ORIGINAL ARTICLE

Evaluation of bacterial profile, antibiotic susceptibility patterns, detection of ESBL and MBL producers and identification of quinolone and colistin resistance genes among the patients of catheter associated urinary tract infection (CA-UTI) in ICU of a tertiary hospital in Bangladesh

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Abstract:

Background: In ICU, catheter associated urinary tract infections (CA-UTI) are considered as principal threats to patient's health and are the leading cause of morbidity and mortality. It is superimposed by the emergence of multi drug resistant (MDR) organisms which ultimately leads to social and economic burden on the community. **Methodology:** Samples were collected from clinically suspected patients of CA-UTI in ICU. Sample screening, bacterial isolation and antibiotic susceptibility were done by standard procedures. Quinolone, colistin, imipenem and tigecycline resistance genes were identified by PCR. **Result:** One hundred fifty urine samples were taken from clinically suspected CA-UTI patients of ICU. CA-UTI was found in 72 (48%) after standard primary screening. Among the common pathogens 30 (41.67%) were E. coli, 20 (27.78%) were Pseudomonas spp., 13 (18.05%) were Klebsiella spp., and 1 (1.38%) coagulase negative Staphylococcus (CoNS) were found. In this study, colistin showed the best sensitivity while ceftriaxone, ciprofloxacin amoxiclav and gentamicin were not promising against the isolated organisms causing CA-UTI in ICU of DMCH. **Conclusion:** The high proportions of CA-UTI in ICU highlights the need of regular antimicrobial surveillance program to prevent emergence of multi drug resistant (MDR) organisms.

Key words: Catheter associated urinary tract infection, Device associated infection.

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Introduction:

Catheter associated urinary tract infection (CA-UTI) has significant clinical and economic consequences and is associated with high mortality, even after controlling the illness and comorbidities.[1] Although, septicemia due to extension of the urinary pathogen into the blood is not common but if occurs, has a fatality of 32.8%.[2] CA-UTI in ICU varies from 8 to 21% of the total nosocomial infections in ICU.[3]

Recognized risk factors for CA-UTI includes older age, female sex, diabetes mellitus and prolonged catheterization.[4] It occurs commonly by bacteria or candida and is almost inevitable in patients who require urinary catheter for more than 5 days.[5]

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Common pathogens responsible for CA-UTIs in ICU includes E. coli, Pseudomonas spp., Proteus spp., Klebsiella spp., Enterobacter spp, CoNS and Candida spp.[6]

Methodology:

This was a cross-sectional study under the Department of Microbiology, Dhaka Medical College, during a period of 1st July 2015 to 30th June 2016. Protocol was approved by the research review committee (RRC) and ethical review committee (ERC) of Dhaka Medical College. Samples were taken from 150 clinically suspected patients of CA-UTI, screened by standard procedure and finally 72 samples were selected for this study. Antimicrobial susceptibility test was done by disk diffusion method following CLSI 2015.[7]

Clinical definition of CA-UTI:[8] Patients with urinary catheter for more than 48 hours plus at least two of the clinical features as fever (>38⁰C), urgency, frequency, dysuria, or suprapubic tenderness with no other recognized cause.

Sample collection:[9] At first, Foley's catheter was clamped 4 to 5 cm distal to its exit from the genital organ and waited for at least 15 minutes for collection of fresh urine. The site of puncture of the catheter was washed properly by 70% alcohol. Then a sterile syringe was inserted into the catheter at 45⁰ angle and 10 ml freshly passed urine was drawn.

Screening of samples:

Microscopic criteria:[10] 50 µl of well mixed uncentrifuged urine sample was placed on a clean, grease free, glass slide and covered with coverslip to examine under the high power (40X) microscope. Presence of 1 pus cell / 7 HPFs of uncentrifuged urine corresponds to 10^4 leucocytes / ml and was included in this study as a case of CA-UTI.

Microbiological criteria (Semi-quantitative culture):[11] Blood agar, chocolate agar and MacConkey's agar (MCA) plates were streaked in 3 sectors by well mixed urine samples using a sterile wire loop (contains .01 ml of fluid) and incubated at 37⁰C overnight. Growths were classified as rare, light, moderate or heavy. Only moderate and heavy growths were considered as significant growth.

Report	Number of colonies		
	1st Sector	2nd Sector	3rd Sector
Rare	<10	0	0
Light	≥10	<5	0
Moderate	≥10	≥5	<5
Heavy	≥10	≥5	≥5

Determination of MIC:[12] It was done by agar dilution method using imipenem stock solution prepared by mixing 100 ml distilled water with 500 mg imipenem injection to make a concentration of 5 mg/ml. Then, 50 ml sterile Mueller-Hinton agar (MHA) was impregnated with 10 µl, 20 µl, 40 µl, 80 µl, 160 µl, 320 µl, 640 µl and 1280 µl of imipenem stock solution to achieve concentration of 1 µg/ml, 2 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml, 32 µg/ml, 64 µg/ml and 128 µl/ml respectively. To obtain 104cfu/spot on the agar surface, 1:10 dilution of 0.5 McFarland turbidity of test inoculums was placed on MHA plates. After incubation at 37°C overnight, the lowest concentration of antibiotic impregnated MHA showing no visible growth was considered as the MIC of imipenem.

Detection of ESBL producers by double disc synergy (DDS) test:[13] Two discs, one containing ceftazidime (30 µ gm) and the other containing amoxicillin plus clavulanic acid (20 µgm + 10 µgm) were placed 20 mm apart (center to center) on MHA plate swiped with 0.5 McFarland turbidity of the test organism and incubated at 37⁰C for 24 hours. A clear extension of the edge of zone of inhibition around the ceftazidime disc, towards the amoxiclav disc was interpreted as ESBL producer.

Detection of MBL producers by Double disc synergy (DDS) test:[14] Two discs, one containing imipenem (10 µ gm) and the other soaked with 20 µl of Tris- EDTA (1 M Tris-HCL and 0.1 M EDTA, P^H 8.0) plus 20 µl of 1:320 diluted 2- mercaptopropionic acid (MPA) were placed 10

mm apart in MHA plate swiped with 0.5 McFarland turbidity of test the organism and incubated at 37^0C for 24 hours. Extension of the edge of inhibition of imipenem disc towards the Tris-EDTA-MPA disc indicates MBL producer.

Detection of MBL producers by combined disc (CD) assay:[15] Two imipenem discs (10 µgm) were placed on MHA plate swiped with 0.5 McFarland turbidity of test organism. One of the 2 discs was impregnated with 5 µl of 0.5 M EDTA solution (750 µgm EDTA) and incubated at 370C for 24 hours. If the diameter of zone of inhibition around the imipenem-EDTA disc is ≥ 6 mm compared to that of the imipenem disc without EDTA, was interpreted as MBL producer.

DNA extraction:[16] Three hundred microliter distilled water was added with pellet in the eppendorf tube and vortexed. Then it was heated at 100^0C for 10 minutes in a heat block and was placed in an ice pack for 5 minutes. Finally, the eppendorf tube was centrifuged at 13000 rpm for 6 minutes at 4^0C and the supernatant was preserved at -20^0C for PCR.

Primers of this study

Quinolone resistance genes

Gene		Sequence (5`-3`)	Size (bp)
QnrA	F	ATTTCTCACGCCAGGATTTG	
	R	GATCGGCAAAGGTTAGGTCA	516
QnrB	F	GATCGTGAAAGCCAGAAAGG	
	R	ACGATGCCTGGTAGTTGTCC	469
QnrS	F	ACGACATTCGTCAACTGCAA	
	R	TAAATTGGCACCCTGTAGGC	417

Colistin resistance genes

Gene		Sequence (5`-3`)	Size (bp)
pmrA	F	ATGACAAAAATCTTGATGATTGA	
	R	TTATGATTGCCCCAAACGGTA	675
pmrB	F	GACTGATTTGGGGCACCTC	
	R	TGTTTCATGTAAATGTAAAACTTT	1304

MBL genes

Gene		Sequence (5`-3`)	Size (bp)
blaIMP	F	GGAATAGAGTGGTTAAYTCT	188
	R	CCAAACYACTASGTTATCT	
blaVIM	F	GATGGTGTTTGGTCGCATA	390
	R	CGAATGCGCAGCACCAG	
blaNDM-1	F	ACCGCCTGGACCGATGACCA	264
	R	GCCAAAGTTGGGCGCGGTTG	
blaKPC	F	CGTCTAGTTCTGCTGTCTTG	498
	R	CTTGTCATCCTTGTTAGGCG	

ESBL genes

Gene		Primer Sequence (5`-3`)	Size (bp)
*bla*TEM	F	TCGGGGAAATGTGCG	966
	R	TGCTTAATCAGTGAGGCACC	
*bla*SHV	F	AGGATTGACTGCCTTTTTG	392
	R	ATTTGCTGATTTCGCTCG	
*bla*CTX M- 15	F	CACACGTGGAATTTAGGGACT	996
	R	GCCGTCTAAGGCGATAAACA	
*bla*OXA- 1	F	ACCAGATTCCAACTTTCAA	598
	R	TCTTGGCTTTTATGCTTG	

Result:

In this study, of 48% CA-UTI cases detected in ICU of DMCH, isolated pathogens were E. coli 30 (41.67%), Pseudomonas spp. 20 (27.78%), Klebsiella spp. 13 (18.05%), Proteus spp. 6 (8.33%), Enterobacter spp. 2 (2.78%) and 1 (1.38%) were CoNS (Table-1 and 2).

Table-1: Bacterial profile among urine samples from catheterized patient of ICU (N = 72)

Bacteria	Total number of each bacteria n (%)
Escherichia coli	30 (41.67)
Pseudomonas spp	20 (27.78)
Klebsiella spp	13 (18.05)
Proteus spp.	6 (8.33)
Enterobacter spp.	2 (2.78)
Coagulase negative staphylococcus (CoNS)	1 (1.38)
Total	72 (100)

Of the 30 isolated E. coli in ICU, 86.67% were resistant to ceftriaxone, gentamicin and amoxiclav, 80% to ciprofloxacin, 70% to ceftazidime and 66.67% were resistant to piperacillin- tazobactam. Of the 20 isolated Pseudomonas spp., all were resistant to ciprofloxacin, 90% were resistant to ceftriaxone and tigecycline, and 85% to gentamicin, ceftazidime and amoxiclav. Among 13 Klebsiella spp. all were resistant to ceftriaxone, ceftazidime and amoxiclav, 76.92% to ciprofloxacin and gentamicin. Of the 6 Proteus spp., all were resistant to ceftriaxone, ciprofloxacin, colistin and tigecycline. Of the 2 Enterobacter spp. all were resistant to ceftriaxone. Only 1 CoNS was isolated

Table 2: Primary screening to detect CA-UTI (N=150)

Samples from suspected CA-UTI patients	Screening of samples				Total number of confirmed CA-UTI cases n (%)
	Microscopic criteria N = 150	Microbiological criteria (Semi-quantitative culture) N = 110			
	Rejected	Selected	Rare and light growth	Moderate and heavy growth	
150	40	110	38	72	72(48%)

which was resistant to ceftriaxone, ciprofloxacin and nitrofurantoin while sensitive to gentamicin, colistin, tigecycline, imipenem and cefoxitin. In this study, colistin showed the best sensitivity while ceftriaxone, ciprofloxacin amoxiclav and gentamicin were almost invalid against the isolated organisms causing CA-UTI in ICU of DMCH (Table-4) .

Table-3: Detection of Quinolone and Colistin resistance genes by PCR

Drug resistance genes	Detected genes		
Quinolone resistance genes	Qnr A N (%)	QnrB N (%)	QnrS N (%)
	13 (20.97)	27 (43.55)	33 (53.23)
Colistin resistance genes	Pmr A N (%)	Pmr B N (%)	Pmr A + Pmr B N (%)
	9(52.94)	5(29.41)	2 (11.76)

Of the 62 quinolone resistant isolates, 53.23% were positive for QnrS gene while 43.55% and 20.97% were positive for QnrB and QnrA genes respectively. Of the colistin resistance genes, pmrA was found in 52.94% while pmrB in 29.41% of the 17 colistin resistant isolates. Of the 60 ESBL producers, blaCTXM-15 was found in 75%, blaOXA-1 in 38.24%, blaTEM in 18.34% and blaSHV in 11.67% of the isolates. Among the different MBL genes, blaNDM-1 was found in 44.33%, blaVIM and blaKPC in 17.24% each and blaIMP in 13.79% of the 29 carbapenemase producers(Table-3 and 5).

Discussion:

This study revealed 48% CA-UTI in ICU which coincides with El-Din Hamdy[17] on 2014 where 46% CA-UTI was reported. Similarly, Safdar[18] on 2001 reported 40 CA-UTI in ICU of USA. In contrast, Dasgupta[19] reported 27.59% CA-UTI on 2015 in ICU of a tertiary hospital in India that differs significantly with this study. This might be due to the fact that, the number of CA-UTI samples was 72 in this study while it was only 29 in the study by Dasgupta[19]. Moreover, the comorbidities of majority of ICU patients of this study might have caused longer period of catheterization and thus greater chance of CA-UTI.

Among the common pathogens causing CA-UTI in ICU, 41.67% were Escherichia coli, 27.78% were Pseudomonas spp. and 18.05% were Klebsiella spp. Similar data were reported by El-Din-Hamdy[17] on 2014 where, 45.68% were E. coli, 29.07% were Pseudomonas spp. and 15.78% were Klebsiella spp. Among 30 isolated E. coli, 86.67% were resistant to ceftriaxone; amoxiclav and gentamicin while 80% to ciprofloxacin. Similar data were reported by Sattar[20] on 2016 from DMCH where 81.13% E. coli were

Table 4: Antibiotic susceptibility pattern of major ICU pathogens (n=72)

Disc code	*E. coli* (N = 30) n (%)	Pseudomonas (N = 20) n (%)	Klebsiella (N = 13) n (%)	Proteus (N = 6) n (%)	Enterobacter (N = 2) n (%)	CoNS (N = 1) n (%)
CRO	26(86.67)	18(90.00)	13(100.00)	6(100.00)	2 (100.00)	1(100.00)
CIP	24(80.00)	20(100.00)	10 (76.92)	6(100.00)	1 (50.00)	1(100.00)
CN	26(86.67)	17(85.00)	10(76.92)	6(50.00)	2(100.00)	0(00.00)
CT	3(10.00)	6(30.00)	2 (15.38)	6(100.00)	0 (00.00)	0(00.00)
CAZ	21(70.00)	17(85.00)	13(100.00)	6(100.00)	2(100.00)	1(100.00)
TGC	3(10.00)	18(90.00)	2 (15.38)	6(100.00)	0(00.00)	0(00.00)
AMC	26(86.67)	17(85.00)	13(100%)	4(66.67)	2(100.00)	0(00.00)
IPM	11(36.67)	9(45.00)	4 (30.79)	4(66.67)	1(50.00)	0(00.00)
FOX	11(36.67)	11(55.00)	7(53.84)	5(83.33)	1(50.00)	0(00.00)
F	8(26.67)	12(60.00)	5 (38.46)	4(66.67)	1(50.00)	1(100.00)
TZP	20(66.67)	2(10.00)	8 (61.15)	4(66.67)	2(100.00)	1(100.00)

CRO- Ceftriaxone CIP- Ciprofloxacin, CN- Gentamicin CT- Colistin, CAZ- Ceftazidime, TGC- Tigecycline, AMC- Amoxicillin-Clavulanic acid, IPM-Imipenem, FOX- Cefoxitin, F- Nitrofurantoin, TZP- Piperacillin-tazobactam

resistant to ceftriaxone followed by 82.36% were resistant to amoxiclav. Of the 20 isolated Pseudomonas spp. 100% were resistant to ciprofloxacin, 90% to tigecycline and ceftriaxone while 85% were resistant to ceftazidime, amoxiclav and gentamicin. It was in agreement with Akter[21] from DMCH on 2014 who reported that 95.83% Pseudomonas spp. were resistant to ciprofloxacin, 83.33% to ceftriaxone and ceftazidime, 91.66% to gentamicin and amoxiclav. In contrast, Goel[22] reported on 2012 that 82.35% Pseudomonas spp. was resistant to ciprofloxacin. The higher resistance of Pseudomonas spp.against ciprofloxacin in the present study might be due to frequent use of quinolones in treatment of UTI.

Present study also observed 45.71% imipenem resistant Pseudomonas spp. which was in agreement with Goel[22] on 2012 where 47.06% Pseudomonas spp. were resistant to imipenem. In this study, 100% of the 13 Klebsiella spp. were resistant to ceftriaxone, ceftazidime and amoxiclav while 72.92% were resistant to ciprofloxacin and gentamicin. Similarly, study by Sattar[20] on 2016 reported that 100% of the isolated Klebsiella spp. was resistant to ceftriaxone, ceftazidime and amoxiclav. 73.33% to ciprofloxacin and 76.19% to gentamicin. This study reported that 30.76% of the 13 Klebsiella spp. were resistant to imipenem which correlates with Khatun[23] on 2014 who reported 27.50% imipenem resistant Klebsiella spp., whereas Mostafa[24] on 2016 reported 15.38% imipenem resistant Klebsiella spp. In this study, 100% of the six Proteus spp. was resistant to colistin This might be due to the fact that the isolated Proteus spp.

in this study were intrinsically resistant to colistin as we know that addition of L-Ara4N (4-amino-4-deoxy-L-arabinose) in the LPS of Proteus spp. is responsible for the development of its intrinsic resistance to colistin.[25]

This study searched for quinolone and colistin resistance genes as well as ESBL and MBL genes among the isolates of ICU causing CAUTI which revealed 53.23% QnrS, 43.55% QnrB and 20.97% QnrA genes among 62 quinolone resistant isolates. In accordance Das[26] on 2016 reported 59.57% QnrS, 49.65% QnrB and 23.40% QnrA. In contrast, Yugendran and Harish[27] on 2016 reported 15% QnrB and 10% QnrS gene. Higher proportion of quinolone genes in the present

Table 5: detected ESBL and MBL genes

Drug resistance genes	Detected genes			
ESBLgenes	blaCTXM-15 N (%) 45 (75)	blaOXA-1 N (%) 23 (43.55)	blaTEM N (%) 11 (18.34)	blaSHV N (%) 7 (11.67)
MBL genes	blaNDM-1 N (%) 13 (44.83)	blaVIM N (%) 5 (17.24)	blaKPC N (%) 5 (17.24)	blaIMP N (%) 4 (13.79)

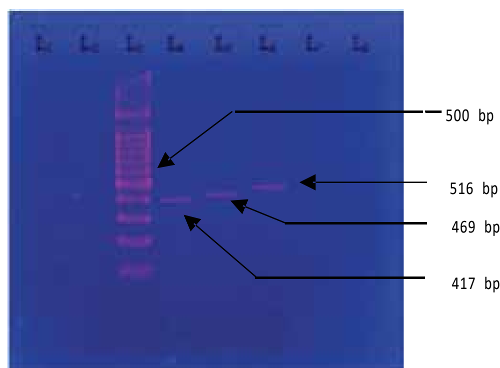

Figure 1: Amplified DNA of 417 bp for QnrS gene (Lane 4), DNA of 469 bp for QnrB gene (Lane 5), 516 bp for QnrA gene (Lane 6), 100 bp DNA ladder (Lane 3), negative control E. coli ATCC 25922. (Lane 8).

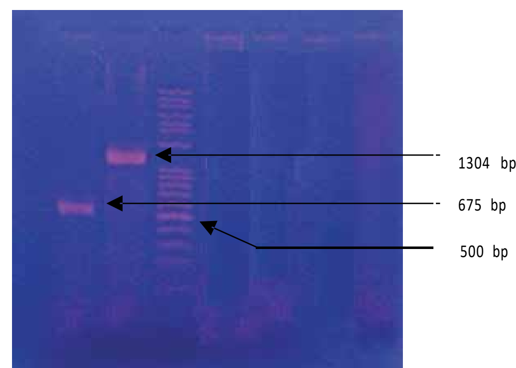

Figure 2: Amplified DNA of 675 bpfor pmrA gene(lane 2), DNA of 1304 bp for pmrB gene (lane 3), 1000 base pair DNA ladder (Lane 4), negative sample (Lane 5, 6 and 7), negativecontrol E. coli ATCC 25922 (Lane 8).

study might be due to irrational use of quinolones specially ciprofloxacin in the recent past.[28] Quinolone resistance among gram positive bacteria occurs due to mutation in glrA and gyrA genes rather than Qnr genes[29] which reveals the reason of non- detection of quinolone resistance genes in the isolated CoNS.

Out of 17 phenotypically detected colistin resistant bacteria, pmrA gene was found in 52.94% while pmrB was found in 29.41% of the isolates. Similarly, Mostafa[24] reported 50% pmrA and 22.72% pmrB genes in her study on 2017. Present study revealed 44.83% blaNDM-1 and 17.24% blaVIM and blaKPC each of the 29 carbapenemase producers. In agreement to this, Sattar[20] reported 43.24% blaNDM-1, 16.22% blaVIM and 21.6% blaKPC on 2016. In Bangladesh, prevalence of blaNDM-1 is increasing as reflected by several studies such as 3.5% blaNDM-1 in 2011, [30] 22.86% in 2012, [31] 43.24% in 2015[20] and 44.83% in the present study. This might be due to irrational use of carbapenem in the recent past due to emergence of resistance against cephalosporin and penicillin.

Conclusion:

In this study, the microbiological causes of CA-UTI in ICU of Dhaka Medical College hospital have been evaluated. Moreover, resistance patterns against commonly used drugs were also searched along with the genes responsible for the resistance. The hidden truth that was revealed by this study is that, the incidence of drug resistance is increasing specially in ICU which might be due to the indiscriminate use of antibiotics.

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