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Biofilm Formation, Antimicrobial Resistance and Biofilm-Related Genes among Uropathogens Isolated from Catheterized Uro-Oncology Patients

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ABSTRACT

Background & Objective: Despite the critical importance of catheter as an indwelling medical device, its prolonged utilization in hospitalized patients may lead to infection. This study aimed to identify distribution of uropathogenic bacteria isolated from catheterized uro-oncology patients, their biofilm production, and antimicrobial resistance patterns to generally used antibiotics.

Materials & Methods: The urine samples of catheterized urology cancer patients were collected for urinalysis and urine culture. Then capability of biofilm production was detected by Congo red agar method, tube method, and microtiter plate assay. Antimicrobial susceptibility test was also performed using the Kirby–Bauer disc diffusion method on Muller–Hinton agar. Subsequently, polymerase chain reaction (PCR) assays were used to detect the biofilm encoding genes.

Results: Of the 100 urinary catheter samples, 76 isolates were recovered from urinary catheters of 52 patients. *Escherichia coli* was established to be the most frequent pathogen isolated from the urine of patients followed by Pseudomonas and Staphylococcus. All of *Pseudomonas aeruginosa* and Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were found to be biofilm producers. All studied isolates were found resistant to ampicillin, amoxicillin, and cephalexin. All biofilm- producer MRSA and Pseudomonas isolates were found to harbor the virulence genes studied. Both imipenem and fosfomycin were the most effective antibiotics against isolated bacteria.

Conclusion: In our study virulent pathogens with highly- resistant profile and potential to form biofilm were isolated from uro-oncology patients. Therefore, the current study highlights the significance of antibiotic resistance which can lead to treatment failure.

Keywords: Prostate cancer, Urinary bladder cancer, Biofilm, Antimicrobial drug resistance

Introduction

Implantable medical devices can be colonized by bacteria (1), therefore a dynamic microbiology of biofilm exists on an indwelling catheter with permanent acquire of new microorganisms at a rate of almost 3–7 percent each day (2). Routine use of catheters in urology practices and their contamination, causes an increasing challenge of catheterassociated urinary tract infections (CAUTIs) with the subsequent enhancement of morbidity and mortality (3), increased length of hospital stay, and increased treatment costs (4). CAUTIs remain among the major complications of indwelling devices (5) and comprise approximately 80% of all nosocomial UTIs. Moreover, UTIs account for about 40 percent of all health care- associated infections (5). Prolonged catheterization up to 30 days leads to CAUTI development in 100% of patients (2). In addition, CAUTIs can lead to more serious complications including bloodstream infections and endocarditis. Around 20% of health care- acquired bacteraemia in intensive care units (ICUs) and over 50% in long term care centers are due to CAUTI (6). Moreover, 13,000 deaths every year are estimated to be linked to healthcare-associated UTIs in USA (7).

Biofilms can be formed on the prostate stones or urothelium and are able to colonize the surfaces of implanted medical devices (8). Biofilm formation initiates immediately after catheterization and involves both the interior and exterior surfaces of catheter (8). Biofilms are organized accumulation of bacterial cells on a surface embedded within a polymeric matrix

created by bacteria (9). Bacterial attachment begins through adherence to surface receptors of host cell or catheter (10). A worrying characteristic of biofilm-related infections is higher resistance of biofilm-embedded bacteria on the inner surface of catheters to antimicrobial agents as well as resistance to phagocytosis and other components of the immune defense system, compared to their free-living counterparts (11). Therefore, recurrent infection occurs after antibiotic treatment is completed (12), making the bacteria to be a serious obstacle for the patient's recovery process (13). In addition, mature biofilms disperse and cause bacterial spread to the whole body (14). The patient's treatment based on antibiotic susceptibility test results of planktonic bacterial cells, which largely differs from the biofilm mode, results in treatment failure (8). Nosocomial infections due to antibiotic-resistant pathogens in cancer patients can increase the mortality rates to much higher rates (15). In spite of the importance of CAUTIs particularly in cancer patients, they have been underestimated in research. This might be due to scant surveillance and lack of regular reporting systems for intervention and prevention activities (13).

To our best knowledge, there has been no report on antimicrobial susceptibility pattern and biofilm production of uropathogenic bacteria from catheterized urology cancer patients in Iran. Therefore, we aimed to evaluate the distribution of uropathogenic bacteria isolated from catheterized uro-oncology patients, their biofilm production, and antimicrobial resistance patterns to generally- used antibiotics.

Materials and Methods

Bacterial isolation

Of the 100 urinary catheter samples, 76 bacterial isolates were recovered from urinary catheters of 52 hospitalized men in urology ward of Imam Khomeini Hospital, affiliated to Tehran University of Medical Sciences from February 2020 to April 2021.

The patients already diagnosed with UTI before catheterization, immunosuppressed patients, and those who had received antibiotic prophylaxis were excluded. Urine samples of eligible cases were collected within the first 48 hours of catheterization using a sterile syringe to puncture the catheter tube. This study was approved by institutional review board (Ethical ID: IR.TUMS.IKHC.REC.1399.014).

In the microbiology laboratory, the urine samples were inoculated directly onto tryptic soy broth (TSB), blood agar, MacConkey agar, and Mannitol salt agar (MSA) (24 hrs. /37° C, aerobic atmosphere).

Then the isolates from urine cultures were recognized using standard microbiological processes (Gram staining and colony morphology on the respective media including MSA, MacConkey agar, blood agar, Enterococcus selective agar, and cetrimide agar, biochemical tests such as catalase production, bacitracin resistance, DNase and coagulase production, fermentation of mannitol for Gram-positive bacteria and, lactose fermentation, nitrate reduction, Simmons' citrate, Methyl red/Voges–Proskauer, urease, triple sugar iron, H2S production, and motility for Gram-negative bacteria) .

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA)

The cefoxitin (30 µg) discs were used for cefoxitin disc diffusion test to detect methicillin resistance in *Staphylococcus aureus* isolates as described earlier (16). Bacterial DNA was extracted by a DNA extraction kit (Qiagen, Valencia, USA) according to the manufacturer's guidelines. Polymerase chain reaction (PCR) assay targeting the *mecA* gene was also performed for methicillin- resistance confirmation (17).

Biofilm formation tests

The capability of biofilm production of microorganisms was detected by tube method, Congo red agar (CRA) method, and microtiter plate assay.

Slime assay on CRA

Bacterial slime production was detected by the CRA method as described before (9). The isolates were inoculated to CRA media (Merck TM) in aerobic condition at 37°C for 72 hours. Based on the colony color, they were differentiated as slime-producers (Black colonies with irregular, dry, and crystal-like appearance) or non-slime producers (pink and smooth and flat colonies with a dark center). All tests were carried out in triplicate.

Colorimetric microtiter plate method

The ability of bacteria to produce biofilm was quantified by their cultivation on a 96-well flat-bottomed polystyrene microtitre plate as described before (18). Briefly, standardized bacterial suspension (0.5 McFarland (1.5×10^8 cfu/mL) was prepared and inoculated in TSB complemented with 1% glucose [TSB was used for dilution of bacterial suspensions (1:100)]. After incubation (48hrs., 37°C), the wells were aspirated and washed three times with 300 µL phosphate-buffered saline (PBS; pH 7.2) to eliminate non-adherent bacterial cells, and attached bacteria were fixed by heat and stained with crystal violet (2%) for 15 minutes. The additional stain was rinsed off with 300 µL distilled water three times. Subsequently, the plates were air dried and resolubilized with 200 µl ethanol (95%) for 30 minutes to extract the purple crystal from biofilms. Finally, the optical densities (ODs) of adherent bacterial films which had been stained were recorded by automated ELISA at a wavelength of 570 nm. All tests were accomplished for each isolate in triplicate. The wells with TSB alone were used as negative control.

Average OD values of negative controls and samples were calculated. Cut-off value (ODc) was described as

a three- standard deviation (SD) above the mean OD of the negative control. For explanation of the adherence ability, strains were classified into four groups: non biofilm producer (0) OD ≤ODc, weak biofilm producer (+ or 1) = ODc <OD ≤2×ODc, moderate biofilm producer (++ or 2) = 2×ODc <OD≤4×ODc, and strong biofilm producer (+++or 3), 4×ODc <OD. In all biofilm formation experiments, *S. epidermidis* ATCC 35984 was used as positive control.

Christensen test tube method (TM)

The TM was performed after the modification of a procedure described by Christensen GD et al **(19)**. Briefly, approximately 10 mL of TSB was inoculated with a loop full of bacteria from overnight cultures and incubated (48 hrs, 37°C). Then the walls of glass test tube were stained with crystal violet for 1 hour and smoothly washed with distilled water three times and then air dried. A detectable film that lined the interior of the tube wall was considered as positive slime formation. A stained ring at the liquid–air edge was reflected as negative result. The test was performed in triplicate.

Antimicrobial susceptibility testing.

Antimicrobial susceptibility test was accomplished using the Kirby–Bauer disc diffusion method on Muller–Hinton agar (Oxoid Ltd.), according to the clinical and laboratory standards institute (CLSI) guideline **(20)**. Multiple-drug resistance (MDR) was described as bacterial resistance to at least one agent in three or more antimicrobial categories (21). The assessed antibiotics were those generally used in treatment of CAUTI and approved for bacterial infection treatment. The susceptibility profiles were determined for gentamicin (10 µg), norfloxacin (10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), amoxicillin (30 µg), ampicillin (10 µg), trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg), tetracycline (30 µg), erythromycin (15 µg), amikacin (30 µg), ceftriaxone (30 µg), imipenem, fosfomycin,and cephalexin (Mast Diagnostics, Mast Group Ltd, Merseyside, UK). Finally, antibiotic susceptibility profiles of biofilm-forming and nonbiofilm-forming bacterial isolates were compared.

Molecular identification of genes encoding biofilm and virulence factors

Genomic DNA of bacterial isolates were extracted by a Dneasy kit (Qiagen, Valencia, CA) according to the manufacturer, and the purified DNA was used for subsequent molecular evaluations. PCR assays were used to detect the biofilm encoding genes separately (*ica*A *and ica*D genes in *Staphylococcus aureus* and *cup A gene* in *Pseudomonas aeruginosa*). Detection of genes encoding virulence factors in *Escherichia coli* that cause urinary tract infection including hemolysin (*hly*), P fimbriae (*pap*C), type 1 fimbriae (*fim*A) was also carried out by the PCR method using specific primers as previously described (22) (Table 1). The genes were amplified on an Eppendorf (Hamburg, Germany) thermocycler (Table 1) in a volume of 25 µL with SinaClon PCR Master Mix 2X containing 1 µl of each primer (20 pMol) (Table1), 2 µl of DNA template,12.5 µL of PCR Master Mix 2X, and H2O to achieve a final reaction volume of 25 µL.

Results

Uropathogen isolation

Totally 76 uropathogen isolates were recovered, of which *Escherichia coli* was shown to be the most frequent pathogen isolated followed by Pseudomonas and Staphylococcus. The frequencies of different bacteria isolated from patients are summarized in Table 2.

Biofilm formation

Slime production by urinary isolates were explored by three mentioned methods. According to microtitre plate method and tube method, the incidence of biofilm- producer bacteria was 82% (63/76). All of *Pseudomonas aeruginosa* and MRSA isolates were found to be biofilm formers. The results of biofilm production in urinary isolates using three different methods are summarized in Table 3.

Table 1. Target genes and their primers

Bacterium	Gene	Primer Sequence	Product size(bp)	PCR program cycle parameters	Standard strain positive for the gene of interest	Reference
Staphylococcus aureus	*mecA*	F -TCCAGATTACAACTTCACCAGG R- CCACTTCATATCTTGTAACG	162	Initial denaturation:94°C for 5 min	ATCC29247	9
Staphylococcus aureus	*Ica/A*	ICAA-F 5'-CCTAACTAACGAAAGGTAG-3' ICAA-R 5'-AAGATATAGCGATAAGTGC-3'	188		ATCC35556	9
Staphylococcus aureus	*Ica/D*	ICAD-F 5'-AAACGTAAGAGACGTGG-3' ICAD-R 5'-GGCAATATGATCAAGATAC-3'	198	35 cycles denaturation (94°C for 2 min), annealing (55-62°C depending on gene for 1:30 min), extension (72°C for 2 min)	ATCC35556	9
Pseudomonas aeruginosa	*cup A*	F -5`- CTACCGCTATTCCACCGAAG-3` R-5`-AGGAGCCGGAAAGATAGAGG-3`	172		ATCC 27835	23
Escherichia coli	*papC*	papC-F:GACGGCTGTACTGCAGGGTGTGGCG papC-R: ATATCCTTTCTGCAGGGATGCAATA	328		ATCC 25922	24
	hly	hly-F: AACAAGGATAAGCACTGTTCTGGCT hly-R: ACCATATAAGCGGTCATTCCCGTCA	1177			22
	fimA	fimA-F: GTTGTTCTGTCGGCTCTGTC fimA-R: ATGGTGTTGGTTCCGTTATTC	447	final elongation at 72°C for 2 min.		24

Table 2. The frequencies of different bacteria isolated from urine samples

Bacterial isolates, (N = 76)		Number (%)
Escherichia coli		29 (38.1)
Staphylococcus aureus	**MSSA**	5 (6.6)
	MRSA	16 (21.0)
Pseudomonas aeruginosa		16 (21.0)
Enterobacter spp.		6 (8)
Klebsiella spp		3 (4)
Proteus mirabilis		1 (1.3)
Total		**76 (100)**

Table 3. Results of biofilm production among bacterial isolates using three different methods

Biofilm Formation										
Method		**Microtitre Plate Method N(%)**				**CRA Method N(%)**				**Tube Method N(%)**
Strains (n)		NBP OD ≤ODc	WBP ODc <OD ≤2×ODc	MBP 2×ODc <OD≤4×ODc	SBP 4×ODc <OD	NBP (Red)	WBP (Almost black)	MBP (Black)	SBP (Very Black)	NBP	BP
***Escherichia coli* (29)**		9 (31.0)	1(3.5)	1(3.5)	18(62.0)	10(34.5)	0	1(3.5)	18(62.0)	9(31)	20(69)
Staphylococcus aureus	**MSSA (5)**	3(60)	2(40)	0	0	3(60)	2(40)	0	0	3(60)	2(40)
	MRSA (16)	0	0	2(12.5)	14(87.5)	0	0	2(12.5)	14(87.5)	0	16(100)
***Pseudomonas aeruginosa* (16)**		0	0	3(18.8)	13(75.0)	1(6.2)	0	3(18.8)	12(75.0))	0	16(100)
Enterobacter spp.(6)		1(16.7)	0	4(66.6)	1(16.7)	1(16.7)	0	3(50.0)	2(33.3)	1(16.7)	5(83.3)
Klebsiella spp (3)		0	0	0	3(100)	0	0	0	3(100)	0	3(100)
***Proteus mirabilis* (1)**		0	0	1(100)	0	0	0	1(100)	0	0	1(100)

NBP: Non-biofilm producer; MBP: Weak biofilm producer; Moderate biofilm producer: MBP; SBP: Strong biofilm producer; BP: biofilm producer

Antimicrobial-resistance profile of bacterial isolates

All studied isolates were found resistant to amoxicillin, ampicillin, and cephalexin. Moreover, a high rate of resistance was found against gentamicin, trimethoprim/sulfamethoxazole, and erythromycin. The results showed that resistance to tetracycline, ceftriaxone, ciprofloxacin and norfloxacin were significantly different between biofilm-producing and non-biofilm-producing bacteria.

All *Pseudomonas aeruginosa* isolates had high frequency of resistance to all of the tested antibiotics and all of them were resistant to gentamicin. According to the MDR definition, all of the *Pseudomonas aeruginosa* isolated were MDR as well.

Additionally, all biofilm- former bacteria in our study were MDR. The resistance to norfloxacin and ciprofloxacin among biofilm-producing isolates was almost similar except for Pseudomonas, where the resistance rates to these two antibiotics were 12.5% and 75%, respectively.

The antibiotic susceptibility patterns of the biofilm-former isolates and nonbiofilm formers are shown in Table 4.

Table 4. The antibiotic resistance patterns of the biofilm-former and nonbiofilm former isolates

	Escherichia coli N (%)			*Staphylococcus aureus* N (%)				*Pseudomonas aeruginosa* N (%)
				MRSA	MSSA			
	BP n=20	NBP n=9	P-value *		BP n=2	NBP n=3	P-value *	
GEN	20(100)	9(100)	-	16(100)	2(100)	3(100)	-	16(100)
NOR	11(55)	2(22.2)	0.130	7(43.7)	1(50)	0	0.4	2(12.5)
NFN	2(10)	0	1	3(18.7)	0	0	-	2(12.5)
CIP	11(55)	2(22.2)	0.130	9(56.2)	1(50)	0	0.4	12(75)
AMX	20(100)	9(100)	-	16(100)	2(100)	3(100)	-	16(100)
AMP	20(100)	9(100)	-	16(100)	2(100)	3(100)	-	16(100)
TMP-SMX	20(100)	9(100)	-	16(100)	2(100)	3(100)	-	12(75)
OXA	-	-	-	16(100)	-	-	-	-
TET	15(55)	2(22.2)	0.014	9(56.2)	0	0	-	16(100)
VAN	-	-	-	0	0	0	-	-
ERY	20(100)	9(100)	-	16(100)	2(100)	3(100)	-	12(75)
AMK	4(20)	1(11.1)	1	7(43.7)	1(50)	0	0.4	2(12.5)
CRO	11(55)	2(22.2)	0.130	9(56.2)	1(50)	0	0.4	12(75)
CLX	20(100)	9(100)	-	16(100)	2(100)	3(100)	-	16(100)
IPM	2(10)	0	1	3(18.7)	0	0	-	2(12.5)
FOF	2(10)	0	1	3(18.7)	0	0	-	0

	Enterobacter spp. N (%)			*Klebsiella spp* N (%)	*Proteus mirabilis* N (%)	All isolates N(%)		
	BP n=5	NBP n=1	P-value*			BP n=27	NBP n=13	P-value**
GEN	5(100)	0	0.167	3(100)	1(100)	27(100)	12(92.3)	0.325
NOR	2(40)	0	1	2(66.7)	0	14(51.9	2(15.4)	0.027
NFN	1(20)	0	1	2(66.7)	1(100)	3(11.1)	0(0)	0.538
CIP	2(40)	0	1	2(66.7)	1(100)	14(51.9)	2(5.2)	0.027
AMX	5(100)	1(100)	-	3(100)	1(100)	27(100)	13(100)	-
AMP	5(100)	1(100)	-	3(100)	1(100)	27(100)	13(100)	-
TMP-SMX	5(100)	0	0.167	2(66.7)	1(100)	27(100)	12(92.3)	0.325
OXA	-		-	-	-	-	-	-
TET	5(100)	0	0.167	2(66.7)	1(100)	20(74.1)	2(15.4)	< 0.001
VAN	-	-	-	-	-	-	-	-
ERY	5(100)	0	0.167	2(66.7)	1(100)	27(100)	12(92.3)	0.325
AMK	-	-	-	1(33.3)	1(100)	5(22.7)	1(8.3)	0.389
CRO	5(100)	0	0.167	2(66.7)	1(100)	17(63)	2(15.4)	0.005
CLX	5(100)	1(100)	-	3(100)	1(100)	27(100)	13(100)	-
IPM	1(20)	0	1	1(33.3)	1(100)	3(11.1)	0(0)	0.538
FOF	0	0	-	2(12.5)	0	2(7.4)	0(0)	1

GEN: Gentamicin; NOR: Norfloxacin; NFN: Nitrofurantoin; CIP: Ciprofloxacin; AMX: Amoxicillin; AMP: Ampicillin; TMP-SMX: Trimethoprim/Sulfamethoxazole; OXA: Oxacillin; TET: Tetracycline; VAN: Vancomycin; ERY: Erythromycin; AMK: Amikacin; CRO: Ceftriaxone; CLX: Cephalexin, IPM: Imipenem; FOF: Fosfomycin

*: Fisher's exact ** : Chi-Square or Fisher's exact Note: Bold numbers indicate statistically significant difference with a p-value less than 0.05

Detection of genes associated with biofilm formation

All biofilm producer MRSA and Pseudomonas isolates were positive for the genes studied. In addition, none of the non-biofilm former *Pseudomonas aeruginosa* strains harbored *cup A* gene. The detailed results are shown in Table 5.

Table 5. Virulence genes among biofilm producer and non-biofilm producers of isolated bacteria

Isolated Bacteria		Gene profile	Biofilm Producer N(%)	Non-Biofilm Producer N(%)	Total N(%)
Escherichia coli		*papC/ hly/ fimA*	20(100)/20(100)/20(100)	2(22.2)/ 2(22.2)/ 2(22.2)	22(75.9)
Staphylococcus aureus	**MRSA**	*mecA/ Ica/A/*	16(100)/16(100)/16(100)	-	16(100)
	MSSA	*Ica/D*	0	0	0
Pseudomonas aeruginosa		*cup A*	12(75)	-	12(75)

Discussion

Nowadays, biofilm- based infections are an emerging problem in hospital settings. According to the National Institutes of Health (NIH), up to 80% of all infections are due to biofilm- producer bacteria and urology is one of the major fields in which biofilm and antibiotic resistance can become problematic **(23)**. Treatment of infections due to MDR organisms especially in cancer patients has become a clinical challenge, since suitable therapeutic choices are often limited. Therefore, anticipating biofilm formation and antibiotic resistance profile of bacteria circulating in the hospital environment allow the selection of a more

appropriate antibiotic at the beginning of the treatment, thereby avoiding the need to change antibiotic in the later stages **(24)**.

In the present study, 82% (63/76) of bacterial isolates were positive for the biofilm production (Table 4), which was higher compared with previous studies (**25, 26**). This might result from the fact that our study was performed only in catheterized patients.

The main biofilm -producer urinary pathogens known as ESKAPE include Enterococcus spp. especially Enterococcus faecalis, MRSA, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus epidermidis, Providencia stuartii, and Morganella morganii **(27, 28)**. In the current study, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus were the most frequent strains isolated from patients. This was consistent with other studies which have reported these strains as the most important pathogens isolated from urine cultures **(28-30)**. Escherichia coli is one of the most common causes of urinary infections **(31)**. Proteus species are also a part of human intestinal flora and are widely distributed in long-term care settings and hospitals **(31)**. Not only can they colonize the skin and mucosa of hospitalized patient but also cause nosocomial infections **(31)**. In our study only one of our cases was Proteus mirabilis and resistant to all of study antibiotics.

Pseudomonas is one of the most important causes of nosocomial infections and is resistant to a wide range of antibiotics **(32)**. Our findings showed that all of *Pseudomonas aeruginosa*, MRSA, and Klebsiella Spp., isolated from urine samples were biofilm producer. All *Pseudomonas aeruginosa* isolates harbored *cup A* gene and had high frequency of resistance to all of the tested antibiotics. All of them were MDR as well. Furthermore, all of *Pseudomonas aeruginosa* isolates were resistant to gentamicin. This was contrary to the previous study in our country **(32)**.

Klebsiella spp. are other uropathogens and have both endogenous and exogenous sources **(32)**. Klebsiella spp comprised 4% of isolates in current study.

Biofilms have major effects on antibiotic resistance, and minimum inhibitory concentrations (MICs) of antibiotics which are essential for effective treatment **(23)**. It is recognized that biofilm formation leads to antibiotic resistance by decreased penetration of the antimicrobial agents and altered growth rate of biofilm microorganisms. In fact, infection due to biofilm-producer bacteria means infection with highly-resistant bacteria. Our results were concordant with previous studies regarding higher antibiotic resistance in biofilm producers compared with non-biofilm producers **(5, 9)**. However, an overall comparison between all biofilm-producing bacteria with non-biofilm producers showed that the resistance to some antibiotics such as ciprofloxacin, tetracycline, ceftriaxone, and norfloxacin was significantly higher among biofilm-producers than non-biofilm producers (Table 4). One of the limitations of the present study was the small sample size. Perhaps if the number of isolates studied were greater, the significance of antibiotic resistance would change between the two groups of biofilm producers and non-biofilm producers.

Regarding *in vivo* and *in vitro* studies, β-lactams and aminoglycosides are able to inhibit the young biofilm development; however, fluoroquinolones affect not only young biofilms but also older biofilms **(33)**. All biofilm- former bacteria in our study were MDR and the resistance to norfloxacin and ciprofloxacin among biofilm-producing isolates was almost similar except for Pseudomonas, where the resistance rates to these two antibiotics were 12.5% and 75%, respectively. High resistance to ciprofloxacin might result from frequent use of this antibiotic as prophylaxis and empiric therapy in the last few years. Furthermore, the frequency of isolates resistant to nitrofurantoin was low. Thus, it can be considered as a treatment choice for UTI in our settings. Resistance of Pseudomonas to imipenem has been reported in the past studies in Iran within the range of 16% - 100%. In this study, the resistance was 12.5%, which was lower than a previous study **(32)**. Thus, antibiotic susceptibility tests are recommended before empirical treatment for management of UTIs in our settings. According to our data, resistance to erythromycin was high among isolates. This is contradictory to a previous study that recommended macrolides as first-line treatment for biofilm-associated UTIs **(34)**. Our results indicated that both imipenem and Fosfomycin had the best effect against isolated bacteria and can be considered as good choices for treatment of UTIs which is consistent with previous studies **(35-38)**. Since the urology cancer patients are at high risk for CAUTIs, the main goal of oncology nursing is to improve patients' safety and reduce infection rates and save their lives. This needs early intervention and adequate information about the causative agents of infections and their characteristics to make proper decision for the treatment or prevention. Therefore, antibiotic prophylaxis particularly in cancer patients, utilization of aseptic techniques and antimicrobial- incorporated catheters can reduce the incidence of CAUTI.

Conclusion

According to this study, virulent pathogens with highly- resistant profile and potential to form biofilm were isolated from uro-oncology patients. To the best of our knowledge, this is the first study that reports a significantly high spread of bacterial isolates with the potential to form biofilm among urology cancer patients in Iran.

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Conflict of Interest

The authors declare no conflict of interests.

References

1. Guggenbichler JP, Assadian O, Boeswald M, Kramer A. Incidence and clinical implication of nosocomial infections associated with implantable biomaterials - catheters, ventilator-associated pneumonia, urinary tract infections. GMS Krankenhaushyg interdiszip. 2011;6(1):Doc18.
2. Parida S, Mishra SK. Urinary tract infections in the critical care unit: A brief review. Indian J Crit Care Med. 2013;17(6):370-4. [PMCID] [DOI:10.4103/0972-5229.123451] [PMID]
3. Gould CV, Umscheid CA, Agarwal RK, Kuntz G, Pegues DA. Guideline for prevention of catheter-associated urinary tract infections 2009. Infect Control Hospital Epidemiol. 2010;31 (4):319-26. [DOI:10.1086/651091] [PMID]
4. Chant C, Smith OM, Marshall JC, Friedrich JO. Relationship of catheter-associated urinary tract infection to mortality and length of stay in critically ill patients: a systematic review and meta-analysis of observational studies. Crit Care Med. 2011;39(5):1167-73. [PMID] [DOI:10.1097/CCM.0b013e31820a8581]
5. Sabir N, Ikram A, Zaman G, et al. Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance. Am J Infect Control. 2017;45(10): 1101-5. [DOI:10.1016/j.ajic.2017.05.009] [PMID]
6. Esposito S, Noviello S, Leone S. Catheter-associated urinary tract infections: epidemiology and prevention. Le Infezioni in Medicina. 2008;16(3):130-43.
7. Klevens RM, Edwards JR, Richards CL, et al. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Public Health Rep. 2007;122(2):160-6. [PMCID] [DOI:10.1177/003335490712200205] [PMID]
8. Lebeaux D, Chauhan A, Rendueles O, Beloin C. From in vitro to in vivo Models of Bacterial Biofilm-Related Infections. Pathogens. 2013;2 (2):288-356. [PMCID] [PMID] [DOI:10.3390/pathogens2020288]
9. Ohadian Moghadam S, Pourmand MR, Aminharati F. Biofilm formation and antimicrobial resistance in methicillin-resistant Staphylococcus aureus isolated from burn patients, Iran. J Infect Develop Count. 2014;8(12):1511-7. [DOI:10.3855/jidc.5514] [PMID]
10. Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. 2008;21(1):26-59. [DOI:10.1128/CMR.00019-07] [PMID] [PMCID]
11. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agent. 2010;35(4):322-32. [PMID] [DOI:10.1016/j.ijantimicag.2009.12.011]
12. Hooton TM, Bradley SF, Cardenas DD, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. Practice Guideline.2010;50(5):625-63. [DOI:10.1086/650482] [PMID]
13. Awoke N, Kassa T, Teshager L. Magnitude of biofilm formation and antimicrobial resistance pattern of bacteria isolated from urinary catheterized inpatients of Jimma university medical center, Southwest Ethiopia. Int J Microbiol. 2019;2019:5729568. [DOI:10.1155/2019/5729568] [PMID] [PMCID]
14. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002; 15(2):167-93. [PMID] [PMCID] [DOI:10.1128/CMR.15.2.167-193.2002]
15. Meunier A, Nerich V, Fagnoni-Legat C, et al. Enhanced emergence of antibiotic-resistant pathogenic bacteria after in vitro induction with cancer chemotherapy drugs. J Antimicrob Chemother. 2019;74(6):1572-7. [DOI:10.1093/jac/dkz070] [PMID]
16. Skov R, Smyth R, Clausen M, et al. Evaluation of a cefoxitin 30 microg disc on Iso-Sensitest agar for detection of methicillin-resistant Staphyloc-occus aureus. J Antimicrob Chemother. 2003;52 (2):204-7. [DOI:10.1093/jac/dkg325] [PMID]
17. Ohadian Moghadam S, Pourmand MR, Douraghi M, Sabzi S, Ghaffari P. Utilization of PFGE as a powerful discriminative tool for the investigation of genetic diversity among MRSA strains. Iran J Public Health. 2017;46(3):351-6.
18. Stepanović S, Vuković D, Hola V, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 2007;115

(8):891-9. [DOI:10.1111/j.1600-0463.2007.apm_630.x] [PMID]

19. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect Immunity. 1982;37(1):318-26. [DOI:10.1128/iai.37.1.318-326.1982] [PMID] [PMCID]

20. Institute CaLS. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-First International Supplement. M100-S21 CLSI: Wayne, PA. 2016.

21. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol. 2012;18(3):268-81. [PMID] [DOI:10.1111/j.1469-0691.2011.03570.x]

22. Fattahi S, Kafil HS, Nahai MR, Asgharzadeh M, Nori R, Aghazadeh M. Relationship of biofilm formation and different virulence genes in uropathogenic Escherichia coli isolates from Northwest Iran. GMS Hygiene Infect Control. 2015;10:Doc11.

23. Soto SM. Importance of biofilms in urinary tract infections: New therapeutic approaches. Adv Biol. 2014; 2014;2014:543974. [DOI:10.1155/2014/543974]

24. Nicolle LE. Catheter-related urinary tract infection. Drug & Aging. 2005;22(8):627-39. [DOI:10.2165/00002512-200522080-00001] [PMID]

25. Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). J Clin Diagn Res. 2012;6(9):1478-82. [DOI:10.7860/JCDR/2012/4367.2537] [PMID] [PMCID]

26. Ponnusamy P, Natarajan V, Sevanan M. In vitro biofilm formation by uropathogenic Escherichia coli and their antimicrobial susceptibility pattern. Asian Pacific J Trop Med. 2012;5(3):210-3. [DOI:10.1016/S1995-7645(12)60026-1]

27. Stickler DJ. Bacterial biofilms in patients with indwelling urinary catheters. Nature Clin Pract Urol. 2008;5(11):598-608. [DOI:10.1038/ncpuro1231] [PMID]

28. Alves MJ, Barreira JCM, Carvalho I, et al. Propensity for biofilm formation by clinical isolates from urinary tract infections: developing a multifactorial predictive model to improve antibiotherapy. J Med Microbiol. 2014;63(Pt 3): 471-7. [DOI:10.1099/jmm.0.071746-0] [PMID]

29. Kucheria R, Dasgupta P, Sacks SH, Khan MS, Sheerin NS. Urinary tract infections: new insights into a common problem. Postgraduate Med J. 2005;81(952):83-6. [PMCID] [DOI:10.1136/pgmj.2004.023036] [PMID]

30. Kirmusaoglu S, Yurdugül S, Metin A, Vehid S. The effect of urinary catheters on microbial biofilms and catheter associated urinary tract infections. Urol J. 2017;14(2):3028-34.

31. Weiner LM, Webb AK, Limbago B, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: Summary of data reported to the national healthcare safety network at the centers for disease control and prevention, 2011-2014. Infect Control Hospital Epidemiol. 2016;37(11):1288-301. [DOI:10.1017/ice.2016.174] [PMID] [PMCID]

32. Ghanbarzadeh Corehtash Z, Khorshidi A, Firoozeh F, Akbari H, Mahmoudi Aznaveh A. Biofilm formation and virulence factors among Pseudomonas aeruginosa isolated from burn patients. Jundishapur J Microbiology. 2015;8(10):e22345. [DOI:10.5812/jjm.22345] [PMID] [PMCID]

33. Reid G, Habash M, Vachon D, Denstedt J, Riddell J, Beheshti M. Oral fluoroquinolone therapy results in drug adsorption on ureteral stents and prevention of biofilm formation. Int J Antimicrob Agents. 2001;17(4):317-9. [DOI:10.1016/S0924-8579(00)00353-8]

34. Delcaru C, Alexandru I, Podgoreanu P, et al. Microbial biofilms in urinary tract infections and prostatitis: Etiology, pathogenicity, and combating strategies. Pathogens (Basel, Switzerland). 2016;5(4). [PMCID] [DOI:10.3390/pathogens5040065] [PMID]

35. Neuner EA, Sekeres J, Hall GS, van Duin D. Experience with fosfomycin for treatment of urinary tract infections due to multidrug-resistant organisms. Antimicrob Agent Chemother. 2012;56(11):5744-8. [DOI:10.1128/AAC.00402-12] [PMID] [PMCID]

36. Kaur P, Sachan RSK, Karnwal A, Devgon I. A Review on Clinical Manifestation and Treatment Regimens of UTI in Diabetic Patients. Iran J Med Microbiol. 2022;16(2):98-115. [DOI:10.30699/ijmm.16.2.98]

37. Kalantari H, Hajizade A, Issazadeh K, Faezi Ghasemi M. A Study on the Prevalence of Vancomycin-resistant Enterococci and Their Antibiotic Resistance Pattern in Recreational Waters in Guilan Province, Iran. Iran J Med Microbiol. 2022; 16 (3):251-258. [DOI: 10.30699/ijmm.16.3.251]

38. Elahi Y, Javdani Shahedin G, Nejati A, Ashrafi I, Asadian M, Mazaheri Nezhad Fard R. Whole-

Genome Sequencing of a Clinically Isolated Antibiotic-Resistant Enterococcus faecium EntfacYE. Iran J Med Microbiol. 2021;15(6) :692-699. [DOI:10.30699/ijmm.15.6.692]

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