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Ashraf Ibrahim Mohammed Hassan¹, Bashar M Al-Hammodi²,
Ramzi Mowffaq Ramzi²

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A comparative study of the identification frequency and composition of bacteria isolated from catheter and urine in urologic patients: A single-center descriptive study

¹Department of Surgery, College of Medicine, University of Mosul, Iraq

²Al-Jamhoori Teaching Hospital, Mosul Health Directorate, Ministry of Health/Environment, Mosul, Iraq

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Abstract. *This study aimed to compare the identification frequency and composition of bacteria isolated from catheter and urine in urologic patients.*

Methods: *Ninety patients with urethral catheters were involved in the study. Urinary and catheter cultures were taken simultaneously from each patient and cultured on MacConkey's agar. Urine culture and sensitivity were performed for all samples in pre- and postoperative periods. Swab culture and sensitivity from the surface of intraluminal urethral catheters were performed for all cases in the post-operative period.*

Results: *The median indwelling period of the catheters was 8 days (range 3 to 21). The overall positive rate of catheter culture was significantly greater than that of urine culture, even in subjects without a recent antibacterial agent history. Urine cultures and catheter cultures did not match each other completely. The percentage of patients who had the same bacterial species isolated from both specimens increased in a time-dependent manner.*

Conclusions: *Not all species of bacteria colonizing the intraluminal surface of the urethral catheter were detected as urinary bacteria. Bacterial colonization on the intraluminal catheter surface could precede the emergence of bacteriuria.*

Keywords: *bacteria, catheter, urine, colonization, urologic patients.*

Conflict of interest statement. The authors declare no competing interest.

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Correspondence should be addressed to Ramzi Mowffaq Ramzi:
medicalresearch20@yahoo.com

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Ашраф Ібрагім Мохаммед Хасан¹, Башар М. Аль-Хаммоді², Рамзі Мовфак Рамзі²

Порівняльне дослідження частоти ідентифікації та складу бактерій, виділених із катетера та сечі урологічних пацієнтів: Одноцентрове описове дослідження

¹Кафедра хірургії, Медичний коледж, Університет Мосула, Ірак

²Навчальний госпіталь Аль-Джамхурі, Управління охорони здоров'я Мосула, Міністерство охорони здоров'я / довкілля, Мосул, Ірак

Резюме. Метою нашого дослідження було порівняти частоту визначення та видуవు належність катетер-колонізуючих та сечових бактерій у урологічних хворих.

Методи. У дослідженні брали участь 90 пацієнтів з уретральними катетерами. Посіви сечі та катетера брали одночасно у кожного пацієнта та культивували на агарі МакКонкі. Ідентифікацію збудників та їх чутливість до антибактеріальних лікарських засобів визначали в сечі пацієнтів у до- та післяопераційному періодах. Культуру мазка з поверхні інтралюмінальних уретральних катетерів досліджували у післяопераційному періоді.

Результати. Середній період перебування катетерів становив 8 днів (діапазон від 3 до 21). Частота бактеріальної колонізації катетерів була значно більшою, ніж сечі, навіть у пацієнтів, які не приймали антибактеріальні засоби. Результати культурального дослідження сечі та уретральних катетерів не повністю співпадали між собою. Відсоток пацієнтів, у яких однакові види бактерій були виділені з обох зразків, збільшувався з часом перебування катетеру.

Висновки. Не всі види бактерій, що колонізують внутрішньосвітлову поверхню уретального катетера, були виявлені як сечові бактерії. Колонізація бактерій на поверхні внутрішньосвітлового катетера може передувати появі бактеріурії.

Ключові слова: бактерії, катетер, сеча, колонізація, урологічні пацієнти.

Introduction. Nosocomial urinary tract infections (NUTI) are associated mostly with bladder catheterization, and to a lesser extent, with other genitourinary procedures [1-3]. The NUTI mainly occurs during an indwelling catheterization, via an ascending route, either intra-luminal, extra-luminal, or periurethral. Whatever the route, the development of biofilm around the foreign body rapidly induces chronic colonization [4, 5]. Catheter-associated urinary tract infections (CAUTI) are caused by a variety of pathogens including *E. coli*, *Klebsiella*, *Proteus*, *Enterococcus*, *Pseudomonas*, *Enterobacter*, *Serratia* and *Candida*. Bacterial colonization of the urinary catheter usually results in biofilms and is one of the most likely explanations for the intractability [6, 7]. Biofilm bacteria are known to be highly drug-resistant and therefore very difficult to eradicate [8, 9]. The detection of a significant density of a variable bacteria as planktonic cells in urine is essential for the diagnosis of NUTI [10]. However, despite the potential importance of CAUTI, the significance of catheter-colonizing bacteria is still unclear. The bacteria colonizing the intra-luminal surface of the catheter

tip, which is located inside the urine, bladder and totally submerged in urine, was selected as the site for detection [10]. The extra-luminal surface is not suitable as a detection site because it has a considerable risk of contamination from meatal or urethral colonization at removal [11]. Bacterial counts as low as 10^2 CFU/mL in urine obtained from a catheter in an aseptic method might be significant. The development of bacterial biofilms confers a degree of antimicrobial resistance to bacteria, including bacteria adherent to Foley's catheter [6]. There is no special preparation required before doing a urine culture test [12]. The patient should avoid urination just before having this test. The first void of the day is preferred to collect a clean-catch midstream urine sample for testing [12]. Urine culture results are usually ready in one to three days. Some organisms take longer to grow in the culture. For this reason, results may not be available for several days [12]. The number of bacteria in a given quantity of urine is usually estimated a count of 10^5 CFU/mL of urine or more may indicate an infection. A count ranging from 10^2 - 10^5 CFU/mL could be due to infection or contamination of the sample (may need to repeat urine culture) [12]. If the count is 10^2 CFU/mL or less, the infection is unlikely or could be seen in antimicrobial agent takers [12]. If the test results indicate an infection, sensitivity testing may be done to determine the best antimicrobial agent to eradicate the bacteria. Choice of the best antimicrobial agents for the specific type of bacteria infecting a specific person. Differences in the genetic material (DNA) in some types of bacteria

Ramzi Mowffaq Ramzi
medicalresearch20@yahoo.com

make them resistant to certain antimicrobial agents. In such cases, these antimicrobial agents cannot eradicate all the bacteria. When an effective antimicrobial agent is chosen its entire course must be completed to prevent bacteria from developing resistance to that antimicrobial agent. Short term administration of antimicrobial treatment kills only the most sensitive bacteria, while more resistant bacteria can multiply and prolong the infection [12, 13].

The study aimed to evaluate the relationship between catheter-colonizing bacteria and urinary planktonic bacteria.

Material and Methods. Design of the study. Ninety patients who have been treated at the Urology Department of Al-Jumhoori Teaching Hospital in Mosul City from September 2015 to the end of October 2016 were included in this prospective clinical study. The most common underlying urological conditions were bladder cancer (n = 27), followed by benign prostatic hyperplasia (n = 25), urethral stricture (n = 11), vesical stone (n = 9) and miscellaneous urological disease (n = 18). Quinolones (Ciprofloxacin), cephalosporins (Cefotaxime) or penicillins (Amoxicillin, Ampiclox, and Ampicillin) were administered for these patients.

Procedures. Urinalysis was performed for all subjects to detect pyuria and/or bacteriuria. Urine culture and sensitivity were performed for all cases by different bacteriologists in pre- and postoperative periods. Swab culture and sensitivity from the surface of intraluminal urethral catheters were performed for all cases by different bacteriologists in the post-operative period. The bacteriological result was reported by the laboratory department.

Data collection. On admission, demographic and clinical information was obtained including age, sex, an underlying condition in the urinary tract, history of pre-operative antibiotic use, and other associated conditions. The indication and the period of urethral catheterization were recorded as postoperative complications, any additional procedures and whether the patient had an internal and/or external stent.

Interventions. Placement of an indwelling latex (siliconized) catheter, the only available type for use in the hospital, was done under aseptic conditions with closed drainage. Immediately before catheter removal, urine was aseptically collected from the distal end of the catheter with a sterile tube. The catheter was then removed and cut off 5cm from the tip. The entire intraluminal surface of the 5cm -long segment was swabbed thoroughly with a sterile cotton swab. The two specimens were sent to the laboratory (urine and swab culture) and the Gram stain test was done for both. After that, the urine was inoculated on blood agar and MacConkey's agar. After incubation in 37° for 24 hours, the blood with O₂ will differentiate Gram-positive cocci (GPC) and Gram negative rods (GN R) spp. whereas the MacConkey's agar will differentiate the Gram-negative like coliform spp.

Biochemical tests. Since the oxidase test was performed on McConkey agar, the same workup was also done for the catheter culture. There were no facilities to detect or isolate the anaerobic spp. and the definitive number of the colonies could not be determined.

The bacterial density was determined according to the facilities available in the laboratory and was classified into scanty (<10²), moderate (10²-10⁴) and heavy growth (≥10⁵). In this study, any density of bacteria isolated from either urine or the catheter was regarded as positive. Antimicrobial agents were withheld one week preoperatively and were only allowed postoperatively.

Ethical clearance of the study. All authors hereby declare that the study was approved by the Ethics Committee from Aljamhoori Teaching Hospital, Iraqi Ministry of Health (code:202032001) and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Written informed consent was obtained from all patients before enrollment.

Statistical analysis. All the data were entered and processed using statistical package SPSS ver. 24 (Chicago Inc., Ill). A descriptive statistical test was used to summarize and tabulate the data.

Results. Ninety patients were included in the study. The patients' age distribution is presented in Table 1.

Table 1

The patients' age distribution

Age groups (Years)	No	%
<20	6	6.6
21 – 40	20	22.2
41 – 60	31	34.5
61 – 80	30	33.4
> 80	3	3.3
Total	90	100%

The silicon catheters used in the study were 8 to 22 Fr; the average indwelling period was 8 days (range from 3 to 21 days). The catheters were placed for varying reasons, mainly bladder irrigation, urine output measurement after urological surgery and bladder outlet obstruction. The closed drainage system was maintained throughout the study period. Cultures of urine and catheter did not completely correspond to each other either in terms of the overall positive indicator or in terms of the isolated types of bacteria.

The preoperative urinalysis revealed pyuria (pus cells more than 10/ hpf) in 55 (61%) patients and bacteriuria in 52 (68.8%) patients. Regarding the pre-operative urine cultures, positive results were obtained in 24 (75%) males and 8 (25%) females, whereas negative cultures were obtained in 51 (87.9%) males and 7 (12.1%) females (Table2).

Table 2

Results of preoperative urine culture in the study participants

Gender	Positive culture		Negative culture		Total	
	No	%	No	%	No	%
Male	24	32	51	68	75	100
Female	8	53.33	7	46.66	15	100
Total	32	35.55	58	64.44	90	100

The postoperative urine culture was positive in 60 (92.3%) men and 5 (7.7%) women and negative in 15 (60%) male patients and 10 (40%) female patients (Table 3).

Table 3

Results of postoperative urine cultures in the study participants

Gender	Positive culture		Negative culture		Total	
	No	%	No	%	No	%
Male	60	80	15	20	75	83.4
Female	5	313	10	66.7	15	16.6
Total	65	100	25	100	90	100

In the postoperative catheter culture, there were positive results in 58 (85.6%) males and 9 (13.4%) females. Negative cultures were obtained in 17 (74%) males and 6 (26%) females (Table 4).

Table 4

Results of postoperative catheter culture in the study participants

Gender	Positive culture		Negative culture		Total	
	No	%	No	%	No	%
Male	58	77.4	17	22.6	75	83.4
Female	9	60	6	40	15	16.6
Total	67	74.5	23	25.5	90	100%

Regarding the density of bacterial colony growth in preoperative urine culture, 3 (10%) cases showed scanty growth, 2 (6.6%) cases showed moderate growth and 25 (83.4%) cases showed heavy growth. In the postoperative urine culture, 5 (7.2%) cases showed scanty growth, 12 (17.3%) cases moderate growth and 52 (75.5%) cases heavy growth. In the postoperative catheter culture, there were 4 (6.2%) cases with sustained scanty growth, 6 (9.2%) cases with moderate growth and 55 (84.6%) cases with heavy growth (Table 5).

Table 5

The density of bacterial colonization according to successive culturing

	Scanty		Moderate		Heavy		Total	
	No	%	No	%	No	%	No	%
Preoperative urine	3	10	3	6.6	26	83.4	32	100
Postoperative Urine	5	7.2	10	17.3	50	75.5	65	100
Postoperative Catheter	4	6.2	7	9.2	56	84.6	67	100

In the preoperative urine culture, mixed growth was found in 4 (12.5%) samples and a single microorganism in 28 (87.5%) samples. On the other hand, mixed growth was identified in 27 (42.5%) samples and a single microorganism in 38 (57.5%) samples of postoperative urine culture. In the postoperative catheter culture, 18 (25.7%) cases showed mixed growth and 45 (74.3%) cases a single microorganism (Table 6).

Table 6

Positive culture results in relation to catheter indwelling period

	Mixed M.O.		Single M.O.		Total	
	No	%	No	%	No	%
Preoperative urine culture	4	12.5	28	87.5	2	100
Postoperative Urine culture	27	42.5	58	57.5	5	100
Post-operative catheter culture	18	25.7	49	74.3	67	100

There is no direct relationship between the bacteria in the urine and the catheter bacteria because it does not appear as a simple linear relationship when the catheter bacteria is plotted against urinary bacteria.

Four species of gram-negative rods (GNR) and one species of gram-positive cocci (GPC) were isolated from a total of 184 isolates. Some samples had multiple bacterial species isolated from urine and cul-

ture. The most common species isolated were *Pseudomonas aeruginosa* (53 isolates). A total of 31 strains, 17 GNR and 14 GPC, were isolated from urine culture preoperatively. In 83 strains isolated from the post-operative urine culture, there were 71 GNR and 12 GPC. A total strain number of 54 GNR and 16 GPC were isolated from the catheter culture post-operatively (Table 7).

Table 7

Bacterial spp. isolated from urinary and catheter samples

Bacterial spp	Preoperative urinary culture		Post-operative cultures				Total	
	No	%	Urine		Catheter		No	%
			No	%	No	%		
<i>Escherichia coli</i>	2	8.4	10	41.6	12	50	24	100
<i>Acinetobacter spp</i>	0	0	12	66.5	6	33.5	18	100
<i>Pseudomonas aeruginosa</i>	7	13.2	26	49	20	37.8	53	100
<i>Staphylococcus aureus</i>	14	33.5	12	28.5	16	35	42	100
<i>unidentified spp</i>	8	17	23	49	16	34	47	100
Total	31	14	53	45	70	38	154	100

Discussion. The present study has demonstrated that not all species of bacteria colonizing the intraluminal surface of the urethral catheter can be detected as planktonic bacteria. The results have also demonstrated that the bacterial isolates from the urine and catheter were not always the same. All the urine samples were obtained from the catheter lumen; the samples, therefore, had direct contact with the intraluminal surface and attached bacteria if present. Despite this, not all the species of bacteria colonizing the lumen were detected in the urine. Our results are similar to the study conducted by Masanori et al [14] and Sauer K [16]. The authors have stated that bacterial biofilm in vitro exhibit several phases (early attachment, a robust structured form and later dislodging) which allows biofilm bacteria to form biofilms without shedding of planktonic counterparts.

The use of antibacterial agents might make the urine culture negative, while the catheter culture remains positive. Moreover, Guy S, have reported that biofilm bacteria are resistant to antimicrobial agents [6]. In the present study, the density of bacterial colonization was higher in catheter cultures. This is similar to the mentioned studies [14, 15]. The most commonly

encountered species in this study was *Pseudomonas aeruginosa* species, both in urine and catheter culture. In Masanori study [14], the most common species was *Enterococcus faecalis*. Sauer et al have found that *Staph. epidermidis* and *Strept. faecalis* were the most common microorganism. More gram-negative rods than gram-positive cocci were isolated from urine cultures. Bacteria might be able to colonize the intraluminal surface of the catheter, while being initially absent in the urine, indicating that they will eventually cause UTI. This is in accordance with the Masanori et al [14] and Riedle CR [17] studies. The origin of intra-luminal colonizing bacteria remains unclear. In addition, the natural course and pathological significance of intra-luminal colonizing bacteria are also unclear, but the results of this study seem to support the general approach of removing or changing a urinary catheter when treating UTI. From this study, it is postulated that bacterial colonization on the intra-luminal surface of the urethral catheter can predispose to the emergence of bacteriuria. The reasons are as follows:

- The growth of bacteria with urinary catheter usually become significant within a few days. This is similar to the Masanori [14] and Stark RP [18] results;

- Rectal and urethral meatal colonization often proceeds catheter-associated bacteria [6, 19, 20].

The bacteria isolated from the urine were not always recovered from the catheter as well [21-24]. The bacteria might have colonized another site, especially a distal portion of the catheter more than 5 cm from the proximal catheter end, which might be the route of intra-luminal bacterial entry. Alternatively, the bacteria might not have been attached to the catheter at all, implying a lack of surface attachment ability or a host inhibitory factors against bacterial attachment. The swab method was applied to minimize the chance of contamination from meatal or urethral colonization. Electron microscopy and staining are both accurate and reliable to detect attached bacteria. It is difficult to identify the microorganism using these techniques [25-27].

Study limitations. Short study time, small sample size and crowding of responsibilities of researchers.

Conclusion. Not all species of bacteria colonizing the intraluminal surface of the urethral catheter can be detected as planktonic bacteria. The urine and catheter bacterial populations differ from each other without

the influence of antibacterial agents. The use of antibacterial agents might make the urine culture negative, whereas the catheter culture remains positive. The density of bacterial colonization was found to be higher in catheter culture. The absence of urinary bacteria does not always indicate the absence of bacteria colonizing in the urethral catheter tip.

Conflicts of Interest. The authors declare no conflict of interest.

Contribution.

Ashraf Ibrahim Mohammed Hassan: conceptualization, data curation, formal analysis, methodology, supervision, validation, visualization, writing, original draft preparation and editing;

Bashar M Al-Hammodi: funding acquisition, investigation, methodology, project administration, review and editing;

Ramzi Mowffaq Ramzi: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, validation, visualization, writing, original draft preparation, review and editing.

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