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Diversity of Bacteria Isolated From Long- and Short-term Catheterized Patients

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ABSTRACT

***Background*:** The objectives of this study were to assess the antibiotic resistance and clonality of the bacteria isolated from patients with long- (LTC) and short-terms catheterizations (STC).
***Methods*:** A total of 31 clinical *Staphylococcus aureus* isolates were collected from Loghman Hospital of Tehran, Iran. In vitro biofilm formation ability was determined by microliter tissue culture plates. All clinical isolates were examined for determination the *ica* locus by using PCR method.
Results: Ninety seven (62%) of the samples were bacterial positive. Positive samples were significantly higher in LTC (95%) than STC (61%) patients. *Escherichia coli* were the predominant microorganism (32%) followed by *Klebsiella pneumoniae* (15%), *Pseudomonas aeroginosa* (11%), *Enterococcus faecalis* (9.2%). From the total isolates, 42% were resistant to 5 or more antibiotics. Furthermore, high prevalence of resistance amongst all isolates to ciprofloxacin (49%) was observed.
***Conclusions*:** Diverse bacterial clones were observed for LTC and STC patients. Overall, the results suggested that catheterization could be a major source for growth and dissemination of highly resistant and diverse bacterial species in the hospitals.

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Introduction

Catheter associated urinary tract infections (CAUTI) are the most common type of nosocomial infection (1) which have become major concern in patient treatment, long hospitalization and health care cost (27). It has been suggested that the rate of infection in individuals with an indwelling urinary catheter increases between 3 to 10% each day and accounting for more than 1 million cases each year in US hospitals (4). Number of factors affect the level and extend of CAUTI such as method and duration of catheterization, the quality of catheter care and host susceptibility (3). The patients with long catheterization (≥30 days), even with an excellent care, may become bacteriuric and majority of short-term catheterized (1-4 days) patients also could become bacteriuric 30 days post indwelling. Among various molecular typing methods, pulsed-field gel electrophoresis (PFGE) has been accepted as a method of choice for confirming bacterial relatedness in outbreaks. The use of PFGE in hospital infections is still under intensive investigations worldwide especially for CAUTI (5). The aim of this study was to determine the bacterial infections in short- (STC) and long-term catheterized (LTC) patients and to determine the antibiotic resistance and clonal diversity of the bacterial isolates and the applicability of PFGE in this setting.

Material and method

Bacterial isolates

The urine samples (156) were collected from LTC (62) and STC (94) patients from December 2009 to August 2010. Patients with STC and LTC were catheterized for at least 24h and 30 days or more, respectively. The isolates were obtained from 5 hospitals, 2 major hospitals and 3 care institutions that are devoted to hospitalization of the veteran patients with spinal cord injuries in Tehran, Iran. Urine specimens were plated onto blood agar and Mac Conkey agar and incubated for 24h at 37ºC the organisms were identified by biochemical tests. All isolates with bacteriuria (≥100,000 colony forming units /ml) containing one or two pure bacteria were considered as uropathogens. The inclusion criterion for patients to enter the study was lack of previous UTI in the last 6 months.

Antibiotic resistance

Antimicrobial susceptibility test was performed and interpreted according to the guidelines of the Clinical Laboratory Standards Institute (5). The following antibiotic disks were used for Gram negative *Enterobacteriaceae*: ampicillin (10 µg), tetracycline (30 µg), cephalexin (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), ceftizoxime (30 µg), cefepime (30 µg), gentamicin (120 µg), chloramphenicol (30 µg), amikacin (30 µg), nitrofurantoin (300 µg). For Pseudomonas sp, ciprofloxacin (5 µg), cotrimoxazole (25 µg), cefepime (30 µg), gentamicin (120 µg), chloramphenicol (30 µg), amikacin (30 µg), carbenicillin (100 µg), cephotaxime (30 µg), tobramycin (10 µg), ceftazidime (30 µg), azlocillin (75 µg), imipenem (10 µg) and for Gram positive cocci, ampicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamicin (120 µg), chloramphenicol (30 µg), vancomycin (30 µg), erythromycin (15 µg), dalfopristin-quinupristin (15 µg), linezolid (30 µg), teicoplanin (30 µg) and oxacillin (30 µg) were used. All of antibiotic discs were purchased from Mast Diagnostics Ltd. (Bootle, UK).

PFGE

PFGE was performed for all of the bacterial species with more than 10 isolates on a CHEF-DR II apparatus (Bio-Rad Laboratories Richmond, USA). Briefly, after digestion with *Xba*I and *Sma*I for Gram negative and positive bacteria, respectively, genomic DNA was separated by electrophoresis (7, 8).

The banding patterns were interpreted by Dice analysis and clustered by the unweighted pair group method with arithmetic averages with Gel compar II version 4.0 (Applied Maths, Sint-Matens-Latem, Belgium).

Statistical Analysis

T- test was used to compare the significance of difference between samples.

Result

Bacterial Isolates

Out of 156 specimens, 97 (62 %) were bacteria positive amongst which 85 (87.6 %) and 12 (12.4%) specimens were single and mixed infections. Bacterial isolates were obtained from LTC and STC constituted about 42 (67.7%) and 55 (58.5%) of cases, respectively. Yeasts were isolated in 20 (12.8%) specimens with no bacterial contamination. Amongst these specimens, 17 (27%) and 3 (3%) yeasts were found in LTC and STC patients. Nineteen different bacterial species were identified in the samples taken from LTC and STC patients. Escherichia coli were the most frequently isolated uropathogens (32.1%), followed by *Klebsiella pneumoniae* (14.7%), Enterococcus sp (14.7%), and *Pseudomonas aeruginosa (*11.1%) (Table 1).

Antibiotic Resistance

Antibiotic susceptibility results showed that *E. coli* strains were highly resistant to ampicillin (91%) and tetracycline (83%). amikacin (20%) and nitrofurantoin (20%) were the most active agents against *E. coli* infections. *K. pneumoniae* isolates demonstrated high antimicrobial resistance to ampicillin (100%). Antibiotic resistance of *K. pneumonia* to other antibiotics was not significant. *P. aeruginosa* showed resistant to carbaornicillin (100%), cotrimoxazole (66.7%) and cefotaxime (75%). All *P. aeruginosa* were sensitive to imipenem. Although the number of isolated E. faecium was low, 40% of the isolates were resistant to vancomycin (Table 2). No resistance to teicoplanin, linezolid and ampicillin were found amongst E. faecalisand *S. epidermidis*. All of S. epidermidis isolates were resistant to tetracycline, ciprofloxacin, ampicillin, and oxacillin. No *S. aureus* resistance to vancomycin, gentamicin, teicoplanin, linezolid and synercid was observed. All of *S. aureus* strains were resistant to ampicillin (100%).

Table 1: Bacteria isolated from LTC and STC patients

Organisms	Short- term	Long- term	Total
	Isolates No. %	Isolates No. %	Isolates No. %
E. coli	14(40.0)	21 (60.0)	35 (32.1)
K. pneumonia	5(31.2)	11 (68.8)	16 (14.7)
P. aeruginosa	9 (75)	3 (25)	12 (11.1)
E. faecalis	4(40)	6 (60)	10 (9.2)
E. faecium	5(83.3)	1 (16.7)	6 (5.5)
Diphteroid	2(33.3)	4 (66.7)	6 (5.5)
M. morganii	0	4 (100)	4 (3.7)
S. aureus	3(100)	0	3 (2.8)
E. cloacae	2(100)	0	3 (2.8)
S. epidermidis	2(100)	0	2 (1.8)
A. baumanii	0	2 (100)	2 (1.8)
K. oxytaca	0	2 (100)	2 (1.8)
P. alkaligenes	0	2(100)	2 (1.8)
E. aerogenes	2(100)	0	1 (0.9)
C. fruendii	1(100)	0	1 (0.9)

PFGE Patterns

From the total of 35 *E. coli* isolates, 26 PFGE patterns were observed. Eight common types (CT) with 2 or 3 members and 18 single types (ST) were seen. Four (57%) and 3 (43%) isolates from LTC and STC, respectively, were in the same CT (H, N, R) (Fig. 1). Pulsotypes G, I, and T for LTC and W and Y pulsotypes for STC patients contained 2 isolates each; the rest of pulsoytpes contained only one isolate. Sixteen *K. pneumoniae* isolates showed 14 PFGE patterns.

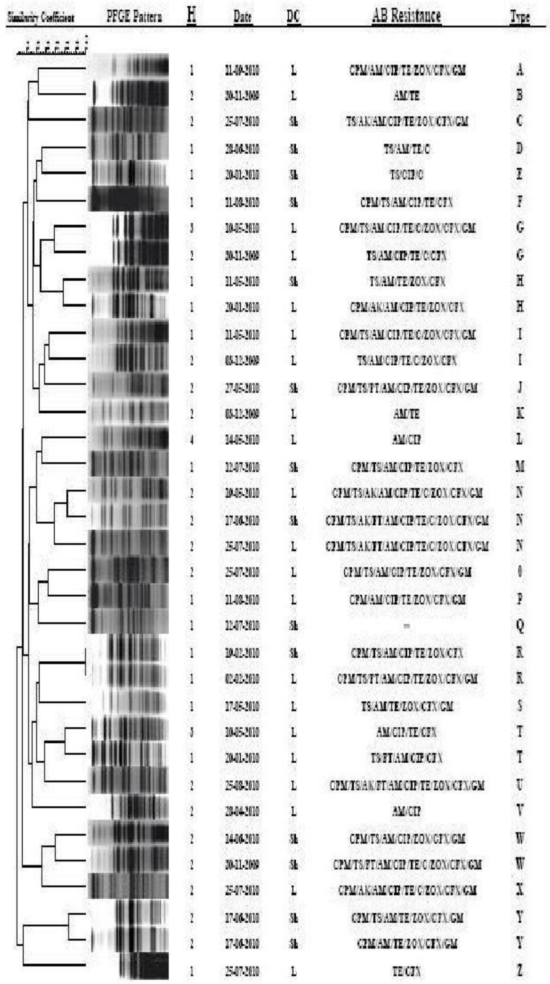

Figure 1. PFGE analysis of *E. coli* strains isolated from LTC and STC patients. Abbreviations: H: Hospitals ; DC: Duration of Catheterization; L: Long-term; Sh: Short-term; Different Hospitals 1-5; CPM: cefepime; TS: cotrimoxazole, AK: amikacin, AM: ampicillin, CIP: ciprofloxacin, Tetracyclin, C: chloramphenicol, ZOX: ceftizoxime, cephalexin, GM: gentamicin, FT: nitrofurantoin.

Two common types (J, O) with 2 isolate each and 12 STs were observed. Amongst the isolates obtained from LTC, 3 and 8 isolates were from CT (J and O) and ST, respectively. In STC, 1 and 4 isolates were from CT (J) and ST, respectively (Figure. 2).

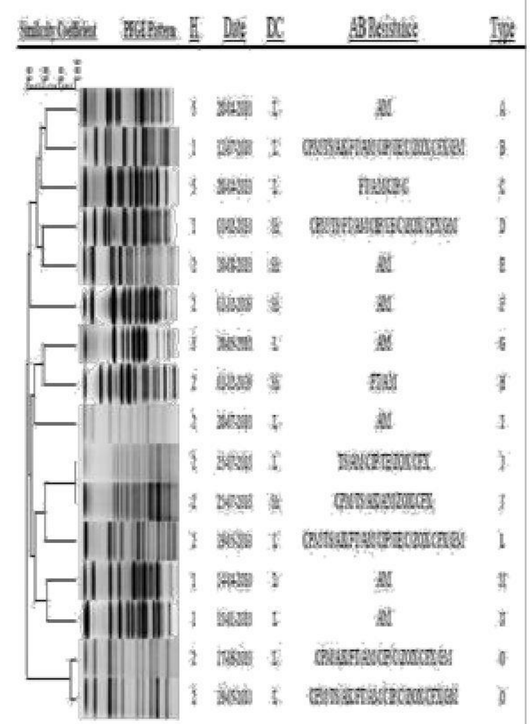

Figure 2. PFGE analysis of *K. pneumonia* strains isolated from LTC and STC patients Abbreviations: H: Hospitals; DC: Duration of Catheterization; L: Long-term; Sh: Short-term Different Hospitals 1-5; CPM: Cefepime, TS: Cotrimoxazole, AK Amikacin, AM: Ampicillin, CIP: Ciprofloxacin TE: Tetracyclin, C: Chloramphenicol, ZOX: Ceftizoxime, CFX: Cephalexin, GM: Gentamicin, FT: Nitrofurantoin.

Table 2: Clinical characteristics and different organ involvements of the patients with EPTB referred to Tabriz Tuberculosis and Lung Disease Research Center from 2007 to 2011

	E. coli		***K. pneumoniae***		***P. aeruginosa***		***E. faecalis***		***E. faecium***	
Antibiotics	Short-term	Long-term	Short-term	Long-term	Short-term	Long-term	Short-term	Long-term	Short-term	Long- term
Ampicillin	12(85.7)	20(95)	5(100)	11(100)	—	—	0	0	5(100)	1(100)
Tetracycline	11(78.6)	18(85.7)	1(20)	3(27.3)	—	—	4(100)	5(83.3)	4(80)	1(100)
Cephalexin	11(78.6)	17(81.0)	2(40)	5(45.4)	—	—	—	—	—	—
Ciprofloxacin	9(64.3)	17(81.0)	1(20)	6(54.5)	4(44.4)	0	3(75)	2(33.3)	5(100)	1(100)
Cotrimoxazole	12(85.7)	11(52.4)	2(40)	4(36.4)	6(66.7)	2(66.7)	—	—	—	—
Ceftizoxime	10(71.4)	13(62.0)	2(40)	5(45.4)	—	—	—	—	—	—
Cefepime	9(64.3)	11(52.4)	2(40)	4(36.4)	5(55.6)	0	—	—	—	—
Gentamicin	7(50)	10(47.6)	1(20)	4(36.4)	5(55.6)	0	3(75)	1(25)	3(60)	1(100)
Chloramphenicol	4(28.6)	7(33.3)	1(20)	5(45.4)	4(44.4)	0	1(25)	0	4(80)	1(100)
Amikacin	2(14.3)	5(23.8)	1(20)	4(36.4)	5(55.6)	0	—	—	—	—
Nitrofurantoin	3(21.4)	4(19.0)	2(40)	5(45.4)	9(100)	3(100)	—	—	—	—
Carbenicillin	—	—	—	—	7(77.8)	2(66.7)	—	—	—	—
Cefotoxime	—	—	—	—	5(55.6)	0	—	—	—	—
Tobramycin	—	—	—	—	5(55.6)	0	—	—	—	—
Ceftazidime	—	—	—	—	5(55.6)	0	—	—	—	—
Azlocillin	—	—	—	—	0	0	—	—	—	—
Imipenem	—	—	—	—	—	—	—	—	4(80)	—
Erythromycin	—	—	—	—	—	—	4(100)	4(66.7)	2(40)	1(100)
Vancomycin	—	—	—	—	—	—	0	0	2(40)	0
Teicoplanin	—	—	—	—	—	—	0	0	0	0
Lynezolid	—	—	—	—	—	—	0	0	0	0
Synercid	—	—	—	—	—	—	0	0	0	0
Oxacillin	—	—	—	—	—	—	—	—	—	—

Type J was shared between LTC and STC. Among 12 *P. aeruginosa* isolates, 8 PFGE patterns were observed. Four CTs with 2 members each (A, B, C, F) and 4 STs were observed. Six isolates obtained from STC fell in CTs of B, C and F pulsotypes (2 each) (fig. 3), whereas only 3 isolates from LTC was found in A, G, F (1 each) pulsotypes. All E. faecium and 80% of E. faecalis isolates obtained from STC and LTC were STs (data not shown).

Discussion

In this study, 68% out of 94 specimens from LTC patients were bacterial positive culture and 27% were positive for yeast. In STC patients 58.5 and 3% of the specimens were positive for bacteria and yeasts, respectively. It was apparent that the patients with LTC were more susceptible to yeast infections. In addition, the results showed that LTC patients were more significantly ($P<0.05$) prone to bacterial infections than STC patients. Moreover, a significant number of mixed bacterial infections (92%) were isolated from LTC ($P<0.005$) as compared to STC (8%) patients. Many investigators have reported that only 5% of urine specimens from LTC patients were single infection and up to 95% were mixed infections (9). In this report, on the other hand, we found only 20% of our LTC patients to carry mixed infections. Such difference between various reports could be, in part, due to prevalence of hospital infections, hospital care and personal hygiene. However, similar to other studies, our results from STC patients showed that the majority of specimens were single infection. This may suggest that the short-term CAUTI were not subjected to variations that affected LTC patients. Comparison of the bacterial species between LTC and STC showed that *E. coli*, *Enterococcus*, *Pseudomonas*, *K. pneumoniae* were common in both patients. As expected, these normal bacteria flora dominated the infections caused in both groups of catheterized patients. Other species such as *M. morganii*, *Acinetobacter* and *K. oxytoca* were found only in LTC patients. The data show that even rare hospital infections such as *K. oxytoca* could colonize in patients with long term catherization. Moreover, our results showed that *S. aureus* were found in STC patients. The reason(s) that *S. aureus* could not be isolated from LTC patients is unclear. However, it could be hypothesized that *S. aureus* may not be able to colonize the urinary tract for a long period of time where it may be in competition with other bacterial infections such as *E. coli* which are more compatible in colonization of the urinary tract and 2) our *S. aureus* isolates may lacked biofilm characteristics. Similar to other studies, we also report here that *E. coli* (32%) were the most frequent isolate in LTC and STC patients (10, 11). No significant difference was observed in the number of *E. coli* Isolated from LTC and STC patients. However, contrary to other published reports (12), our *E. coli* (80%) were resistant to at least four antibiotics. Such a high survival rate of *E. coli* could be due, in part, to use of heavy antibiotic treatment regimens for the hospitalized patients in Iran and the circulation of bacteria within various hospital wards including catheterized patients. Moreover, 52 and 48% of our *E. coli* isolates were single (ST) and common clonal types (CT) as shown by PFGE, respectively. Amongst the 8 CTs, 3 CTs (G, I, T) were found only in LTC patients. These isolates were obtained from different hospitals at different time intervals, suggesting of inter-hospital bacteria dissemination. In addition, the results showed that catheterization, LTC and STC, may act as a suitable milieu where *E. coli* persisted and colonized and eventually they disseminated as the multi-resistant bacteria elsewhere. Three CTs (H, N, R) were shared by the LTC and STC patients and were obtained from the same hospital. The results suggested that these clonal types may continue to be remained in-hospital infecting catheterized and non-catheterized patients likewise. The present study showed that multiple drug resistant (MDR) strains of *E. coli* and *K. pneumoniae* were more prevalent in LTC than STC patients. In addition, the strains resistant with ≥ 3 antibiotics were more common in LTC than STC patients. MDR strains of *K. pneumoniae* (45%) were prevalent more significantly in LTC than STC patients (P<0.005). PFGE analysis showed that 75% of isolates were clonally diverse, suggesting the increased potential of the turnover of these bacteria into becoming MDR strains. In accordance to other studies (5, 13) that have reported more isolation of *P. aeruginosa* strains in STC than LTC patients, we observed similarly. The results showed that the antibiotic susceptibility pattern of *P. aeruginosa* was different in patients with LTC and STC. Some of the isolates obtained from STC were resistant to 9 antibiotics. Such difference could be due to the fact that the STC patients were infected with MDR strains of *P. aeruginosa* circulating in the hospitals, whereas LTC patients may have been infected by the antibiotic susceptible non-nosocomial sources. This analysis is supported by PFGE where it was shown that *P. aeruginosa* isolates from STC patients were highly diverse in comparing with the isolates obtained from LTC. Our previous report (14) suggested the prevalence of vancomycin resistant enterococci (VRE) in non-catheterized hospitalized patients to be around 6%. Although the VRE sample size obtained in this study was small, the results showed that the level of VRE in catheterized patients was about 40%. This significant number of VRE may show the potential of enterococci to rapidly become VRE. In one patient, VRE was obtained within a short period of time (one week) after catheterization. In addition, we determined that 100% of the catheterized, LTC and STC, and non-catheterized (14) patients infected with *E. faceium* were resistant to ciprofloxacin. This may suggest that the resistant genes related to the ciprofloxacin resistant *E. faecium* were not affected by the environmental setting linked to LTC and STC patients.

Conclusion

The results showed that even with excellent care, all patients that were catheterized for a long time could become bacteriuric. Nevertheless, decreasing mixed infections could make CAUTIs easier to treat, especially in LTC patients. The high percentage of MDR bacteria and clonality in our study is alarming. The diverse bacteria clonality suggested that there were different sources for CAUTI.

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Conflict of interest

None declared conflicts of interest.

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