study were A) to investigate microorganisms isolated from patients with UTI in a maternal and child health care based hospital and evaluate their in vitro susceptibility patterns to commonly used antimicrobial agents and B) to provide proper updates to the clinician and the hospital management about current antibiotic sensitivity pattern and help them updating antibiotic usage guidelines and policy.

Materials and Methods

A retrospective study on Urinary tract infection was done at the Microbiology laboratory of Ad-Din Women's Medical College Hospital (AWMCH) based on available laboratory data. This study was conducted from January 2017 to December 2017 on 7139 patients with requisition of urine culture and sensitivity from outdoor and indoor of different departments of the hospital.

Sterile disposable containers were used for collection of clean catch midstream urine samples (MSU) (4-5 ml) and transported immediately to the laboratory. Urine culture was done by semi quantitative method on MacConkey, 5% blood agar and cystine lactose electrolyte deficient medium (CLED) agar (Oxoid Ltd, Bashingstore, Hampire, UK) by using calibrated loops[14] and incubated in aerobic condition for 24 hours at 37°C. Routine urine microscopy of all urine specimens was done for counting white blood cell (WBC). Growth of microorganisms from culture was compared with the report of routine microscopy for diagnosis of UTI. If after 24 hour incubation no colony appears on culture media, they were further incubated for 48 hours. The isolates were identified and confirmed by using standard microbiological and biochemical tests like Gram staining, growth on selective media, colony morphology on culture media, lactose fermentation, indole, and citrate utilization, H_2S production, catalase, coagulase, oxidase, and urease test according to guidelines of WHO.[15]

The bacterial colonies were counted and multiplied by 100 to find out the number of bacteria present per milliliter of urine.[8] Growth of more than 10^5 organisms per millilitre is considered as infection. However, if the patient have prominent symtoms, a smaller number of single type of bacteria (10^2 to 10^4/ml) is also reported as infection. In urine specimens obtained by suprapubic aspiration or from patient with an indwelling catheter, few colonies of bacteria per millilitre has been reported as UTI. On the other hand, colony counts>10^5/ml of multiple species (≥3 types) are reported as contamination [16] and the samples were repeated.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (Merck, Germany) using disk diffusion (Kirby Bauer's) technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotic discs of ampicillin (Amp), cephradine (Ceph), cotrimoxazole (Cot), ciprofloxacin (Cip), nitrofurantoin (Nit), levofloxacin (Lev), nalidixic acid (NA), ceftriaxone (CTR), amoxiclav (AMC), cefixime(CXM), cefotaxime (CTX), gentamicin(Gen), amikacin (AK), ceftazidime (CAZ), meropenem (Mero), piperacillin-tazobactam (PIT), colistin (Col) were used for Gram negative bacteria and ampicillin (Amp), cephradine (Ceph), cotrimoxazole (Cot), ciprofloxacin (Cip), nitrofurantoin (Nit), levofloxacin (Lev), nalidixic acid (NA), cefotaxime (CTX), ceftriaxone (CTR), amoxiclav (AMC), gentamicin (Gen), ceftazidime (CAZ), amikacin (AK), meropenem (Mero), cefixime(CXM), oxacillin (Ox), cloxacillin (Clox), erythromycin (Ery), doxycycline (Do), vancomycin (Van), linezolid (Lz) were used for Gram positive bacteria. All antibiotic discs are obtained from Oxoid Ltd, Bashingstore, Hampire, UK.

Result

In this study, majority of the patients (88.5%, 6315/7139) were female (Fig. 1). Out of 7139 patients, 1664 (23.3%) showed positive urine cultures (Fig. 2) of which there were 1551 (93.2%) females and 113 (6.8%) males (Table-1). Among the growth positive cases, 61.7% (1028/1664) were infected by Gram negative bacilli while 636 (38.3%) cases were infected by Gram positive cocci (Table 3). Distribution of Gram reactive organisms isolated from urine samples are illustrated in Table 3. *E. coli* was the predominant isolates 712 (42.8%), followed by Coagulase negative Staphylococcus (CONS) 589 (35.4%), Acinetobacter 126 (7.5%), *Enterobacter* spp. 72 (4.3%), *Klebsiella* spp. 62 (3.7%), *Enterococcus* spp. & *Proteus* spp. 16 (1.9%).

The rates of susceptibility to 19 selected antimicrobial agents against Gram positive cocci and to 17 antimicrobial agents against Gram negative bacilli are summarized in Table 4 and Table 5, respectively.

In this study, Staphylococci were responsible for about 36% of UTIs cases; among these, *CONS* isolates were most frequently isolated. Gram positive bacteria showed high resistance to cephradin, nalidixic acid, erythromycin, and high sensitivity to nitrofurantoin, gentamicin, meropenem, vancomycin and linezolid. But interestingly, sensitivity of CONS towards ampicillin has a rising tendency (42.1%). (Table 4)

E. coli isolates, the predominant cause of UTIs, showed higher sensitivity to imipenem (92%), amikacin (83.8%), piperacillin- tazobactum (85.4%), gentamycin (69.4%), levofloxacin (65.6%), ciprofloxacin (62.1%) and nitrofurantoin (60%) and high resistance to ampicillin (17%), cephradin (11.8%), cotrim (26%) and amoxiclav (28%). *Klebsiella* strains displayed almost similar sucseptibility pattern as for *E. coli* and showed high susceptibility to imipenem, amikacin, gentamycin, levofloxacin and cotrimoxazole and high resistance to ampicillin, cephradin, amoxiclav and nalidixic acid, ceftazidime and cefuroxime. Sensitivity pattern observed in Enterobacter species are almost similar of the sensitivity pattern of *Klebsiella* spp. (Table 5).

Acinetobacter species show higher susceptibility to colistin (94%), imipenem (79%), piperacillin tazobactum (73%) and gentamicin (72%) and lower susceptibility to cephradine (10%), nalidixic acid (22%) and cefuroxime (19%). But an increased sensitivity of Acinetobacter spp to ampicillin is observed in the present study (41.3%).

On *Proteus* strains, cephradin, ampicillin, nitro-furantoin, and amoxyclav showed poor (6.5%, 25%, and 22%, respectively) and meropenem, amikacin, ciprofloxacin, levofloxacin, ceftazidime, cefotaxim, ceftriaxone showed good sensitivity (93%, 81%, 71%, 68% and, 64%, respectively). On the other hand *Pseudomonas* species is much more sensitive to colistin, meropenem, piperacillin-tazobactam, amikacin and gentamycin (88%,75%, 69% & 63% respectively) but less susceptible to cotrimoxazole, cefuroxime and ciprofloxacin (25%, 31% & 37%).

Table 1: Distribution of samples received (7139) from UTI Patients on the basis of sex and growth of micro organisms

Sex	Growth Positive	Percentages	Growth negative	Percentages
Male	113	6.8	711	12.9
Female	1551	93.2	4764	87.1
Total	1664	100	5475	100

Table 2: Distribution of isolated Gram reactive microorganisms from total (7139) urine samples in 2017

Gram Reaction	Microorganisms	No	Percentages
Gram positive organism	CONS spp	589	35.4
	Enterococci	31	1.9
	Staphylococcus aureus	16	1
Total Gram positive organisms			**38.3**
Gram negative organism	E. coli	712	42.8
	Acinetobacter	126	7.5
	Enterobacter	72	4.3
	Klebsiella	62	3.7
	Proteus	31	1.9
	Pseudomonas	16	1
	Citrobacter	9	.5
Total Gram negative organisms			**61.7**

Table 3: Susceptibility pattern of Gram positive organisms causing Urinary tract infection

Antibiotics	CONS N=(589)	Enterococci N=(31)	Staph. Aureus N=(16)
	S (%)	S (%)	S (%)
Ampicillin	248 (42.1)	3(9.7)	0(0)
Cotrimoxazole	306 (52)	11(35.5)	9(56.3)
Ciprofloxacine	308 (52.3)	15(48.4)	8(50)
Nitrofurantoin	332(56.4)	12(38.7)	8 (50)
Nalidixic acid	34 (5.8)	10 (32.3)	5 (31.1)
Cefepime	154 (26.1)	435 (73.9)	5 (31.1)
Levofloxacine	320 (54.3)	12 (38.7)	11 (68.8)
Ceftriaxone	287(48.7)	15(48.4)	10 (62.5)
Cefotaxime	315(53.5)	4(12.9)	9 (56.3)
Amoxyclav	394(66.9)	17(54.8)	11(68.8)
Oxacillin	203(34.5)	(NU)	7(43.8)
Cloxacillin	184 (31.2)	(NU)	7(43.8)
Doxycycline	274 (46.5)	(NU)	8(50)
Erythromycin	82(13.9)	(NU)	5(31.3)
Amikacin	139(23.6)	17(54.8)	8(50)
Meropenem	407(69.1)	19(61.3)	10(62.5)
Gentamicin	392(66.6)	12(38.7)	8(50)
Vancomycin	572(97.1)	26(83.9)	14(87.5)
Linezolid	581(98.6)	31(100)	16(100)

NU= Not used

Table 4: Susceptibility pattern of Gram negative organisms isolated from Urinary tract infection

Antibiotics	**E. Coli (N=712)**	**Acinetobacter (N=126)**	**Klebsiella (N=62)**	**Enterobacter (N=72)**	**Proteus (N=31)**	**Pseudomonas (N=16)**	**Citrobacter (N=9)**
	S(%)	S(%)	S(%)	S(%)	S(%)	S(%)	S(%)
Ampicillin	124(17.4)	52(41.3)	13(21)	11(15.3)	8(25.8)	(NU)	1(11.1)
Cephradine	84 (11.8)	13 (10.3)	12 (19.4)	4 (5.6)	2 (6.5)	(NU)	(NU)
Cotrimoxazole	189 (26.5)	68 (54)	42 (67.7)	38 (52.8)	15 (48.4)	4 (25)	4(44.4)
Ciprofloxacin	442 (62.1)	63(50)	35(56.5)	34(47.2)	22(71)	6(37.5)	2(22.2)
Levofloxacin	467 (65.6)	75 (59.5)	44 (71)	44 (61.1)	25 (80.6)	7 (43.8)	6(66.7)
Nitrofurantoin	428 (60.1)	56(44.4	24(38.7)	35(48.6)	7(22.6)	(NU)	6(66.7)
Nalidixic acid	232 (32.6)	28(22.2)	14(22.6)	14(19.4)	15(48.4)	(NU)	3(33.3)
Ceftriaxone	397 (55.8)	56(44.4)	21(33.9)	24(33.3)	20(64.5)	(NU)	7(77.8)
Cefotaxime	332 (46.6)	62(49.2)	28(45.2)	29(40.3)	21(67.7)	(NU)	5(55.5)
Amoxyclav	208 (29.2)	45(35.7)	29(46.8)	22(30.6)	7(22.6)	(NU)	(NU)
Cefuroxime	257 (36.1)	24(19)	14(22.6)	11(15.3)	14(45.2)	5(31.3)	2(22.2)
Amikacin	597 (83.8)	95(75.4)	51(82.3)	65(90.3)	25(80.6)	11(68.8)	7(77.8)
Meropenem	654 (91.9)	98(77.8)	58(93.5)	69(95.8)	29(93.5)	12(75)	8(88.9)
Gentamicin	495 (69.5)	91(72.2)	48(77.4)	52(72.2)	13(41.9)	10(62.5)	6(66.7)
Ceftazidime	310 (43.5)	53(42.1)	17(27.4)	18(25)	22(71)	7(43.8)	5(55.5)
Piperacillin Tazobactam	608 (85.4)	92(73)	(NU)	(NU)	(NU)	12(75)	(NU)
Colistin	(NU)	119 (94.4)	(NU)	(NU)	(NU)	14(87.5)	(NU)

NU= Not Used

Discussion

The complications related to urinary tract infection with the rising resistance against antimicrobial agents, are matter of worldwide concern. This study shows the distribution of microorganisms isolated from patients with UTI and their susceptibility pattern to various antimicrobial agents at Ad-din Women's Medical College Hospital (AWMCH) which provides mainly Maternal and Child health care.

In our study isolation rate of microorganisms from suspected UTI patients is 23.3% which is in agreement with report by Sheikh et al who have found 28.5% incidence rate, higher than the rate 8.06% reported in Iran[17] and lower than the rate of 31.35% and 66.78% significant bacteriuria recorded in India.[18,19] In Bangladesh Rezwana Haque found 42.66% and Khanam et al found 55.4% Growth positive cases in their study.[4,20]

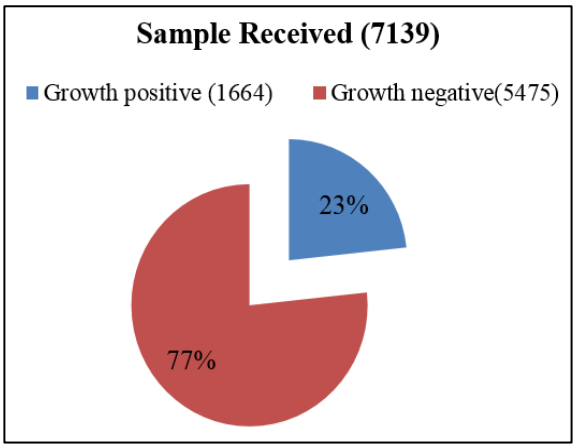

Fig. 1: Distribution of urine samples received in Year 2017

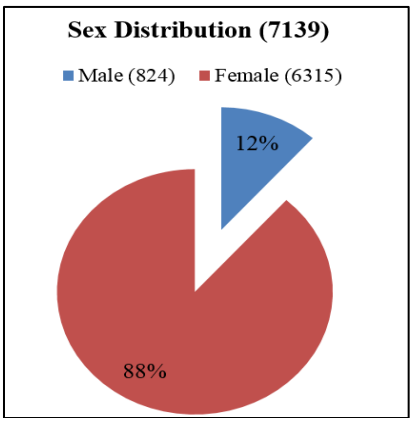

Fig. 2: Sex distribution of the received sample in 2017

Rate of UTI in our hospital is lower than other reports from different Hospitals of our country. As majority of the patients in the hospital are female and urine culture is advised here routinely as antenatal check up to pregnant women[21] this may be one reason of lower rate in our hospital.

Furthermore, 93.2% of the Growth positive cases are females in the present study. The sex distribution of patients in our study is consistent with those in other studies[18,22,23] showing a predominance of females (88.69% of the positive cultures). It has thought that, ascending infection occur in female patient because of the short urethra. Moreover, women used some bad practices such as cleaning perineum forward from the anus to the vulva[22] that can also cause urinary tract infection. Sexual activity has also been reported as a causative factor for higher prevalence of UTI in females.[25] Males have longer urethra and some antimicrobial substances in prostatic fluid, so they are less prone to UTIs.[26]

Our study indicates that *E. coli* is still the most common cause of UTI in Bangladesh. This corresponds with the data obtained by other investigators.[4,19,26] In addition, coagulase negative *Staphylococcus spp.* was the most common cause of UTI among Gram positive bacteria. Some recent studies have illustrated the importance of *coagulase negative Staphylococcus spp.* in urinary tract infections.[28,29]

Acinetobacter with the rate of 7.5% was the third species that caused UTI. The increasing trend of UTI by Acinetobacter spp. indicates hospital acquired infection specially in those patients who have urinary catheter in situ because of their strong biofilm production along the catheter that ascends into the bladder along both the internal and external catheter surfaces.[30,31] A feasible hospital antibiotic policy and strict maintenance, rigorous surveillance and good hospital infection control program are needed to control the increasing incidence of highly resistant Acinetobacter infections.

In Our study, as with previous studies, *E. coli* demonstrated a very high microbial resistance to antibiotics. The analyzed results of antibiotic susceptibility test showed that *E. coli* was least sensitive to ampicillin (17%), cephradin (11.8%), cotrimoxazole (26%) and amoxiclav (28%). On the other hand, the organism is highly sensitive to imipenem (92%), amikacin (83.8%), piperacillin-tazobactum (85.4%), gentamycin (69.4%), levofloxacin (65.6%), ciprofloxacin (62.1%) and nitrofurantoin (60%). This is similar to previous studies in Bangladesh, India, pakistan and Iran.[8,18,29] Nitrofurantoin is still showing good susceptibility (60%) but it has a decreasing trend than other studies.[4,19] *Klebsiella and Enterobacter* strains displayed almost similar sucseptibility pattern as for *E. coli* but with a decreasing sensitivity to nitrofurantoin and ciprofloxacin, but good sensitivity to levofloxacin (Table-4). But other studies in our country show variable susceptibility of Gram negative organisms.[4,20,27]

In the present study Gram negative bacteria showed increased susceptibility towards Aminoglycosides than other reports in our country.[4,20] As gentamycine is available in only injectable form it is not suitable for empirical therapy in UTI. This may be the cause of increasing gentamycine sensitivity. Moreover amoxicillin-clavulanic acid is found less effective among Gram negative organisms which co-inside with study done by Jafri et al.[33]

In the present study, the *coagulase negative Staphylococcus* showed increased resistance to cephradin, nalidixic acid and erythromycin, respectively. Results showed that these bacteria was highly susceptible to gentamicin, amoxicillin-clavulanic acid, meropenem, vancomycin and Linezolid that corresponds with other study in our region and other parts of the world.[8,20,29,34] So the Vancomycin and linezolid can be used as drug of choice in against UTI caused by Gram positive cocci. But interestingly, sensitivity of CONS towards ampicillin has a rising tendency (42.1%) which is probably attributed to prolonged cessation of use and no available commercial oral preparation in our country. All isolated Gram positive organisms shows good sensitivity to vancomycin and Linezolid.

The effective drugs for UTI are levofloxacin, amikacin and imipenem and piperacillin-tazobactum in our country.[20] They are also recommended in some other studies.[33,35] Low resistance to these drugs was observed because they're relatively expensive compared to others and are not readily available. Thus, these drugs could be considered as alternative options in empirical treatment of UTIs.[35]

In clinical practice, the β-lactamase inhibitors are often administered in combination with β-lactam antibiotics to extend the spectrum of antibacterial activity of the antibiotics. Tazobactam inhibits a broad range of plasmid mediated and chromosomal bacterial β-lactamases and is the most active of currently available β-lactamase inhibitors. The combination of this agent with piperacillin, a β lactamase-sensitive antibiotic, expands the activity of piperacillin to β-lactamases producing microorganisms, including Enterobacteriaceae.[35] Isolates in this study were highly sensitive to PIT (75-85%).

Ciprofloxacin and Nitrofurantoin were considered as a remedy to UTI but recently efficacy of these drugs are decreasing day by day. So, careful uses of these drugs should be ensured so that, resistance rates to these antibiotics for UTIs do not increase.

All uropathogens in the present study showed a high resistance to cephalosporins (Cephradin, and ceftriaxone) (50-90%), while other studies have reported a comparatively lower resistance.[36-38] The high resistance to cotrimoxazole, amoxyclave and cephalosporins in the present study indicate the easy access and indiscriminate use of these drugs for all kinds of infections.

Selective drug pressure is responsible for emergence of drug-resistant mutants. Moreover, use of antibiotics (Cotrimoxazole, Ciprofloxacin) in livestock for growth promotion is another important cause for development of resistance towards commonly used antimicrobials.[39] So these drugs should not be considered as first-line therapy for the empiric treatment of UTI.

However in the current study we observed a generalized drug resistance to commonly used antibiotics particularly among Gram negative isolates. Increasing tendency of UTI is observed by known nosocomial pathogens like Acinetobacter and CONS. So careful monitoring of their antibiotic susceptibility pattern is necessary for reduction of treatment failure rate.

There are significant geographic differences in the susceptibility of commonly used antimicrobials against uropathogens.[40,41] So, accurate knowledge on local epidemiology and antimicrobial resistance pattern of organisms causing UTI is essential to design effective therapy. Annual determination of bacterial sensitivity pattern in a particular area as a guideline is also recommended. In Bangladesh, dispensing of antibiotics are not restricted to prescription only, rather they are available over the counter.[42] Evidence suggests that, if any antibiotic is used for a short time in the locality and withdrawn for some time, it brings remarkable changes in resistance

pattern.[43] Besides, gradual increase in antibiotic resistance demands establishment of Antimicrobial stewardship programs (involving pharmacists, physicians and other healthcare providers).[43,44]

One of the important limitation of our study is we could not confirm the hospital acquired and community acquired UTI. Species of all bacteria could not be identified because of inadequate laboratory settings. Moreover, MIC method was not done for antibiotic susceptibility testing.

Conclusion

In a nutshell, E,coli and CONS are the common pathogen causing UTI in our hospital. But infection with Acinetobacter is increasing day by day. Levofloxacin, amikacin, meropenem and piperacillin tazobactam are potential drug for UTI caused by Gram negative bacteria whereas, vancomycin and linezolid are effective against UTI caused by Gram positive cocci.

Acknowledgements

We are grateful to all Technician and the staffs of Microbiology Department, Laboratory Division, AWMCH.

Conflict of Interest: None.

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How to cite this article: Saha R, Afzalunnessa Binte Lutfor, Arpita Deb, Taskina Akhter, Tamanna Sultana. Current antimicrobial susceptibility pattern of uropathogens in a maternal and child health care hospital in Bangladesh. *Indian J Microbiol Res* 2019;6(2):135-41.