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Original Article

Antibiotic resistance patterns of uropathogens isolated from catheterized and noncatheterized patients in Dhaka, Bangladesh

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Objectives: Catheter-associated urinary tract infection (CAUTI) is the most common device-associated nosocomial infection worldwide. Bacteria, which exist as a biofilm inside catheters, show higher antimicrobial resistance when compared to non-CAUTI pathogens. The present study was conducted to determine the antibiotic susceptibility patterns of CAUTI and non-CAUTI bacteria.

Materials and Methods: The antibiotic susceptibility patterns of 102 uropathogens from noncatheterized patients and 100 uropathogens from catheterized patients were compared using the disc diffusion method.

Results: A higher incidence of uropathogens was correlated with catheter use in male patients. *Escherichia coli* was the predominant isolate obtained from catheterized (81%) and noncatheterized (67%) patients. This was followed by *Pseudomonas aeruginosa*, with rates of 28% and 15% in non-CAUTI and CAUTI patients, respectively. Overall, the *E. coli* isolates from CAUTI patients showed significantly higher resistance ($p < 0.05$) than those from non-CAUTI patients against all antibiotics tested, except for trimethoprim/sulfamethoxazole and gentamicin. Catheter-associated *P. aeruginosa* isolates showed significantly higher resistance ($p < 0.05$) against most antibiotics tested compared to non-catheter-associated isolates.

Conclusion: Uropathogens from CAUTI patients exhibit significantly higher resistance to most antibiotics than non-CAUTI isolates. This is an important factor to take into consideration when choosing correct treatment options for patients with urinary tract infection.

1. Introduction

Urinary tract infection (UTI) is a serious health problem affecting millions of individuals worldwide. It is estimated that about 150 million cases of UTI occur in the world every year [1–3]. UTI is one of the most common bacterial infections in many developing countries, including Bangladesh. UTIs affect all age groups and are diagnosed in both hospitalized patients and outpatients. This type of infection causes a serious burden on the socioeconomic life of individuals and leads to the consumption of a large proportion of all antibacterial drugs used in the world [4]. Women are more susceptible to UTIs than men due to the anatomical structure of their genitourinary tract [5]. It has been observed that up to one-third of all women experience a UTI at some point during their lifetime [6]. The rate of resistance to antibiotics among community-acquired UTIs is increasing and shows significant geographical variations [7]. Updated knowledge on the diverse etiology of UTIs and the resistance against antibiotics of the causative organisms is important to clinicians when treating such patients.

UTI is the most common infection among patients who have a chronic indwelling bladder catheter, which may cause bacteriuria [8–10]. Catheter-associated urinary tract infection (CAUTI) remains the commonest nosocomial infection worldwide. It is also the most common nosocomial infection in hospitals and nursing homes, accounting for >40% of all institutionally acquired infections [11,12]. The presence of a biofilm plays a central role in the pathogenesis of CAUTI [13]. CAUTIs are a cause for concern because catheter-associated bacteriuria comprises a huge reservoir of resistant pathogens in the hospital environment [14].

Conflicts of interest: none.

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This study was performed in order to isolate and identify the common pathogenic bacteria present in catheterized and noncatheterized patients hospitalized in Dhaka city, Bangladesh and to determine the antibiotic sensitivity patterns of these isolates against commonly prescribed antibiotics.

2. Materials and methods

2.1. Collection of samples

Urine samples were collected from patients who reported to Sohrawardi Hospital and Dhaka Medical College Hospital, Dhaka City, Bangladesh. Two types of patients, with and without an indwelling catheter, were included in this study when they reported to the abovementioned hospitals. In case of noncatheterized patients, mid-stream urine samples were collected. Among the catheterized patients, the help of a health technician was needed to collect the urine sample.

2.2. Isolation of pathogenic bacteria

A measured loop was used to streak 10 μL of each sample separately on blood agar and MacConkey agar plates, which were then incubated at 37°C overnight. Suspected bacterial colonies were identified using standard biochemical tests. All samples with a bacteriuria of $>10^4$ colony-forming units/mL urine of one or two organisms were analyzed to determine the causative uropathogens. From a total of 202 samples, 102 uropathogens were isolated from noncatheterized patients and 100 from catheterized patients.

2.3. Biochemical identification of isolated bacteria

Isolates were identified by standard biochemical tests, including the oxidase test, the Simmon's citrate agar test, the motility iodole urease test, growth on Kligler's iron agar, the coagulase test, and the catalase test [15].

2.4. Antibiotic sensitivity test

Three to five well-isolated colonies were transferred into a tube containing 4–5 mL of Trypticase Soy Broth (TSB, Oxoid, UK) and incubated at 35°C until the turbidity reached the 0.5 McFarland standard. A sterile cotton swab was dipped into the adjusted suspension. The dried surface of a Müeller–Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. Commercially available antibiotic discs were used, namely amikacin 30 μg, ciprofloxacin 5 μg, clindamycin 2 μg, rifampicin 5 μg, cefuroxime 30 μg, trimethoprim/sulfamethoxazole 1.25/23.75 μg, erythromycin 15 μg, gentamycin 10 μg, penicillin 10 units, amoxicillin 500 μg, amoxicillin/clavulanate 20/10 μg, cefixime 30 μg, cefeprime 30 μg, vancomycin 30 μg, linezolid 30 μg, ceftriaxone 30 μg, ceftazidine 30 μg, meropenem 10 μg, pipercillin–tazobactam 100/10 μg, nitrofurantoin 300 μg, carbenicillin 100 μg, tobramycin 10 μg, aztreonam 30 μg, and colistin 10 μg, all with a correct diameter and potency. Stocks of antibiotic discs were stored at −20°C. The plates were incubated at 35°C for 18–24 hours. The sizes of the zones of inhibition were interpreted according to the Clinical Laboratory Standards Institute guidelines (2011).

2.5. Novobiocin disc sensitivity test

Colonies from a pure culture diluted into TSB to a 0.5 McFarland standard was inoculated by swabbing on Müeller–Hinton agar. When the surface was dry, a novobiocin disc was placed in the center. The plate was incubated for 24 hours at 37°C. A zone size ≤16 mm indicates resistance to novobiocin. A zone size of 17 mm or larger indicates susceptibility to novobiocin [16].

2.6. Statistical analysis

Statistical analysis was carried out using the online software available at http://www.socscistatistics.com/. Tests of proportions were performed using the z test. The level of significance in the two-tailed z test was set at $\alpha = 0.025$.

3. Results

A total of 202 samples, 102 from noncatheterized patients with UTI (Category A, non-CAUTI) and 100 from catheterized patients with UTI (Category B, CAUTI), were included in this study to determine any significant correlation between the drug resistance patterns of these two groups. The ages of participants ranged from 1.5 years to 89 years for the former category and from 15 years to 85 years for the latter category.

3.1. Distribution of UTI across the two sexes

Table 1 depicts the occurrence of the isolates across the two genders. A significant difference was found with respect to the occurrence of antibiotic-resistant pathogens between males and females in the noncatheterized group ($p < 0.05$), with females having a greater incidence rate than males. By contrast, there was no significant difference between the occurrence of multidrug resistant (MDR) uropathogens in males and females in the catheterized group ($p > 0.05$). A significantly higher incidence of UTI with MDR uropathogens was observed in females who were noncatheterized than in those who were catheterized ($p < 0.05$). By contrast, males who were catheterized showed a significantly higher occurrence of MDR uropathogens than those who were not catheterized ($p < 0.05$).

3.2. Presence of pathogenic bacteria in CAUTI and non-CAUTI patients

The overall distribution of the uropathogens is presented in Fig. 1A and B for non-CAUTI and CAUTI patient samples, respectively. In both cases, *E. coli* was the predominant isolate, making up 67% and 81% of the total isolates from non-CAUTI and CAUTI patients, respectively. This was followed by *P. aeruginosa*, which was found in 28% and 15% of the total isolates from the non-CAUTI and CAUTI patients, respectively. Gram-positive organisms made up only 2% and 3% of the total isolates from the non-CAUTI and CAUTI patients, respectively (Fig. 1A and B). *Streptococcus agalactiae* was isolated only from noncatheterized patients, and *Staphylococcus saprophyticus* was unique to catheterized patients.

The two predominant isolates, *E. coli* and *P. aeruginosa*, were tested for their susceptibility pattern to 11 commonly used

Table 1
Distribution of infection in the study samples with respect to sex.

Sample type	Sex	No. of UTI cases	% of UTI cases	p (z test)[a]
Noncatheter	Female	57	55.88 (a)	<0.05 (between a and b)
	Male	45	44.12 (b)	<0.05 (between b and d)
Total		102	100%	
Catheter	Female	46	46 (c)	<0.05 (between a and c)
	Male	54	54 (d)	>0.05 (between c and d)
Total		100	100	

UTI = urinary tract infection.
[a] The proportions (%) of males and females were compared using a z test. The level of significance in the two-tailed z-test was set at $\alpha = 0.025$.

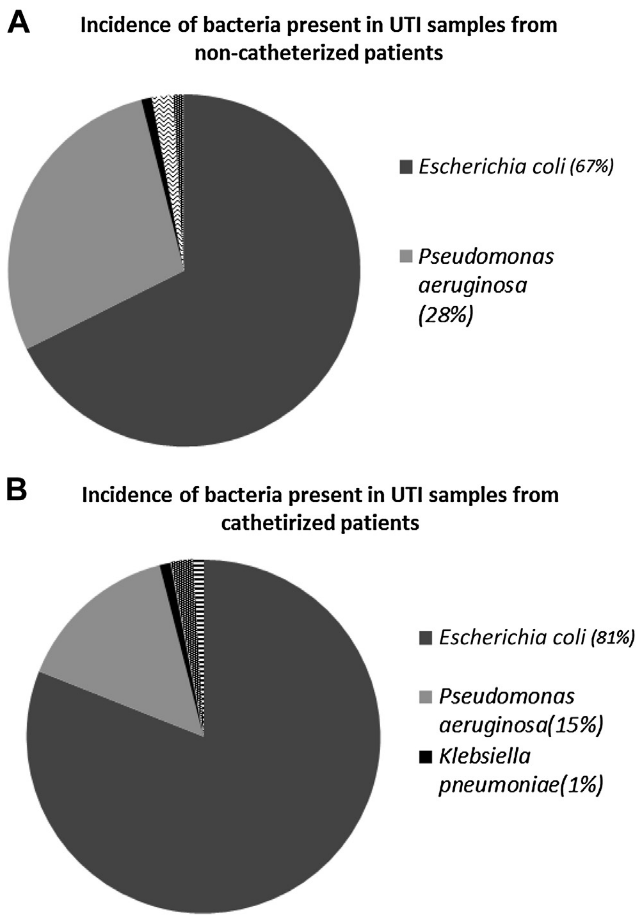

Fig. 1. Distribution of uropathogens in the clinical samples from (A) non-CAUTI and (B) CAUTI patients. CAUTI = catheter-associated urinary tract infection; UTI = urinary tract infection.

antibiotics. Overall, *E. coli* isolates from the CAUTI patients showed significantly higher resistance than those from the non-CAUTI patients when tested against all antibiotics, except for amikacin (Table 2). In contrast to *E. coli*, catheter-associated *P. aeruginosa* showed significantly higher resistance among the non-catheter-associated isolates when tested against all antibiotics (Table 2).

3.3. Distribution of MDR bacteria across different age groups of CAUTI and non-CAUTI patients

MDR *E. coli* and *P. aeruginosa* were present among isolates belonging to all age groups (Figs. 2A, B and 3A, B). The highest number of MDR *E. coli* was found in the age group of 21–30 years for both catheterized and noncatheterized patients. The highest proportion of MDR *P. aeruginosa* was found in CAUTI-patients aged 21–30 years, and a similar trend was observed for *E. coli*. However, the number of MDR *P. aeruginosa* isolates was highest among 31–40-year-old noncatheterized patients.

3.4. Occurrence of MDR bacteria in CAUTI and non-CAUTI patients

Resistance against 10 antibiotics was found to occur at a rate of 1% among the 70 catheter-associated *E. coli* isolates. Of the MDR isolates from the same category, 7% were resistant to a minimum of two antibiotics (cefexime and ciprofloxacin) tested. A maximum of 26% of the isolates from this group was resistant to seven out of the 10 antibiotics tested. Among the catheter-associated *P. aeruginosa* isolates, resistance to a maximum of 10 and to a minimum of two antibiotics (cefexime and ceftazidime) was also examined. A maximum of 27% ($n = 15$) of *P. aeruginosa* isolates was resistant to nine antibiotics and a corresponding a minimum of 7% was found to be resistant to two of the antibiotics tested. In case of non-CAUTI *E. coli* isolates, resistance to a maximum of 10 and a minimum of two antibiotics (cefexime and ciprofloxacin) was observed; the highest proportion of MDR *E. coli* in noncatheterized patients was 36% (resistant to 4 antibiotics) and the lowest was 2% (resistant to 2 antibiotics). In case of *P. aeruginosa* from the same source, resistance to up to 10 antibiotics was observed in 7% ($n = 9$) of the isolates. The maximum and minimum proportions of MDR *P. aeruginosa* were 21% and 7%, which were resistant to three and 10 antibiotics, respectively.

4. Discussion

UTI is considered to be a common health problem and inflicts 150 million people worldwide every year [1]. Infections may or may not be associated with the use of a catheter. The urinary catheter is one of the most frequently used invasive medical devices. Patient exposure may be exceedingly short, involving only a single catheterization, or may be continued for years in individuals with chronic indwelling catheters. Biofilm formation along the catheter means that bacteriuria develops in a predictable manner if the

Table 2
Antibiotic resistance patterns of *Escherichia coli* and *Pseudomonas aeruginosa* isolates from catheterized and noncatheterized patients.

Antibiotic	Resistant/total isolate (%) of *E. coli*		p[a]	Resistant/total isolate (%) of *P. aeruginosa*		p[a]
	CAUTI	Non-CAUTI		CAUTI	Non-CAUTI	
Amikacin	14/81 (17.28)	35/68 (51.47)	<0.05	6/15 (40.00)	6/28 (21.43)	<0.05
Amoxicillin/clavulanate	68/79 (86.07)	38/68 (55.88)	<0.05	12/14 (85.71)	24/28 (85.71)	<0.05
Azithromycin	20/32 (62.5)	22/48 (45.83)	<0.05	2/3 (66.67)	8/19 (42.11)	<0.05
Cefixime	60/80 (75.00)	44/70 (62.86)	<0.05	15/15 (100)	20/28 (71.42)	<0.05
Ceftazidime	61/81 (75.31)	37/67 (55.22)	<0.05	11/15 (73.33)	17/28 (60.71)	<0.05
Ceftriaxone	57/81 (70.37)	35/68 (51.47)	<0.05	11/15 (73.33)	17/28 (60.71)	<0.05
Ciprofloxacin	46/74 (62.62)	32/68 (47.05)	<0.05	10/13 (76.92)	14/28 (50.00)	<0.05
Trimethoprim/sulfamethoxazole	52/81 (64.19)	35/69 (50.72)	<0.05	13/15 (86.66)	19/27 (70.37)	<0.05
Doxycycline	53/80 (66.25)	38/69 (55.07)	<0.05	12/15 (80.00)	17/26 (65.38)	<0.05
Gentamicin	35/64 (54.68)	34/68 (50.00)	<0.05	8/15 (53.33)	13/29 (44.83)	<0.05
Meropenem	14/23 (60.87)	1/6 (14.29)	<0.05	6/6 (50.00)	7/21 (33.33)	<0.05

CAUTI = catheter-associated urinary tract infection.
[a] The proportions of antibiotic resistance (%) were compared between the catheter- and noncatheter-associated isolates using the z test. The level of significance in the two-tailed z test was set at $\alpha = 0.025$.

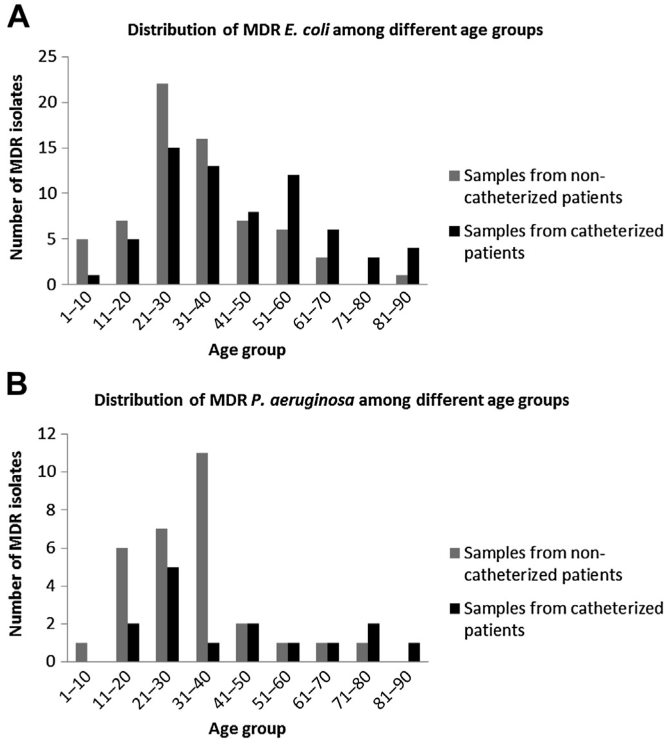

Fig. 2. Distribution of (A) MDR *Escherichia coli* and (B) MDR *Pseudomonas aeruginosa* in the urinary tract infection samples. MDR = multidrug resistant.

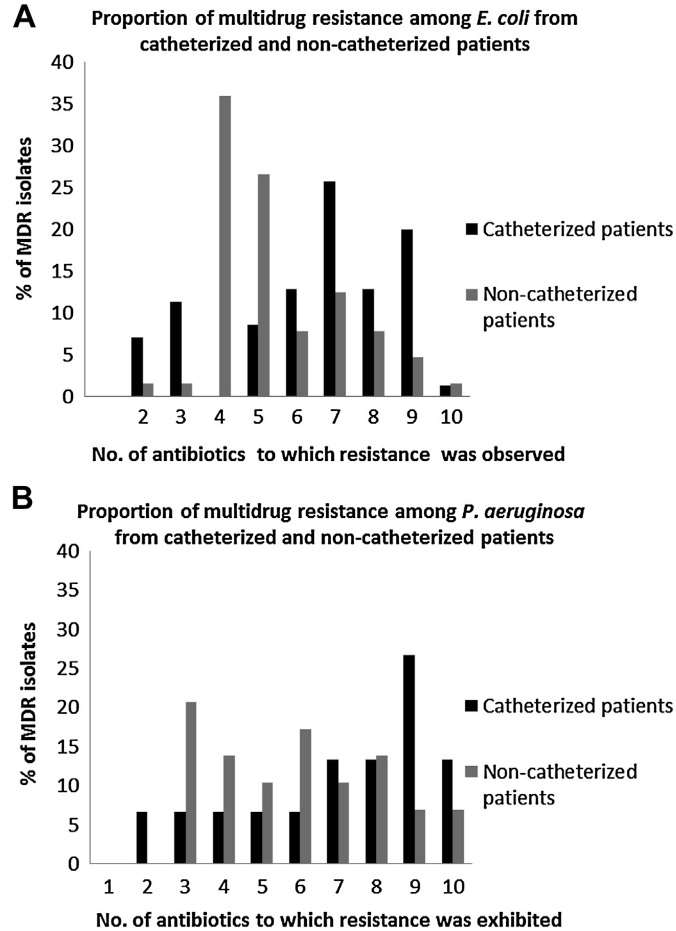

Fig. 3. Distribution of (A) MDR *Escherichia coli* and (B) MDR *Pseudomonas aeruginosa* among catheterized and noncatheterized patients. MDR = multidrug resistant.

indwelling catheter remains *in situ*. However, symptomatic infection is relatively uncommon among patients who are being managed with short-term catheters. The patterns of antimicrobial resistance associated with UTI vary across the different regions of the world. UTI is common in Bangladesh and has been reported to affect all age groups of individuals, both hospitalized patients and outpatients [17].

The occurrence of antibiotic-resistant pathogens at a higher frequency in females than in males may be due to the fact that females are more susceptible to UTI because of their anatomy than males, irrespective of the use of a catheter [5,6]. No significant difference was observed between the occurrence of MDR uropathogens in males and females of the catheterized group ($p > 0.05$). Additionally, males who were catheterized showed a significantly higher occurrence of MDR uropathogens than those who were not catheterized ($p < 0.05$). The higher incidence of MDR uropathogens in males who were catheterized compared to the incidence in either females or males in the other category possibly indicates the effect of catheterization on the presence of MDR isolates. A catheter provides an artificial surface for biofilm formation, and this has been reported to bring about increased drug resistance [13,14,18]. However, a similar effect was not evident in females. In this study, the incidence of MDR bacteria was significantly higher ($p > 0.05$) among noncatheterized patients in the age group of 1–40 years. This was different from the situation among catheterized patients, where the pattern showed a significantly higher incidence in the age group of 41–90 years ($p > 0.05$). This could be due to the administration of antibiotics during catheter use.

The types of uropathogens examined in this study varied slightly between the noncatheterized and catheterized patients. Among the non-catheter-associated isolates *E. coli* was the most predominant uropathogen (67%), followed by *Pseudomonas* spp. (28%), *Klebsiella pneumoniae* (1%), and *S. agalactiae* (2%). Among the catheter-associated isolates, *E. coli* again was the major pathogen (81%), followed by *Pseudomonas* spp. (15%), *S. agalactiae* (2%), and *K. pneumoniae* (1%). The prevalence of uropathogenic organisms has been found to be remarkably consistent, with Gram-negative organisms accounting for most infections [19]. A number of other studies have also found that *E. coli* is usually the predominant uropathogen, for example, *E. coli* made up 70% of isolates in a study conducted in India [13], 38.7% in a study conducted in Korea [20], 31.5% in a study conducted in Tamil Nadu, India [21], 21.5% in a study conducted in Nigeria [22], 53.24% in a study conducted in South Jordan [23], 80% in a study conducted in Canada [24], and 74% in a study conducted in Bangladesh [25]. Baron et al [26] also reported that *E. coli* is the most common organism associated with UTI with respect to both community-acquired and hospitalized cases. In accordance with the present investigation, another recent study conducted in Bangladesh has also found *E. coli* to be the major pathogen associated with UTI [17].

The rate of UTI, particularly that caused by *E. coli*, was higher in patients of reproductive age (21–30 years) irrespective of catheterization. A higher percentage of *P. aeruginosa* was also found among catheterized patients in the same age group. Similar findings have been reported in other studies [17,27,28] conducted in Bangladesh, as well as elsewhere in the world [29]. The high occurrence of UTI at this age may be due to the initial exposure of these people to sex and a lack of sufficient knowledge of hygiene practices [17].

Antibiotic resistance is a major clinical problem when treating infections caused by microorganisms. Resistance to antimicrobials has increased over the years. Resistance rates vary from country to country [30,31]. Overall, isolates from Latin American countries have shown the highest resistance rates against all antimicrobial agents, followed by Asian-Pacific isolates and European strains, whereas the strains from Canada exhibit the lowest resistance pattern [30,31]. In this study, antibiotic resistance was significantly higher among catheterized patients than among noncatheterized patients for both *E. coli* and *P. aeruginosa* ($p < 0.05$). Previous studies conducted in Bangladesh also indicated increasing resistance of uropathogens to most antibiotics [17,27,32–34]. As with the present study, a pattern of higher resistance was observed for CAUTI uropathogens than for non-CAUTI isolates [13,35].

With regard to the treatment of UTI, the optimal duration of antimicrobial therapy is still an unresolved question [36]. Levofloxacin has been suggested to be as effective for clinical outcomes as ciprofloxacin at the right dose [37]. The efficacy of beta-lactam antibiotics has not been investigated adequately. They are said to be less effective than trimethoprim. Quinolones and nitrofurantoin require further study prior to when any conclusion can be drawn with regard to their treatment efficacy [38]. In the present study, all the *E. coli* isolates from CAUTI cases showed resistance to all antibiotics tested, except amikacin. In case of *P. aeruginosa*, all the catheter-associated isolates exhibited resistance to all antibiotics tested. Based on the present study, uropathogens from CAUTI and non-CAUTI patients showed variations in their resistance pattern to different antimicrobials, indicating that the same treatment cannot be applied to all cases. The antibiogram also varies depending on the causative organism. From the present study, it appears that new guidelines and treatment regimens need to be identified for UTI patients irrespective of catheter use. Whether levofloxacin or ciprofloxacin can still remain the antimicrobial of choice for effective treatment of UTI remains enigmatic. It is expected that the results from studies similar to the present research will aid in the development of guidelines for the prevention of UTIs. With emerging knowledge on antibiotic resistance and health care-associated infection, guidelines need to be updated to reflect the need to prescribe narrow-spectrum agents when available and avoid empirical use of broad-spectrum antibiotics.

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