

Identification and antimicrobial susceptibility of aerobic and facultative anaerobic pathogenic bacterial species contaminated urinary catheter in patients admitted to Misrata Hospital

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Received: 28.06.2020

Published: 25.02.2021

Abstract:

Urinary tract catheterization is widely used to admit patients and recognized as a risk factor predispose to urinary tract infection (UTI). Different pathogenic bacterial species were found associated with UTI and Urinary catheterization. This study aimed to identify contaminated bacterial species of internal, external urinary catheter parts and urinary bags, as well as their susceptibility profiles to the most commonly used antimicrobial agents.

Fifty-one patients admitted to Alnokhpah private hospital in Misurata city were included in this study (51 samples of urinary catheter internal part (UCIP), urinary catheter external part (UCEP), and Urinary bag). Aseptically collected samples were sent to the hospital microbiology lab and bacterial species were identified according to standard microbiology methods and their susceptibility to antimicrobials estimated by disk diffusion test.

Of 153 sample collected, 81 (53%) showed bacterial colony growth, whereas 28% were polymicrobial and 72% monomicrobial cultures. Total bacterial isolates obtained from 81 positive cultures were 106 (49 Gram-positive and 72 were gram-negative). The most common identified species was *Enterococcus faecalis* 25.8%. High resistance reported in gram negative isolates collection, however the most effective antimicrobial was AMK 30%. Acinetobacter

baumannii was 100% resistant to all tested antimicrobials. Gram positive isolates showed extreme resistance against E 98%, CRO 90%, and CTT 88%.

Contamination of urinary catheter with pathogenic bacterial species preload to UTI. The High contamination rate of urinary catheter in this study reveals the risk to preload to UTI. Gram positive and negative isolates showed high resistance against tested antimicrobial agents which enhance treatment challenges.

Keywords: Pathogenic bacteria, Urinary catheter, Misurata Hospital.

تعريف أنواع البكتيريا الهوائية واللاهوائية الاختيارية الممرضة الملوثة للقسطرة البولية في المرضى بمستشفى مصراتة وقياس مدى حساسيتها للمضادات الميكروبية

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الملخص:

تستخدم القسطرة البولية بشكل واسع للمرضى داخل المستشفيات وتعتبر عامل خطر مساهم في حدوث التهابات القناة البولية. العديد من أنواع البكتيريا الممرضة مرتبط بحدوث التهاب القناة البولية وإدخال القسطرة للجهاز البولي. هذه الدراسة تهدف لتعريف أنواع البكتيريا الممرضة الملوثة للجزء الداخلي والخارجي للقسطرة البولية وكذلك كيس البول بالإضافة إلى تحديد مدى حساسية هذه الأنواع المعرفة للمضادات الميكروبية الأكثر استخداماً بالمستشفى.

شملت هذه الدراسة واحد وخمسون مريضاً من داخل مستشفى النخبة الخاص بمدينة مصراتة (51 عينة من الجزء الداخلي للقسطرة البولية والجزء الخارجي منها وكذلك كيس البول). العينات المجمعة بشكل معقم أرسلت إلى معمل الأحياء الدقيقة بمستشفى النخبة حيث

عرفت الأنواع البكتيرية طبقاً للطرق القياسية المعمول بها بعلم الأحياء الدقيقة كما تم قياس مدى حساسية هذه العزلات المعرفة للمضادات الميكروبية طبقاً لطريقة الأقراص والانتشار خلال الاجار (Disk diffusion test).

من 153 عينة مجمعة أظهر 81 (53%) منها نمو مستعمرات بكتيرية وتبين أن 28% كانت متعددة الأنواع (polymicrobial) و72% كان وحيدة النوع (monomicrobial). عدد العزلات البكتيرية المتحصل عليها من 81 عينة موجبة للنمو البكتيري كان 106 (49) موجبة الجرام و72 سالبة الجرام). أكثر أنواع البكتيريا انتشاراً كان *Enterococcus faecalis* (25.8%). سجل انتشار عزلات عالية المقاومة للمضادات الميكروبية ضمن البكتيريا سالبة الجرام وعموماً كان AMK هو الأكثر تأثيراً على عموم العزلات البكتيرية حيث كان مؤثراً في 30. *Acinetobacter baumannii* كان 100% مقاومة لكل المضادات الميكروبية المختبرة. عزلات البكتيريا موجبة الجرام كانت ذات مقاومة عالية لكل من E 98% و CRO 90% و CTT 88%.

تلوث القسطرة البولية بأنواع البكتيريا الممرضة عامل مساعد لحدوث التهاب القناة البولية وما وجد بهذه الدراسة من تلوث عالٍ للقسطرة يشكل عامل خطورة لحدوث ذلك. كما أن شدة المقاومة المسجلة للأنواع البكتيرية المعزولة من عينات الدراسة في كل من موجبة وسالبة الجرام يجعل علاج التهاب القناة البولية الناجمة عن الإصابة بها تحدياً كبيراً.
الكلمات المفتاحية: البكتيريا، الممرضة، القسطرة البولية، مستشفى مصراتة.

Introduction:

Catheter associated-urinary tract infection (CA-UTI) considered one of the most common health care-associated infection as a result of widespread of catheterization, whereas Indwelling urethral catheter was responsible for 70-80% of UTI [1, 2]. Using of indwelling urine catheter was performed in 17.5% of patient admitted in 66 European hospitals[3] and 23.6% in 183 US hospitals[2]. Urine catheter preload to UTI because pathogens pass through catheter and urine residue remains in bladder increase bacterial residence[4, 5]. Serious complication can occurs from CA-UTI such as orchitis, epididymitis, and prostatitis in male and pyelonephritis, cystitis and meningitis in all patients[6].

Bacterial species associated with indwelling catheter were variable between different studies. In Poland, bacteria were found in 69% of 156 men urine

sample collected from 2011-2015[7]. A comparative study conducted in Nepal found CA-UTI occurrence (25%) was significantly higher than community-acquired UTI (com-UTI) (18%). Also *Enterococcus faecalis* (*E. faecalis*) was the most commonly identified species in com-UTI while *Staphylococcus aureus* (*S. aureus*) was the dominant species in CA-UTI[8]. Urine catheter was responsible for device-associated nosocomial infections (DANIs) in 19% of 79 included patients between January and December 2014 in the trauma/surgical intensive care unit at Abusalim Trauma Hospital, Tripoli, Libya. The most common identified bacterial species from urine catheter cultures were *Acinetobacter baumannii* (*A. baumannii*) followed by *Klebsiella pneumonia* (*K.pneumonia*)[9]. This study will investigate the pathogenic bacterial species contaminated UCIP, UCEP, urinary bag and their susceptibility patterns to commonly used antimicrobial agents.

Material and methods:

Study setting and sample selection:

This study was conducted in Alnokhpah private hospital in Misurata city, Libya. Fifty one Admitted patients were included (male; 45 and female: 6). From each patient 3 samples were collected; urinary catheter internal part (UCIP) (1 centimeter (cm) of catheter tips), Urinary catheter external part (UCEP) (1cm of catheter protruded outside the urethral orifice) and 5ml of urine from urine bag were aseptically collected and sent immediately to hospital microbiology laboratory.

Sample processing and Bacterial Identification:

Collected samples (153; 51 of each 1cm of UCIP, 1cm of UCEP and 5ml urine bag) were placed in 5ml of sterile normal saline and vortexed for 5 minutes. Loop full of normal saline of internal and external parts of catheter and urine plated on blood and MacConkey agar and incubated 37°C for 24 hours. Colony morphology and gram stain were performed and bacterial isolates identified into two groups; gram positive cocci and gram negative bacilli. All gram negative bacilli isolates identified to species level by using Multi-biochemical test analytical profile index 20E (API 20E) (bioMérieux's).

Catalase test used to distinguish between staphylococcus and streptococcus bacterial genus. Further biochemical tests were used in isolates identified as staphylococcus like coagulase which separate *S. aureus* from other coagulase negative staphylococci (CNS). Mannitol Salt agar also used to confirm *S. aureus* identification as mannitol fermenter and CNS as mannitol non-fermenter bacterial species. Isolates identified as CNS furtherly identified by using novobiocin test whereas *Staphylococcus saprophyticus* (*S. saprophyticus*)

showed resistance results and *Staphylococcus epidermidis* (*S. epidermidis*) were susceptible.

Catalase negative gram positive cocci identified as Streptococcus and/or *E. faecalis* and group D streptococci, to distinguish between both bacterial groups; bile-esculin agar hydrolysis performed and isolates gave positive results identified as *E. faecalis* and others as streptococcus group. Blood hemolysis was performed to all isolates identified as streptococci and classified as non-hemolytic, α hemolytic, β hemolytic and γ hemolytic group[10].

Bacterial isolates storage:

One single isolated colony of well identified pure culture inoculated onto blood or MacConkey agar plates. Colonies grew in subculture were stored in 20% glycerol at -20°C for further analysis (antimicrobial susceptibility and biofilm formation).

Antimicrobial susceptibility:

Isolated species were tested for their susceptibility to antimicrobial agents according to Clinical and Laboratory Standards Institute guidelines (CLSI)[11]. Discs of following antimicrobial agents (OxoidTM) were used; gentamicin (CN, 10 μg), augmentin and clavulanic acid (AMC, 30 μg), ceftriaxone (CRO, 30 μg), cefotetan (CTT, 30 μg), nitrofurantoin (F, 300 μg), nalidixic acid (NA, 30 μg), erythromycin (E, 15 μg), vancomycin (VA, 30 μg), azithromycin (AZM, 15 μg), ciprofloxacin(CIP, 5 μg), amikacin(AMK, 30 μg) and imipenem (IMP, 10 μg).

Data analysis:

The data were analyzed using SPSS 22 (SPSS Inc., Chicago, USA). Data were presented in terms of numbers and percentages and analyzed using Chi-square test and independent sample t-test were employed to calculate the significance. Statistical significance was set at P-value ≤ 0.05 .

Result:

In this study, 153 sample were collected from 51 patients (51 each, UCIP, UCEP and urine bag). Samples showed bacterial growth were 53% (81/153). The source of 81 positive bacterial growth cultures was 30, 25 and 26 from urine bag, UCIP and UCEP respectively (figure 1). The difference between sources of positive bacterial culture was not significant ($P\text{-value}=0.772$).

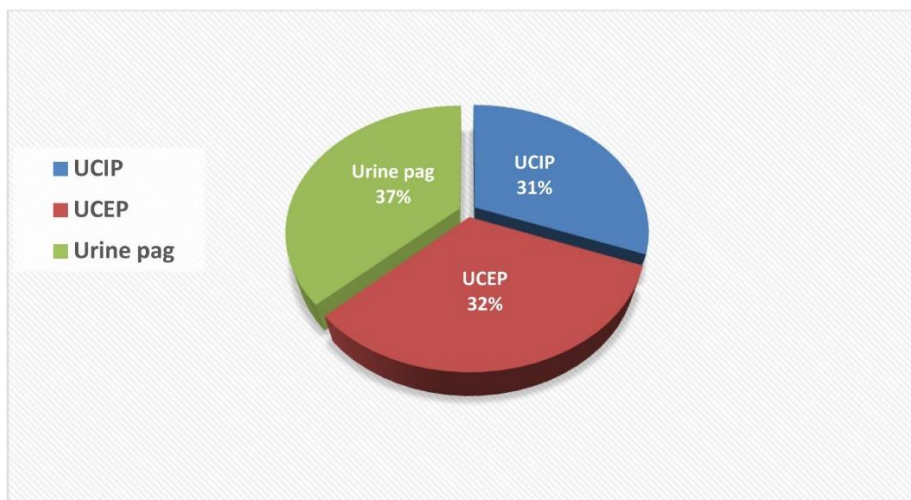


Fig 1: Source of positive bacterial cultures growth

Some cultures showed growth of more than one bacterial species (poly microbial contamination) resulted in 106 bacterial isolates were identified from 81 positive bacterial growth cultures. Of 106 isolates were identified, 49 gram-positive and 57 gram-negative as explained in table 1 (No difference between the two proportions P -value = 0.497). Results' finding revealed 100% of samples collected from female were contaminated while 77.8% (35/45) of male samples were showed positive bacterial growth.

The most commonly identified bacterial species were *E. faecalis* 25.8% (27/106), *Escherichia coli* (*E. coli*) 16.9% (18/106), *S. aureus* 13.3% (14/106) and *K. pneumonia* 12.3% (13/106). Other bacterial species were identified like *pseudomonas aeruginosa* (*P. aeruginosa*) 6.5% (7/106) and *A. baumannii* 4.7% (5/106). (P -value=0.000)

Table1: Prevalence and source of identified isolates

Group of isolate N (%)	Bacterial species	Isolate source			Total N (%)
		UCIP N (%)	UCEP N (%)	Urine bag N (%)	
Gram positive 49 (46)	<i>Enterococcus faecalis</i>	10 (9.6)	11 (10.5)	6 (5.7)	27 (25.8)
	<i>Staph aureus</i>	6 (5.7)	8 (7.6)	0	14 (13.3)
	<i>Staph epidermidis</i>	3 (2.8)	2 (1.9)	0	5 (4.7)
	<i>Streptococcus spp</i>	1 (0.9)	1 (0.9)	1 (0.9)	3 (2.7)
Gram negative 57 (54)	<i>E. coli</i>	5 (4.7)	4 (3.7)	9 (8.5)	18 (16.9)
	<i>Klebsiella pneumonia</i>	2 (1.9)	5 (4.7)	6 (5.7)	13 (12.3)
	<i>Pseudomonas aeruginosa</i>	1 (0.9)	3 (2.8)	3 (2.8)	7 (6.5)
	<i>Serratia marcescens</i>	1 (0.9)	2 (1.9)	2 (1.9)	5 (4.7)
	<i>Serratia .liquefaciens</i>	0	0	2 (1.9)	2 (1.9)
	<i>Acinetobacter baumannii</i>	1 (0.9)	2 (1.9)	2 (1.9)	5 (4.7)
	<i>Proteus mirabilis</i>	1 (0.9)	1 (0.9)	2 (1.9)	4 (3.7)
	<i>Citrobacter Freundii</i>	0	0	2 (1.9)	2 (1.9)
	<i>Enterobacter cloacae</i>	0	0	1 (0.9)	1 (0.9)
Total N (%)		31(29.2)	39 (36.8)	36 (34)	106 (100)

More than one bacterial species were identified in 28% (23/81) (sample (poly microbial infection), while mono microbial infection was 72% (58/81). Bacterial group identified from poly microbial samples exhibited in table 2, whereas gram positive + negative group was the highest 60% (14/23) followed by Gram negative +gram negative 31% (7/23) and gram positive + gram positive was the lowest 9% (2/23) (table 2). Data analysis was showed high significance of the gram-positive + gram-negative group compared to the other two groups (P -value=0.009).

Table 2: Source and grouping of poly microbial infection

Bacterial Group	UCIP N (%)	UCEP N (%)	Urine Bag N (%)	Total N (%)
Gram positive + Gram positive	1 (4.5)	1 (4.5)	0	2 (9)
Gram Positive + Gram Negative	4 (17)	6 (26)	4 (17)	14 (60)
Gram Negative + Gram Negative	1 (4.5)	1 (4.5)	5 (22)	7 (31)
Total N (%)	6 (26)	8 (35)	9 (39)	23 (100)

Gram-negative bacterial isolates revealed high resistance against nitrofurantoin 70% (40/57), AMC 100% and CTT 68%. In contrast, showed low resistance to AMK 30% (17/57). *A. baumannii* resistance to tested antibiotic was 100% except AMK (60%) and AZM (40%). Included isolates of *Citrobacter freundii* (*C. freundii*) resistance to used antibiotic was 100% and

highly susceptible to AMK (table 3). Amikacin effectivity against *C. freundii* compared to the other antimicrobials were highly significant (P -value=0.000).

Table 3: Antimicrobial resistance of identified gram-negative bacterial isolates

Gram -ve isolates N=57	Antimicrobial agents N (%)									
	F	AMK	CRO	CIP	CN	IMP	CTT	AMC	AZM	NA
<i>E.coli</i> n=18	3 (17)	0	16 (89)	13 (72)	4 (22)	2 (11)	3 (17)	18 (100)	13 (72)	18 (100)
<i>K. pneumonia</i> n=13	11 (85)	9 (69)	12 (93)	11 (85)	9 (69)	7 (54)	11 (85)	13 (100)	5 (39)	11 (85)
<i>P. aeruginosa</i> n=7	7 (100)	0	7 (100)	0	0	7 (100)	7 (100)	7 (100)	0	0
<i>Ser. Marcescens</i> n=5	5 (100)	5 (100)	0	0	0	0	5 (100)	5 (100)	0	0
<i>Acin. baumannii</i> n=5	5 (100)	3 (60)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	2 (40)	5 (100)
<i>Pro. Merabilis</i> n=4	4 (100)	0	0	2 (50)	4 (100)	1 (25)	4 (100)	4 (100)	4 (100)	4 (100)
<i>Ser. Liquefaciens</i> n=2	2 (100)	0	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0
<i>Citrobacter Freundii</i> n=2	2 (100)	0	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
<i>Enterobacter cloacae</i> n=1	1 (100)	0	0	0	0	0	0	1 (100)	0	0
Total n=57	40 (70)	17 (30)	44 (77)	35 (61)	26 (46)	26 (46)	39 (68)	57 (100)	46 (81)	40 (70)

In this study, collected gram-positive isolates showed high resistance to Erythromycin 98%, ceftriaxone 90% and cefotetan 88%. Nitrofurantoin was 100% effective against all gram-positive isolates but *E. faecalis* whereas 48% were susceptible (table 4)(P -value=0.000).

Table 4: Antimicrobial resistance of identified gram-positive bacterial isolates

Gram +v isolates	Antimicrobial agents N (%)									
	F	AMK	CRO	CIP	CN	IPM	CTT	AMC	VA	E
<i>Enterococcus faecalis</i> n=27	14 (52)	26 (96)	27 (100)	25 (93)	20 (74)	23 (86)	27 (100)	1 (3.7)	12 (44)	26 (96)
<i>Staph. aureus</i> n=14	0	6 (43)	14 (100)	13 (98)	12 (86)	3 (21)	13 (98)	14 (100)	3 (21)	14 (100)
<i>Staph. epidermidis</i> n=5	0	0	0	2 (40)	2 (40)	0	0	1 (20)	0	5 (100)
<i>Strept. Spp</i> n=3	0	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	0	3 (100)	3 (100)
Total n=49	14 (29)	35 (71)	44 (90)	43 (88)	37 (76)	29 (59)	43 (88)	16 (33)	18 (37)	48 (98)

Discussion:

Prevalence of bacterial pathogen among this study collected samples were 53% which higher than 27.7% the finding reported in a study investigated the prevalence of pathogenic bacterial species in urine samples collected from patients suffering from UTI admitted to the red-crescent private clinic, Tripoli, Libya[12]. Similar result was reported in previous study; Matsukawa and others, 2005, found 53.5% of intraluminal catheter surface was positive for

bacterial culture growth while 30.2% of urine samples showed the presence of bacterial pathogens[13]. Detection of bacterial pathogen in collected sample from urinary catheter and urine bag in this study and Matsukawa 2005 was higher than other investigation due to the difference in kind of collected samples. The study conducted at the Red-crescent private clinic, Tripoli, Libya investigated collected urine samples from patients had UTI symptoms, while Matsukawa and this study investigated urinary catheter and urine bag bacterial contamination.

It has been reported that females are more susceptible to urinary tract infection than males as a result of anatomical differences and hormonal changes in females[5, 14]. Similarly, our study noted all samples obtained from females were positive for bacterial culture growth. Poly microbial detection in one sample complicates prevention and treatment of UTI, when the combined group have different sensitivity to antimicrobial agents or gram-positive and negative isolates identified in one sample.

Gram-positive isolates prevalence was 46% while 54% were gram-negative isolates. In contrast, a study conducted in Bushra Medical laboratory, Tripoli, Libya only 0.7% of identified isolates were gram positive[15]. Niveditha and his colleagues reported 8% of urine samples were yielded gram-positive bacteria[16]. Geographical and patient healthcare management leads to variable bacterial groups and species isolated from patients' urine and catheter samples.

The highest gram-negative bacterial species in this study collection was *E.coli* (16.9%), *K. pneumonia* (12.3%) and *P. aeruginosa* (6.5%), respectively. A study conducted in Abusalim Trauma Hospital, Tripoli found the highest identified gram-negative species associated with urine catheter were *A. baumannii* 31.5% followed by *K. pneumonia* 26.3%[9]. In this study collection *A. baumannii* detection was 4.7%. Similar to this study finding, in Turkey, *E. coli* was the more identified species among gram-negative bacterial isolates and Enterococci the highest identified gram-bacteria associated with urinary tract catheter[17]. Different geographical area, Hospitals and patients population more likely to show variable results of the most common bacterial species associated with urinary catheter device.

Gram-negative isolates were highly susceptible to AMK 70%, CN 54% and IMP 54%, respectively. Similar results reported in the study screened uropathogenes antimicrobial profile done in Messalata Central Hospital, Libya whereas, AMK was the highest effective drug against gram-negative identified species[18]. Milud Ahmed and his colleague found Bushra Medical Laboratory, Tripoli, Libya reported highly resistant gram-negative bacilli to Cefoperazone/Sulbactam 96.1% followed by Ceftazidime 5.1% then Levofloxacin 94% while pseudomonas isolates very susceptible to AMK and

Norfloxacin[15]. Our data support the previous results that reported the emergence of *C. freundii* as highly resistant pathogen in Venezuela, whereas it was ESBLs producer holding CTX-M-14 β -lactamase[19]. In China from one patient isolates of *E. coli*, *A. baumannii* and *C. freundii* were isolated, they all were New Delhi metallo-lactamase (NDM-1) producing, showing strong evidence of transmitting resistance gene between *Enterobacteriaceae* species and from bacterial this family to *A. baumannii*[19].

The most effective antimicrobials against gram-positive bacteria were nitrofurantoin 71% followed by 67%. While they showed high resistance against CRO 90%, E 98% and CTT and CIP 88%. In contrast, Elsayah and his colleague found *S. aureus* isolated from urine culture in Tripoli was very sensitive to CIP 87.7%, and E was 81%[12]. Prevalence of high antimicrobial resistance among identified species in the present study might due to antibiotic abuses and lack of effective infection control polices in the hospital.

Contamination of UCIP and UCEP and urine bag with a pathogenic bacterial species is a risk factor for urinary tract infection. This study results reported high detection of pathogenic bacterial species in collected UCIP, UCEP and urine bag samples. Both gram-positive and negative bacteria were identified including *A. baumannii* and *C. freundii* which exhibited extreme resistance to test antimicrobials. Detection of highly resistant isolates highlights the need to implement an effective plan to control and prevent the spreading of these isolates to reduce antimicrobial resistant gene spreading to other bacterial species.

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