Comparative study of antimicrobial resistance and biofilm formation among Gram-positive uropathogens isolated from community-acquired urinary tract infections and catheter-associated urinary tract infections

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Background: Gram-positive cocci have emerged to be an important cause of urinary tract infection (UTI) both in community-acquired UTI (Com-UTI) and catheter-associated urinary tract infection (CA-UTI). The objective of this study was to investigate the frequency of Gram-positive cocci urinary tract infections, their susceptibility patterns to commonly used antimicrobial agents and the biofilm forming property with respect to catheter-associated UTI and community-acquired UTI.

Methods: A total of 1,360 urine samples from indwelling catheter and 10,423 from mid-stream urine were obtained during a 6-month period and processed following standard microbiological guidelines. Biofilm formation was detected using congo red agar (CRA), tube method (TM) and tissue culture plate (TCP) method. Chi-square test and independent sample t-test were employed to calculate the significance. Statistical significance was set at P-value ≤ 0.05.

Results: The infection rate was significantly higher in CA-UTI as compared to Com-UTI (25% vs 18%, p=0.0001). Among 2,216 organisms isolated, 471 were Gram-positive cocci; 401 were obtained from Com-UTI while 70 were from CA-UTI. Enterococcus faecalis was the most common organism isolated from Com-UTI, while Staphylococcus aureus was commonest among CA-UTI. Multi-drug resistance, methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococci were also significantly higher in CA-UTI as compared to Com-UTI. Biofilm-forming property was significantly higher in CA-UTI than Com-UTI. The sensitivity of congo red agar method and tube method was 79% and 81.9% respectively and specificity was 98.5% each. Antimicrobial resistance was significantly higher in biofilm-formers as compared to non-formers.

Conclusion: Gram-positive bacteria are a significant cause of both CA-UTI and Com-UTI with Enterococcus faecalis and Staphylococcus aureus as common pathogen. Biofilm formation and multi-drug resistance is significantly higher in CA-UTI than Com-UTI. Routine surveillance of antimicrobial resistance and biofilm formation is necessary in all cases of UTI to ensure the proper management of patients.

Keywords: CA-UTI, multi-drug resistant, biofilm, MRSA

Background
Urinary tract infections (UTIs) are the most common infections in both community and hospital settings infections.1 Although Gram-negative bacteria cause the majority of UTI, Gram-positive bacteria have emerged to cause UTI, particularly among
individuals who are elderly, pregnant, or who have other risk factors.\textsuperscript{2,3} Catheter-associated urinary tract infection (CA-UTI) is one of the most common healthcare-acquired infections; 70–80\% are attributable to use of an indwelling urethral catheter.\textsuperscript{4} Gram-positive bacteria like \textit{Staphylococcus aureus}, \textit{Enterococcus faecalis} and \textit{Enterococcus faecium} are responsible for a minority of community-acquired UTI (Com-UTI), but together cause 30–45\% of catheter-associated UTIs and are the third leading cause of hospital-acquired UTIs.\textsuperscript{5} Bacterial biofilms play an important role in UTIs, being responsible for both acute and persistent infections. Biofilm-forming bacteria involved up to 80\% of all infections, with urology being one of the main fields in which biofilm can become a serious problem.\textsuperscript{6} Biofilms can not only develop into urethral stents but they can also form on catheters, causing their blockage. One of the most important concerns of biofilm is the antimicrobial resistance shown by these structures. Biofilm can be up to 1,000-fold more resistant to antibiotics than planktonic cells due to several mechanisms.\textsuperscript{6,7}

The objective of this study was to compare the frequency of Gram-positive cocci urine infection, their susceptibility patterns to commonly used antimicrobial agents and the biofilm forming property between Com-UTI and CA-UTI.

\section*{Methods}

\subsection*{Case definition}

CA-UTI is defined as an infection in a patient with a urinary catheter meeting the National Healthcare Safety Network definition of UTI.\textsuperscript{8} Com-UTI is defined as an infection of the urinary tract that occurs in the community or within less than 48 h of hospital admission and was not incubating at the time of hospital admission.\textsuperscript{9,10} Multidrug resistance (MDR) is defined as resistance to at least one agent in three or more classes of antimicrobials.\textsuperscript{11}

\subsection*{Study population and bacterial isolates}

This is a prospective study carried out at Department of Microbiology, from 1 January 2018 to 30 June 2018. Ethical clearance was obtained from institutional review committee before starting the research. A total of 10,423 clean-catch midstream urine and 1,360 catheter urine samples were collected from the same number of clinically suspected patients of UTI. Once the sample was collected, it was transferred to the laboratory immediately and inoculated on cysteine lactose electrolyte deficient agar using a standard calibrated loop. Isolates from cases with significant bacteriuria (10\textsuperscript{5} colonies/mL) were identified based upon standard microbiological procedures involving morphological characteristics, Gram’s stain, rapid tests (catalase, oxidase, coagulase, bile solubility), and biochemical tests like indole, methyl red, Voges–Proskauer and citrate, triple sugar iron, oxidation/fermentation, urease and nitrate reduction.\textsuperscript{12}

\subsection*{Antimicrobial susceptibility testing}

Antimicrobial susceptibility testing was performed on Mueller–Hinton agar using disk diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines.\textsuperscript{13} The isolates were tested against the following antimicrobial discs (HiMedia, Mumbai, India): amikacin (10 \(\mu\)g), cephalxin (30 \(\mu\)g), ceftriaxone (30 \(\mu\)g), gentamicin (10 \(\mu\)g), nitrofurantoin (50 \(\mu\)g), cotrimoxazole (25 \(\mu\)g), penicillin (10 \(\mu\)g), ofloxacin (5 \(\mu\)g), vancomycin (30 \(\mu\)g), and linezolid (30 \(\mu\)g).

\subsection*{Minimum inhibitory concentration (MIC)}

Resistance to methicillin and vancomycin in \textit{S. aureus} and vancomycin-resistant \textit{Enterococci} were confirmed by calculating the MIC of the antibiotics using broth dilution method.\textsuperscript{14} \textit{S. aureus} isolates with MIC to vancomycin \(\leq 2\) \(\mu\)g/mL was considered susceptible, 4–8 \(\mu\)g/mL intermediate and \(\geq 16\) \(\mu\)g/mL resistant. For methicillin-resistant \textit{Staphylococcus aureus}, MIC to oxacillin \(\leq 2\) \(\mu\)g/mL was considered susceptible and \(\geq 4\) \(\mu\)g/mL considered resistant. For \textit{E. faecalis}, MIC to vancomycin \(\leq 4\) \(\mu\)g/mL was considered susceptible, 8–16 \(\mu\)g/mL intermediate and \(\geq 32\) \(\mu\)g/mL resistant.\textsuperscript{13,14}

\subsection*{Biofilm formation}

Biofilm formation was detected by congo red agar method.\textsuperscript{15} Biofilm formation was detected by congo red agar method.\textsuperscript{15} This method was proposed by Freeman et al. Congo red agar was prepared by mixing brain heart infusion broth, sucrose, congo red dye and agar (HiMedia) in 1 L distilled water. The organisms were plated on it and incubated aerobically at 37 \(^\circ\)C for 24 h. The observation of black colored colony was considered as biofilm positive and red colored colony as negative.
**Tube method**

Test tubes containing trypticase soy broth with 1% glucose (HiMedia) were prepared. Bacterial suspension was inoculated and incubated overnight at 35°C. After incubation, tubes were decanted and washed properly with phosphate buffer solution of pH 7.3 (HiMedia). Upon drying, the tubes were stained with 0.1% crystal violet (HiMedia) and washed several times with water. Test tubes with uniform stain deposits on the walls were considered positive for biofilm formation.

**Tissue culture plate method**

For the TCP method the test organism was inoculated in trypticase soy broth with 1% glucose (HiMedia) and incubated at 35°C aerobically. Culture (0.2 mL) was added into individual wells of 92-well flat-bottom tissue culture plates; the plate was then incubated for 24 h. The plates were first tapped gently and then cleaned with PBS (pH 7.3) four times. After that, the plate was fixed with sodium acetate (2%) (HiMedia) and stained with 0.1% crystal violet. The wells with uniformly stained floor and walls were considered biofilm formers.

**Quality control**

*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were used for quality control of the biochemical tests, MIC and antimicrobial discs. For biofilm formation, *S. epidermidis* ATCC 35984 was used as positive control and *S. epidermidis* ATCC 12228 was used as negative control.

**Data analysis**

The data were entered in Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA) and interpreted using SPSS version 16 (SPSS Inc., Chicago, IL, USA). Data were expressed in terms of numbers and percentages and analyzed using chi-square test and t-test. *P*-value < 0.05 was considered significant.

**Table I** Total number of samples and infection rate in Com-UTI and CA-UTI

| Type of UTI | Total urine samples submitted | No of positive growth | Growth % | Number of GPC | GPC %  |
|-------------|-------------------------------|----------------------|----------|---------------|--------|
| Com-UTI     | 10,423                        | 1,876                | 18%      | 401           | 21.37% |
| CA-UTI      | 1,360                         | 340                  | 25%      | 70            | 20.58% |
| Total       | 11,783                        | 2,216                | 18.8%    | 471           | 21.25% |

**Ethical approval and consent**

Ethical clearance was obtained from Institutional Review Committee, B. P. Koirala Institute of Health Sciences, before starting the research (code no: IRC/1011/017). Written informed consent was obtained from each patient.

**Results**

During the study period, a total of 10,423 urine samples from mid-stream urine and 1,360 from indwelling catheter and were obtained. The infection rate was significantly higher in CA-UTI as compared to Com-UTI (n=340, 25% vs n=1876, 18%, *p*=0.0001) (Table 1). Among 2,216 organisms isolated, 1,745 (78.74%) were Gram-negative bacilli and 471 (21.25%) were Gram-positive cocci (GPC). Among the GPC isolates, 401 were obtained from Com-UTI and 70 from CA-UTI. *E. faecalis* (n=217, 54.11%) was the most common organism isolated from community-associated UTI, followed by *S. aureus* (n=157, 39.15%). However, in case of catheter-associated UTI, *S. aureus* (n=37, 52.85%) was the most common isolate (Table 2).

**Discussion**

The present study demonstrates a significantly high infection rate in catheter-associated urinary tract infection as compared to community-acquired UTI. The infection rate of 25% in CA-UTI is similar to the study done by Dougnon et al in West Africa. However, some studies like Zarb et al (17.2%) and Prashamsa et al (12.5%) have suggested a lower incidence of CA-UTI. Higher incidences of CA-UTI (35.6%) has been reported by Iwuafor et al. The high infection rate of CA-UTI in our study might be attributed to the fact that this hospital is a tertiary care referral hospital; most patients admitted here usually received treatment elsewhere and might have been catheterized elsewhere as well. Several studies have suggested that the use of indwelling urethral catheters increases the risk of UTI occurrence by up to 14-fold.

In the present study, *E. faecalis* was the most common Gram-positive coccus isolated from community-acquired UTI while *S. aureus* was the most common GPC isolated from...
Table 2 Gram-positive cocci isolated from Com-UTI and CA-UTI

| Organism           | Com-UTI | CA-UTI | Total |
|--------------------|---------|--------|-------|
|                    | N       | %      | N     | %     |
| Enterococcus faecalis | 217     | 54.11  | 24    | 34.28 |
| Staphylococcus aureus    | 157     | 39.15  | 37    | 52.85 |
| Staphylococcus saprophyticus | 26   | 6.48   | 2     | 2.85  |
| Staphylococcus epidermidis | 1      | 0.25   | 7     | 10    |
| Total               | 401     | 100    | 70    | 100   |

Multi-drug resistance (77% vs 24%, P-value 0.0001), methicillin resistant Staphylococcus aureus (82% vs 13%, P=0.0001) and vancomycin resistant Enterococci (33% vs 3%, P=0.0001) were significantly higher in CA-UTI as compared to Com-UTI (Table 3). In addition, biofilm-forming property was also significantly higher in CA-UTI than Com-UTI (75% vs 21%, P=0.0001).

Abbreviations: CA-UTI, catheter-associated urinary tract infection; Com-UTI, community-acquired urinary tract infection.

CA-UTI. The result is in agreement with study done by Lewis et al in South Africa. In contrast to our finding, the study done by Baral et al demonstrated S. aureus as the most common Gram-positive bacterium causing Com-UTI. Although the study was conducted in the same setup, it was 9 years before and did not differentiate between community-acquired UTI and catheter-associated UTI. A study done by Bardoloi et al concluded that S. aureus was the most common organism isolated both from Com-UTI and CA-UTI. S. aureus and E. faecalis are the most common Gram-positive bacteria causing UTI. The increased number of enterococcal UTI in this part might be due to the rapid surge in number of diabetic patients; diabetes mellitus is one of the important risk factors of enterococcal UTI. The increased incidence of enterococcal UTI is alarming; resistance to most commonly used antimicrobial agents is a typical characteristic of these bacteria. It is far more difficult to treat enterococcal UTI as compared to UTI caused by other bacteria due to intrinsic resistance to many antimicrobials and rapidly increasing acquired resistance.

Antimicrobial resistance was compared between CA-UTI and Com-UTI. A significant rise in resistance to the usually prescribed antimicrobials was noted among patients with CA-UTI compared to those with Com-UTI. Multi-drug resistance was much higher in CA-UTI in comparison with Com-UTI. The finding is similar to the study done by Bardoloi et al in Kerala, India and Michno et al in Poland. The result suggests that catheterization increases the degree of drug resistance in bacteria. Previous hospitalization, long-term broad-spectrum antimicrobial therapy, co-morbidity, frequent instrumentation, and cross-transmission of pathogens in catheterized patients might explain the higher antimicrobial resistance.

The present study suggested that incidences of MRSA and VRE were much higher in patients with CAUTI than that of Com-UTI; these results were not different from those found by Bardoloi et al and Shrestha et al.

Table 3 comparison of biofilm formation and multi-drug resistance among CA-UTI and Com-UTI

| Organisms isolated from | CA-UTI (n=70) | Com-UTI (n=401) | P-value |
|-------------------------|---------------|-----------------|---------|
| Biofilm formation       | 75% (n=53)    | 24% (n=89)      | 0.0001  |
| MDR                     | 77% (n=54)    | 24% (n=98)      | 0.0001  |
| MRSA                    | 82% (n=29 out of 37) | 13% (n=21 out of 157) | 0.0001  |
| VRE                     | 33% (n=8 out of 24) | 3% (n=7 out of 217) | 0.0001  |

Note: The sensitivity of CRA and TM for biofilm formation was calculated using TCP as reference method. The sensitivity and specificity of CRA was 79% and 98.5% respectively, while that of TM was 81.9% and 98.5% (Table 4).

Abbreviations: CA-UTI, catheter-associated urinary tract infection; Com-UTI, community-acquired urinary tract infection; CRA, Congo red agar; TCP, tissue culture plate; TM, tube method; VRE, vancomycin-resistant Enterococci.

Table 4 Statistical analysis of Congo red agar and tube method using tissue culture plate method as gold standard

|              | Sensitivity | Specificity | PPV | NPV | Accuracy |
|--------------|-------------|-------------|-----|-----|----------|
| Congo red agar (CRA) | 79%         | 98.5%       | 95.6% | 91.9% | 92.7%    |
| Tube method (TM)    | 81.9%       | 98.5%       | 95.8 | 92.9% | 93.63%   |

Notes: The antimicrobial susceptibility was compared between the Com-UTI and CA-UTI, biofilm formers and non-formers and statistical significance was calculated (Table 5). Antimicrobial resistance was significantly higher in biofilm formers as compared to biofilm non-formers for amikacin (50% vs 27%, P=0.0001), ceftriaxone (53% vs 3%, P=0.0001) and nitrofurantoin (36% vs 4%, P=0.0001). Similarly, comparison between Com-UTI and CA-UTI also showed highly significant difference for amikacin (54% vs 31%, P=0.0001), ceftriaxone (50% vs 12%, P=0.0001), ofloxacin (51% vs 19%, P=0.0001) and penicillin (80% vs 52%, P=0.0001).

Abbreviations: CA-UTI, catheter-associated urinary tract infection; Com-UTI, community-acquired urinary tract infection; PPV, positive predictive value; NPV, negative predictive value.
published by Mody et al in Michigan and Iwuafor et al in Nigeria. The higher incidence of MRSA and VRE from patients with catheter-associated UTI might be due to previous antimicrobial therapy, biofilm formation and previous hospitalization in those patients. Catherization is the most important risk factor for MRSA and VRE associated UTI.33,34

Biofilm formation was noted in 75% of isolates from CA-UTI and 24% of isolates from Com-UTI. Similar results were obtained in the study conducted by Sabir et al. Discordanly, Bardoloi et al concluded biofilm formation was higher in Com-UTI (76% vs 60%). The higher prevalence of biofilm in catheters is probably due to the survival advantage provided by the catheter to the microorganisms. Catheter create an environmental condition on its surface that make it an ideal site for bacterial attachment and formation of biofilm structures.

Even though the principle of CRA, TM and TCP method is the same, which is based on the enhancement of exopolysaccharide production using enriched media (TSB with glucose/sucrose), they detect biofilm with variable sensitivity. The sensitivity of TM was slightly higher than CRA for the detection of biofilm; the finding is similar to several other studies. However, some studies also suggest that CRA is superior to TM in detection of biofilm formation. The results of our finding gave us enough evidence that these phenotypic methods can be used routinely for the detection of biofilm formation. Even though tissue culture plate method is considered as the gold standard for the detection of biofilm formation, congo red agar and tube method are easy, inexpensive and reliable method for the detection of biofilm formation.

The antimicrobial resistance pattern, when compared with respect to their biofilm forming property, showed significant differences. The level of resistance to antibiotics was higher in biofilm formers than non-formers. The results are consistent with findings of Bardoloi et al and Soumya et al. This might be due to the fact that biofilm makes it notoriously difficult for the antibiotics to penetrate them and act upon the microorganisms. Hence, detection of biofilm production by uropathogens is crucial, and it can help in initiating the appropriate intervention, especially in cases of symptomatic UTI.

The limitation of our research includes the inability to follow up catheterized patients to collect history of prior hospital admissions, intake of antimicrobials, type of catheter used and clinical outcome.

**Conclusion**

Gram-positive bacteria are a significant cause of both CA-UTI and Com-UTI with *Enterococcus faecalis* and *Staphylococcus aureus* as common pathogen. The most worrisome finding is the high prevalence of multidrug resistant uropathogens. Biofilm formation and multi-drug resistance is significantly higher in CAUTI than Com-UTI. Since the management of UTI with biofilm-forming bacteria is different and difficult, routine surveillance of biofilm formation and antimicrobial

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**Table 5** Antimicrobial resistance and comparison between biofilm formers and non-formers, Com-UTI and CA-UTI

| Antimicrobial agents | Class of antimicrobial agents | Resistance expressed in percentage (%) | P-value | Resistance expressed in percentage (%) | P-value |
|----------------------|-------------------------------|----------------------------------------|---------|----------------------------------------|---------|
|                      |                               | Biofilm-former | Biofilm non-former | | Com-UTI | CA-UTI |
| Amikacin             | Aminoglycosides               | 50            | 27                | 0.001  | 31             | 54     | 0.001  |
| Gentamicin           | Aminoglycosides               | 54            | 12                | 0.0001 | 20             | 60     | 0.0001 |
| Cephalaxin           | Cephalosporins                | 54            | 10                | 0.0001 | 17             | 52     | 0.0001 |
| Ceftriaxone          | Cephalosporins                | 53            | 3                 | 0.0001 | 12             | 50     | 0.0001 |
| Ofloxacin            | Fluoroquinolones              | 34            | 19                | 0.001  | 19             | 51     | 0.0001 |
| Co-trimoxazole       | Folic acid synthesis inhibitors| 23            | 7                 | 0.0001 | 7              | 38     | 0.0001 |
| Nitrofurantoin       | Nitrofurans                   | 36            | 4                 | 0.0001 | 11             | 25     | 0.05   |
| Penicillin           | Penicillins                   | 57            | 56                | 0.419  | 52             | 80     | 0.0001 |
| Vancomycin           | Glycopeptides                 | 10            | 1                 | 0.0001 | 2              | 11     | 0.0001 |
| Linezolid            | Oxazolidones                  | 0             | 0                 | NA     | 0              | NA     | NA     |

**Abbreviation:** CA-UTI, catheter-associated urinary tract infection; Com-UTI, community-acquired urinary tract infection; NA, not applicable.
resistance is necessary in all cases of UTI to ensure the optimum management of patient and for epidemiological surveillance.

Disclosure

The authors report no conflicts of interest in this work.

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