Comparative study of isolates from community-acquired and catheter-associated urinary tract infections with reference to biofilm-producing property, antibiotic sensitivity and multi-drug resistance

Vishwajeet Bardoloi* and K. V. Yogeesha Babu

Abstract

Purpose. Urinary tract infection (UTI) can be community-acquired (Com-UTI) or catheter-associated (CAUTI) and may be associated with biofilm-producing organisms. A comparative analysis of biofilm-producing property (BPP), antibiotic-sensitivity and multi-drug resistance (MDR) and their relation with the BPP of isolates from Com-UTI and CAUTI has not yet been performed and necessitated this study.

Methodology. Objectives: (1) isolation of bacteria from CAUTI and Com-UTI and identification of their BPP, antibiotic-sensitivity and MDR status; (2) comparison of the isolates from CAUTI and Com-UTI as regards BPP, MDR status and their relation with BPP. Method: isolates from 100 cases each of Com-UTI and CAUTI were subjected to Congo red agar (CRA) and Safranin tube tests. Antibiotic susceptibility was investigated using the disc diffusion method. Both groups were compared regarding BPP, drug sensitivity and MDR status. Statistical analyses were performed using $\chi^2$ and Fisher's exact tests.

Results. 76.19 % of isolates from Com-UTI and 60.72 % from CAUTI had BPP ($P=0.0252$; significant). The Safranin tube test detected more isolates with BPP than the CRA test. MDR is greater in CAUTI than Com-UTI (83.33 % versus 64.76 %; $P=0.0039$; significant). MDR is greater in isolates with BPP in both Com-UTI and CAUTI (76.47 and 62.35 %; non-significant).

Conclusions. BPP was found in both Com-UTI and CAUTI. When used together, the Safranin tube test and the CRA test increased the sensitivity of detecting BPP. MDR was higher in CAUTI than Com-UTI. MDR and BPP are not interrelated or associated, especially in settings where it is not certain that isolates were obtained from a well-formed biofilm. However, this does not rule out a higher incidence or prevalence of MDR in isolates with BPP taken directly from the biofilms.

INTRODUCTION

Urinary tract infection (UTI) can be community-acquired (Com-UTI) or nosocomial [1]. The prevalence of Com-UTI varies in different parts of the world, accounting for 35 000 hospitalizations in the USA, 3.15 % of infections in England and 2.4 % in Italy [2–4]. Reliable data for India are lacking in this regard.

Catheter-associated UTI (CAUTI) is defined as an infection in a patient with an urinary catheter meeting the National Healthcare Safety Network (NHSN) definition of a UTI. CAUTI accounts for up to 40 % of all nosocomial infections and 80 % of all nosocomial UTIs [5, 6]. Escherichia coli, Klebsiella spp., Staphylococcus spp., Enterobacter spp., Acinetobacter spp., Proteus spp. and Pseudomonas aeruginosa are the commonest bacterial causes of both Com-UTI and CAUTI [7].

Most cases of CAUTI are biofilm-associated infections usually composed of multi-drug resistant (MDR) microorganisms [8]. Reports of non-device-related infections with almost an equal prevalence of bacteria having a biofilm-producing property (BPP) from the nasal passage and healthy skin, and various non-device-related wound infections, bloodstream infections, UTI and surgical site infections have been also been published [9, 10]. Thus, biofilms can be associated with both device-related as well as non-
device-related infections and therefore both CAUTI and Com-UTI may be biofilm-associated infections.

Biofilms are composed of clusters of diverse micro-organisms and extracellular matrices (primarily polysaccharide materials) formed on both the extraluminal and intraluminal surfaces of urinary catheters [8, 11] and in infections without indwelling devices like cystic fibrosis, infected kidney stones, dental caries, gingivitis, osteomyelitis and otitis media or on the surfaces of living tissues such as the urinary bladder [12–17]. Microbial attachment to surfaces was first described by Zobell in 1935, but the word ‘biofilm’ made its first appearance in scientific literature in the 1970s[11]. Bacteria in biofilms are protected from antimicrobial chemotherapy as well as host defence mechanisms, causing chronic persistent infections, septicemia and death if not treated.

Scanning electron microscopy is the gold standard method used for the demonstration of biofilms [18]. Several phenotypic methods (namely, the tissue culture plate method, the Congo red agar (CRA) method and the tube method) and genotypic methods have been used to demonstrate BPP as indirect evidence of the presence of biofilms [18, 19].

Several studies of different bacterial isolates from CAUTI with relation to BPP, antibiotic sensitivity and multi-drug resistance (MDR) have been conducted [5, 20]. Comparative studies of isolates from CAUTI and Com-UTI (without an indwelling urinary catheter) as regards BPP, antibiotic sensitivity and MDR status are very few and this necessitated the present study [21].

Objectives of study

(1) Isolation of bacteria from CAUTI and Com-UTI and identification of BPP, antibiotic sensitivity and MDR status of the bacterial isolates.
(2) Comparison of BPP, MDR status and relation of BPP with the MDR of the bacterial isolates from CAUTI and Com-UTI.

METHODS

Source of data

Our prospective observational study consisted of 100 patients admitted to different intensive care units (ICUs) with CAUTI and 100 patients with Com-UTI (without an indwelling urinary catheter) confirmed by semi-quantitative urine culture.

Case definition

CAUTI is defined as an infection in a patient with a urinary catheter meeting the National Healthcare Safety Network (NHSN) definition of a UTI [22].

Inclusion criteria

(1) Patients with nosocomial CAUTI admitted to different ICUs.
(2) Patients with Com-UTI without an indwelling urinary catheter.

Exclusion criteria

(1) Isolates of CAUTI from Com-UTI.
(2) Isolates from patients of nosocomial CAUTI admitted to hospital wards other than ICUs.

Specimen and data collection

Specimen collection

Urine from patients with Com-UTI
Specimens of early morning midstream urine (5 to 10 ml) collected with aseptic precautions as per standard laboratory procedures from 100 Com-UTI patients were included in the present study [23].

Urine from patients with CAUTI
All non-duplicated urine specimens from CAUTI were collected as per standard laboratory procedures [23].

Isolation and identification of the bacteria

Bacterial isolates from urine patients with an indwelling catheter and midstream urine specimens from Com-UTI were isolated and identified by using standard laboratory procedures [23, 24]. Antibiotic susceptibility testing was done by using the disc diffusion method (Kirby–Bauer test) as per Clinical and Laboratory Standards Institute (CLSI) guidelines [25]. Quality control of the discs was done according to CLSI guidelines and standard procedures [23, 25].

MDR

The determination of MDR was done according to the interim guidelines incorporating Centers for Disease Control and Prevention (CDC), EUCAST and Food and Drug Administration (FDA) criteria [26]. According to the criteria used, MDR was determined when an organism was non-susceptible to greater than or equal to one agent in greater than or equal to three antimicrobial categories [26].

Qualitative determination of BPP

Bacterial isolates were subjected to two qualitative phenotypic methods for BPP determination.

Tube method

The tube method [27] comprises inoculating 10 ml of brain–heart infusion broth with three to four colonies of bacterial isolates from a blood agar plate and incubating the broth culture tube overnight (18 h) at 37°C. The culture tubes were then emptied, washed with deionized water several times and stained with 0.1% safranin. Slime production is judged to have occurred if a visible film lined the walls of the tube and the isolate was then interpreted as a biofilm producer. Ring formation at the liquid–air interface was not considered indicative of slime production (Fig. 1).

Congo red agar method

Biofilm-forming colony morphology was detected for organisms on CRA [19, 28] plates. Bacteria were cultured in 10 ml brain–heart infusion broth at 35°C for 24 h without shaking and then plated onto CRA plates. Incubation was carried out at 35°C for 24 h and an additional 24 h at room temperature before recording the colony morphology. Crusty black colonies with a dry filamentous appearance
were recorded as biofilm producers, smooth pink colonies as non-producers and intermediate colony morphology (pink with dark centres resembling bull’s eyes) as potential biofilm producers (Fig. 2).

Controls for biofilm-forming property
Biofilm-producing reference strains of *Acinetobacter baumannii* (ATCC 19606) and *Pseudomonas aeruginosa* (ATCC 27853) and non-biofilm-forming reference strains of *Staphylococcus aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used [29].

Statistical analysis
Data are shown in terms of numbers and percentages, and analysed by $\chi^2$ and Fisher’s exact tests using a Microsoft Excel worksheet.

RESULTS
A total of 100 total patients had significant bacteriuria taken in Com-UTI. They yielded 105 isolates. There were 45 male patients and 55 female patients. The 100 CAUTI patients with significant bacteriuria yielded 102 isolates. There were 58 male patients and 42 female patients.

Eighty out of 105, i.e. 76.19 %, of the isolates in Com-UTI had BPP and 62 out of 102, i.e. 60.78 %, had BPP (Table 1). It is apparent that Com-UTI had a greater number of isolates with BPP than CAUTI. This is statistically significant ($\chi^2=5.008; P=0.0252$).

The total numbers of bacteria having BPP in Com-UTI and CAUTI were 80 and 62, respectively. The percentages of BPP detected by the Congo red dye test alone (i.e. tube test negative) were 12 and 12.90 % in Com-UTI and CAUTI, respectively. The percentages of BPP detected by the tube test alone (i.e. Congo red test negative) were 42.5 and 48.39 %, respectively, for Com-UTI and CAUTI (Table 1) Figs 1 and 2.

Thus the tube test detected more bacteria having BPP than the CRA test in both Com-UTI (42.5 % versus 12.5 %) and CAUTI (48.39 % versus 12.90 %) (Table 1).

In Com-UTI, MDR was highest in *E. coli* (79.55 %) followed by *K. pneumoniae* (73.91 %) (Table 2). In CAUTI, the highest MDRs were seen in *K. pneumoniae* (100 %), *P. aeruginosa* (100 %) and *S. saprophyticus* (100 %), followed by *E. coli* (89.13 %) (Table 2).

Overall MDR was higher in CAUTI (85 out of 102, i.e. 83.33 %) as compared to Com-UTI (68 out of 105, i.e. 64.76 %) (Table 2) and the difference is statistically very significant ($\chi^2=8.317, P=0.0039$).

Fifty-two out of the total 68 (i.e. 76.47 %) organisms with MDR had BPP in Com-UTI (Table 2). Thus MDR was higher in organisms with BPP than without BPP, but this difference is not statistically significant ($\chi^2=0.008, P=0.9272$). In CAUTI, 53 out of the 85 organisms with MDR had BPP, which shows MDR to be higher in organisms with BPP but this too was statistically not a significant difference ($\chi^2=0.206, P=0.6502$) (Table 2).

Organism-wise distribution of BPP in Com-UTI was *E. coli* 90.91 %, *K. pneumoniae* 65.22 %, *S. aureus* 57.89 %, *S. saprophyticus* 57.14 %, *Enterococcus faecalis* 100 % and *P. aeruginosa* 77.78 %. Similarly, in CAUTI it was *E. coli* 73.91 %, *K. pneumoniae* 50 %, *S. aureus* 44.44 %, *S. saprophyticus*...
The present study reports a high prevalence of BPP among bacterial pathogens from both Com-UTI and CAUTI. The bacterial flora of both Com-UTI and CAUTI were comparable with other published studies [7]. Few studies have reported a lower prevalence of BPP in Com-UTI than CAUTI by different genotypic and phenotypic methods [21].

Overall 76.19% of bacteria in Com-UTI and 60.78% in CAUTI had BPP and this difference is statistically significant (P=0.0252, Table 1), meaning that there is a greater number of bacteria in Com-UTI than CAUTI having BPP, which is statistically significant. Our results show a high percentage of bacteria in Com-UTI having BPP which is an interesting finding since there are many studies linking biofilms with catheters and hardly any studies linking BPP with Com-UTI. Thus attention should also be paid to the bacteria from Com-UTI that also have BPP. Although hints of Com-UTI having bacteria with BPP are present in some studies, it is not mentioned or elaborated upon a great deal. The hints that they were cases of Com-UTI, could be made from the method of collection of urine which mentions that midstream clean catch urine was taken. For CAUTI, the collection of urine is from the catheter and not midstream [6, 30–32].

Kaur and Sanjivani found that 46.05% of E. coli, 80.64% S. aureus, 61.53% Klebsiella sp. and 66.66% Pseudomonas
Table 2. Association of MDR with BPP in Com-UTI and CAUTI

<table>
<thead>
<tr>
<th>Organism</th>
<th>Com-UTI</th>
<th>CAUTI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDR in isolates without BPP (and percentage of the total no. of isolates with BPP)</td>
<td>MDR in isolates with BPP (and percentage of the total no. of isolates without BPP)</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>19 (68.42%)</td>
<td>11 (68.75%)</td>
</tr>
<tr>
<td>aureus</td>
<td>7 (14.28%)</td>
<td>4 (66.67%)</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>2 (22.22%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>faecalis</td>
<td>9 (22.22%)</td>
<td>2 (66.67%)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>9 (22.22%)</td>
<td>2 (66.67%)</td>
</tr>
<tr>
<td>aeruginosa</td>
<td>9 (22.22%)</td>
<td>2 (66.67%)</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>freundii</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>baumannii</td>
<td>44 (40%)</td>
<td>31 (40%)</td>
</tr>
<tr>
<td>Escherichia</td>
<td>40 (100%)</td>
<td>31 (100%)</td>
</tr>
<tr>
<td>coli</td>
<td>8 (100%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>35 (79.55%)</td>
<td>29 (79.41%)</td>
</tr>
<tr>
<td>pneumoniae</td>
<td>34 (85.33%)</td>
<td>28 (85.33%)</td>
</tr>
</tbody>
</table>

In both Com-UTI and CAUTI groups, greater than 50% of bacteria had BPP, which are very high results (Table 1). This was also reported in studies by Murugan et al. and Soto et al. [6, 36]. Is this a natural phenomenon? Eftekhar and Mirmohamadi have reported an equal prevalence of BPP in bacterial isolates from healthy people and from different infections, suggesting it to be a universal phenomenon [9]. Jeetendra Gurung et al. also reported similar findings [10].

Biofilms are responsible for over 65% of human infections and are associated with indwelling devices like urinary catheters and also with chronic infections without indwelling devices like cystic fibrosis, infected kidney stones, etc., acting as niduses for infections leading to bacteraemia, septicaemia and death [12–17, 36, 37]. The presence of BPP in vitro gives an indication of the potential of the organism to produce biofilm but does not indicate whether a biofilm has actually formed in vivo or not, which can only be confirmed by electron microscopy [18]. In spite of this, several studies have linked the BPP of bacterial isolates with the presence of biofilm without demonstration by electron microscopy [6, 10, 32, 34]. Phenotypic and genotypic methods can be used to demonstrate BPP. In the present study, two phenotypic tests, namely the CRA method and the tube test were used to detect BPP. They were selected as they are easy to perform, cost-effective, not cumbersome and bear reproducible results. The tissue culture plate method, although accurate, sensitive and bearing reproducible results, was not used in the study due to its cumbersome procedure and the requirement for spectrophotometric measurements of density of stained bacterial biofilms adhered to plastic plates. Electron microscopy was not adopted for the study due to inaccessibility and high cost.

The tube test was done in triplicate and observed by three different people to avoid observer variance and high false positive results. Safranin was used for the tube test in this study. Crystal violet can also be used but it gives inconsistent results with greater observer variation, leading to the preference for safranin in this study [38].

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Table 3. Antibiotic resistance among Gram-positive cocci in Com-UTI and CAUTI

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of isolates the drug was tested on</th>
<th>No. of organisms resistant to the drug</th>
<th>Resistance in organisms with BPP (denominator is number of organisms with BPP)</th>
<th>Resistance in organisms without BPP (denominator is number of organisms without BPP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>28*</td>
<td>19/28 (67.87 %)</td>
<td>13/17 (76.47 %)</td>
<td>6/11 (54.54 %)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>5/28 (17.86 %)</td>
<td>3/17 (17.65 %)</td>
<td>2/11 (18.18 %)</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>12/28 (42.86 %)</td>
<td>6/17 (35.29 %)</td>
<td>6/11 (54.54 %)</td>
<td>16/24 (65.32 %)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>11/28 (39.28 %)</td>
<td>5/17 (29.41 %)</td>
<td>6/11 (54.54 %)</td>
<td>16/24 (65.32 %)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>7/28 (25 %)</td>
<td>4/17 (23.53 %)</td>
<td>3/11 (27.27 %)</td>
<td>14/24 (58.33 %)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>5/28 (17.86 %)</td>
<td>3/17 (17.65 %)</td>
<td>2/11 (18.18 %)</td>
<td>12/24 (48.96 %)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>26†</td>
<td>13/26 (50 %)</td>
<td>8/15 (53.33 %)</td>
<td>5/11 (45.45 %)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>9/26 (34.62 %)</td>
<td>4/15 (26.66 %)</td>
<td>5/11 (45.45 %)</td>
<td>8/20 (40 %)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>15/26 (57.69 %)</td>
<td>9/15 (60 %)</td>
<td>6/11 (54.54 %)</td>
<td>10/20 (50 %)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>10/26 (38.46 %)</td>
<td>8/15 (53.33 %)</td>
<td>2/11 (18.18 %)</td>
<td>12/20 (60 %)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>24‡</td>
<td>0</td>
<td>0</td>
<td>4‡</td>
</tr>
</tbody>
</table>

*Tested on all the Gram-positive cocci isolates.
†Not tested on Enterococci.
‡Tested only on Enterococci [25].

**Com-UTI**

**CAUTI**

No. of isolates the drug is tested on | No. of isolates resistant to the drug | Resistance in organisms with BPP (denominator is number of organisms with BPP) | Resistance in organisms without BPP (denominator is number of organisms without BPP)

24* | 18/24 (78.72 %) | 8/10 (80 %) | 10/14 (71.42 %)

2/24 (12.77 %) | 2/20 (20 %) | 0

16/24 (55.32 %) | 8/10 (80 %) | 8/14 (57.14 %)

16/24 (55.32 %) | 8/10 (80 %) | 8/14 (57.14 %)

14/24 (46.81 %) | 8/10 (80 %) | 6/14 (42.86 %)

12/24 (38.30 %) | 6/10 (60 %) | 6/14 (42.86 %)

12/20 (60 %) | 8/10 (80 %) | 4/10 (40 %)

8/20 (40 %) | 4/10 (40 %) | 4/10 (40 %)

10/20 (50 %) | 6/10 (60 %) | 4/10 (40 %)

12/20 (60 %) | 8/10 (80 %) | 4/10 (40 %)

4‡ | 0 | 0 | 0
In Com-UTI, the CRA test detected 12.5 % isolates with BPP which were missed by the tube test. Similarly, the tube test detected 42.5 % isolates with BPP which were missed by the CRA test (Table 1). Thus both tests should be used together to detect a higher number of isolates with BPP. This was similar in CAUTI.

Oliveira and Cunha have reported that, taking PCR as the reference, the tube test shows the best correlation with detection of the ica genes, showing high sensitivity (100 %) and high specificity (100 %). They recommend the test for the routine detection of BPP due to its low cost, easy application, guarantee of reliable results, and high sensitivity and specificity [39].

However, Aricola et al. have reported a better association between the CRA method and ica gene carriage than the tissue culture plate method and tube method [19].

The detection of genes responsible for biofilm production, such as the ica ACD operon and others, could specify the potential to produce biofilm rather than a specific test to predict biofilm formation [19].

Although Enterococcus faecalis and C. freundii showed 100 % isolates having BPP, no conclusion can be drawn due to the very low number of isolates. In Com-UTI, E. coli thus topped the list of isolates having BPP (90.90 %) followed by P. aeruginosa (77.77 %). In CAUTI, A. baumanii had the highest number of isolates with BPP (80 %) followed by E. coli (73.9 %).

All the coagulase-negative staphylococci isolated were S. saprophyticus and interestingly all of them were isolated from female patients. Hovelius and Mardh, in their article, also mention S. saprophyticus being a common cause of UTI in females [40].

MDR in Com-UTI was found to be highest in E. coli (79.55 % of all E. coli isolated were MDR). In CAUTI, the highest MDR was found in S. saprophyticus (100 %), P. aeruginosa (100 %) and K. pneumoniae (100 %) followed by E. coli (89.13 %) (Table 2).

In Com-UTI, MDR seemed to be higher in organisms with BPP than in organisms without BPP (Table 2), but this difference was not statistically significant since BPP was also similarly distributed in non-MDR strains. Similar results were obtained by Abdallah et al. [21]. Contradictory results were obtained by Murugan et al. and Shashikala et al. who reported a high association of MDR with bacteria having BPP [6, 41].

In Com-UTI, it is seen that MDR is almost equally distributed in organisms with and without BPP (65 % versus 64 %). Similarly, in CAUTI, MDR is almost equally distributed in organisms with and without BPP (85.48 % versus 80 %) (Table 2).

### Table 4. Antibiotic resistance among Gram-negative bacilli in Com-UTI

Resistance was more among the bacterial isolates with BPP than without BPP but the difference was statistically non-significant (P>0.05).

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of isolates the drug was tested on</th>
<th>No. of isolates resistant to the drug</th>
<th>Resistance in organisms with BPP (denominator is number of organisms with BPP)</th>
<th>Resistance in organisms without BPP (denominator is number of organisms without BPP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin–tazobactam</td>
<td>77*</td>
<td>16/77 (20.78 %)</td>
<td>9/63 (14.28 %)</td>
<td>7/14 (50 %)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>5/77 (6.49 %)</td>
<td>2/63 (3.17 %)</td>
<td>3/14 (21.43 %)</td>
<td>3/14 (21.43 %)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>7/77 (9.09 %)</td>
<td>4/63 (6.35 %)</td>
<td>3/14 (21.43 %)</td>
<td>3/14 (21.43 %)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>14/77 (18.18 %)</td>
<td>10/63 (15.87 %)</td>
<td>4/14 (28.57 %)</td>
<td>4/14 (28.57 %)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>14/77 (18.18 %)</td>
<td>10/63 (15.87 %)</td>
<td>4/14 (28.57 %)</td>
<td>4/14 (28.57 %)</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>10/77 (12.99 %)</td>
<td>6/63 (9.52 %)</td>
<td>4/14 (28.57 %)</td>
<td>4/14 (28.57 %)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>17/77 (22.08 %)</td>
<td>13/63 (20.63 %)</td>
<td>4/14 (28.57 %)</td>
<td>4/14 (28.57 %)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>52/77 (67.53 %)</td>
<td>42/63 (66.66 %)</td>
<td>10/14 (71.43 %)</td>
<td>10/14 (71.43 %)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>45/77 (58.44 %)</td>
<td>38/63 (60.32 %)</td>
<td>7/14 (50 %)</td>
<td>7/14 (50 %)</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>41/77 (53.25 %)</td>
<td>31/63 (49.21 %)</td>
<td>10/14 (71.43 %)</td>
<td>10/14 (71.43 %)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>38/77 (49.35 %)</td>
<td>30/63 (47.62 %)</td>
<td>8/14 (57.14 %)</td>
<td>8/14 (57.14 %)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>54/77 (70.13 %)</td>
<td>44/63 (69.84 %)</td>
<td>10/14 (71.42 %)</td>
<td>10/14 (71.42 %)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>68†</td>
<td>56/68 (83.33 %)</td>
<td>8/12 (66.66 %)</td>
<td>8/12 (66.66 %)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>43/68 (63.24 %)</td>
<td>35/56 (62.5 %)</td>
<td>8/12 (66.66 %)</td>
<td>8/12 (66.66 %)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>49/68 (72.06 %)</td>
<td>40/56 (71.43 %)</td>
<td>9/12 (75 %)</td>
<td>9/12 (75 %)</td>
</tr>
<tr>
<td>Amoxicillin–clavulanic acid</td>
<td>43/68 (63.24 %)</td>
<td>33/56 (58.93 %)</td>
<td>10/12 (83.33 %)</td>
<td>10/12 (83.33 %)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>4/68 (5.88 %)</td>
<td>3/56 (5.36 %)</td>
<td>1/12 (8.33 %)</td>
<td>1/12 (8.33 %)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>45†</td>
<td>36/45 (80 %)</td>
<td>32/41 (78.09 %)</td>
<td>4/4 (100 %)</td>
</tr>
</tbody>
</table>

*Tested on all isolates.
†Not tested on P. aeruginosa.
‡Tested only on E. coli 25.

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This finding in the present study proves that MDR and BPP have no relation to each other and they do not influence each other. Lihua Qi et al. also found that robust biofilm producers had a larger proportion of non-MDR strains [42]. Yuan Hu et al. found that MDR isolates of A. baumannii had significantly lower biofilm formation [43]. An important aspect to be noted is that a person who is catheterized, already has stasis of urine which causes bacteria to grow and have antibiotic selection pressure and drug resistance [44, 45]. Moreover, a person in an ICU will have been admitted to the ICU for some debilitating condition and has thus been exposed to various drugs. This alone can be a factor that causes drug-selective pressure in bacteria. These are individual risk factors for development of MDR strains and may not be related to biofilms.

There is also the possibility that a person may not respond to antibiotics even though the bacterial strain may be a sensitive strain. This may be due to the biofilm encasing these bacteria and not allowing penetration of the drug through the biofilm. Thus biofilm formation and MDR may not be related at times, as in this study. The urine sample could have MDR strains even before the formation of biofilms and the MDR strains may just be protected by the biofilms. A difference may not be statistically significant but it may be clinically significant. It cannot be negated that though not statistically significant, it can be clinically significant since it contributes to patient outcome, disease severity, cost and length of hospital stay. Of more concern is the treatment of the patient and how biofilms affect drug sensitivity and patient response to the antibiotic. The biofilm is to be targeted along with the organisms since drugs may not penetrate the biofilms even though the bacterial strain maybe a sensitive one. Testing for biofilm production should be done simultaneously and in a routine manner along with identification of the bacteria and antimicrobial sensitivity to improve patient response to drugs.

A difference that was statistically significant, i.e. MDR is higher in organisms with BPP than without BPP, has been found by many studies [5–7, 9, 19, 21, 41]. On the other hand, no statistical difference was found in a few studies, such as those by Lihua Qui et al. and Yuan Hu et al. [42, 43]. So there is a chance that MDR and BPP may not be related. Moreover, having a BPP does not guarantee that a biofilm is present. The bacteria may have the ability to produce biofilms but may not express it. The relationship between MDR and biofilm should be proved through

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### Table 5. Antibiotic resistance among Gram-negative bacilli in CAUTI

Resistance was higher among the bacteria with BPP than without BPP but statistically non-significant (P>0.05).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of isolates the drug was tested on</th>
<th>No. of isolates resistant to the drug</th>
<th>Resistance in organisms with BPP (denominator is number of organisms with BPP)</th>
<th>Resistance in organisms without BPP (denominator is number of organisms without BPP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin–tazobactam</td>
<td>78*</td>
<td>36/78 (46.15 %)</td>
<td>20/52 (38.46 %)</td>
<td>16/26 (61.54 %)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>18/78 (23.08 %)</td>
<td>12/52 (23.08 %)</td>
<td>6/26 (23.08 %)</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>18/78 (23.08 %)</td>
<td>12/52 (23.08 %)</td>
<td>6/26 (23.08 %)</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>22/78 (28.21 %)</td>
<td>16/52 (30.78 %)</td>
<td>6/26 (23.08 %)</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>36/78 (46.15 %)</td>
<td>24/52 (46.15 %)</td>
<td>12/46 (46.15 %)</td>
<td></td>
</tr>
<tr>
<td>Netilmicin</td>
<td>40/78 (51.28 %)</td>
<td>28/52 (53.85 %)</td>
<td>12/46 (46.15 %)</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>44/78 (56.41 %)</td>
<td>28/52 (53.85 %)</td>
<td>16/26 (61.54 %)</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>64/78 (82.05 %)</td>
<td>42/52 (80.77 %)</td>
<td>22/42 (84.62 %)</td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>50/78 (64.10 %)</td>
<td>32/52 (61.54 %)</td>
<td>18/26 (69.23 %)</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>64/78 (82.05 %)</td>
<td>44/52 (84.62 %)</td>
<td>20/26 (76.92 %)</td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>58/78 (74.36 %)</td>
<td>38/52 (73.08 %)</td>
<td>20/26 (76.92 %)</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>72†</td>
<td>66/72 (91.67 %)</td>
<td>46/50 (92 %)</td>
<td>20/22 (90.91 %)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>60/72 (83.33 %)</td>
<td>42/50 (84 %)</td>
<td>8/22 (36.36 %)</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>68‡</td>
<td>58/68 (85.29 %)</td>
<td>36/44 (81.82 %)</td>
<td>22/24 (91.67 %)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>42/68 (61.76 %)</td>
<td>24/44 (54.54 %)</td>
<td>18/24 (75 %)</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>62§</td>
<td>60/62 (96.77 %)</td>
<td>40/42 (95.22 %)</td>
<td>20/20 (100 %)</td>
</tr>
<tr>
<td>Amoxicillin–Clavulanic acid</td>
<td>52/62 (83.87 %)</td>
<td>34/42 (80.95 %)</td>
<td>18/20 (90 %)</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>12/62 (19.35 %)</td>
<td>8/42 (19.05 %)</td>
<td>4/20 (20 %)</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>46</td>
<td></td>
<td></td>
<td>40/46 (86.96 %)</td>
</tr>
</tbody>
</table>

*Tested on all isolates.
†Not tested on P. aeruginosa.
‡Not tested on A. baumanii.
§Not tested on P. aeruginosa and A. baumanii.
||Tested only on E. coli.
demonstration of an actual biofilm using methods such as electron microscopy.

However, the overall percentages of MDR strains were higher in CAUTI (83.33%) than in Com-UTI (64.76%), which was found to be statistically highly significant ($\chi^2 = 8.317; P=0.0039$; Table 2). This suggests catheterization might influence the growth of MDR strains. In a catheterized patient, there is already stasis of urine which contributes to bacterial overgrowth and also drug selection [44, 45]. Data on previous history of catheterization, treatment, procedures or antibiotic usage were not available to probe into the matter. Retrograde investigation of the patient and also long-term follow up is not planned.

If individual drugs and organisms are taken into consideration, higher resistance among isolates with BPP than without BPP in both Com-UTI and CAUTI is seen. Here again the difference is not statistically significant (Tables 3, 4 and 5).

Among the Gram-positive cocci in both Com-UTI and CAUTI, and also among the Gram-negative bacilli in both Com-UTI and CAUTI, the organisms were least resistant to nitrofurantoin. There was no resistance observed for vancomycin and linezolid (Tables 3, 4 and 5).

Findings by Bahadin et al. and Gupta et al. also show nitrofurantoin to be the drug against which there is the least resistance by the bacteria [30, 46]. Thus nitrofurantoin emerged to be a very good drug for treating UTI and can also be used for empiric treatment of UTI.

The findings in the present study were achieved with the help of in vitro analysis of the BPP of the bacterial isolates using simple techniques. What the picture is in vivo and in the actual presence of a biofilm needs to be confirmed by large-scale multicentric studies using electron microscopy and molecular techniques.

Conclusions

1. MDR and BPP of isolates from Com-UTI and CAUTI are not interrelated or associated with each other, especially in a setting where biofilm is not demonstrated with specific methods.

2. The lack of association of MDR and BPP in the present study does not rule out the higher incidence or prevalence of MDR status in isolates with BPP obtained directly from the biofilm.

3. Tube and CRA tests should be used together to detect a higher percentage of bacteria having BPP rather than using either of the tests alone.

4. Nitrofurantoin can be the drug of choice for isolates from Com-UTI and CAUTI as least resistance is observed. It can be suggested for empirical treatment of patients.

Future directions

The association of MDR of isolates with biofilm can only be determined with certainty after investigation of the biofilm by electron microscopy followed by collection and processing of isolates directly from the biofilm.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

Ethical Committee clearance had been given for the study. Patients' consent was taken prior to the study. No animals were used or harmed during the study.

References


32. Neupane S, Pant ND, Khatiwada S, Banjara MR. Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic Escherichia coli isolated from the patients suspected of urinary tract infections visiting Shree Bir德拉 Hospital, Chhauni, Kathmandu, Nepal. Antimicrob Resist Infect Control 2016;5:5.


