Evaluation of Different methods for Diagnosis of Catheter Related Blood Stream Infection

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Abstract: Introduction: Intravascular catheters are crucial in modern medical practice, particularly in intensive care units (ICUs). However, vascular catheter-related bloodstream infections (CRBSIs) have become a leading cause of health-care-associated bloodstream infections and are associated with substantial morbidity and mortality. Aim of the work: to determine whether the Gram stain-acridine orange leucocyte cytospin (AOLC) test could offer accuracy comparable to other methods for the diagnosis of catheter-related blood stream infection and to avoid inconvenient, unnecessary and costly central venous catheter removal. Material and methods: This study included 36 patients with central venous catheterization. All patients with CVC were clinically suspected to have CR-BSI, as suggested by their physicians. They were 24 males and 12 females with their ages ranging between 20 years and 68 years with the mean age 51.3 ± 14.9 years. The samples were subjected to two major diagnostic strategies: the first implemented catheter sparing approach, while the other strategy required catheter removal. Catheter sparing diagnostic methods included paired quantitative blood cultures, pour-plate technique and acridine orange leucocyte cytospin/Gram test (AOLC/G test), while methods that required device removal were semiquantitative catheter segment culture (roll-plate method) and quantitative catheter segment culture (tip-flush method). Results: Taking the roll-plate technique as the reference method, there was fair agreement (66.7%) between the results of pour plate and roll plate techniques (kappa=0.226), there was moderate agreement between the results of paired quantitative blood culture (83.3%), tip flush method (83.3%) and AOLC/G (80.6%) and the reference method (kappa=0.429, 0.429,0.40 respectively). The diagnostic validity tests for AOLC showed that the specificity of AOLC was 100% and the sensitivity was 78.8%. Conclusion: From this study, it was concluded that the Gram stain-AOLC test is a simple, rapid, sensitive and specific test that could be used as a first line screening test for the in situ diagnosis of CRBSI. This policy can prevent the unnecessary removal of uninfected catheters and significantly extends the life span of catheters and lowers the risks and costs of mechanical complications associated with new catheter placement.

Key word: Catheter related blood stream infection; CRBSI; cytospin; AOLC

1. Introduction

Intravascular catheters are crucial in modern medical practice, particularly in intensive care units (ICUs). Even though such catheters provide necessary vascular access, their use puts patients at risk for local and systemic complications, including catheter related infection (CRIs), particularly catheter related bloodstream infections (CR-BSIs) which are associated with increased morbidity, mortality rates of 10% to 20%, prolonged hospitalization, and increased medical costs (1).

(CRBSIs) are considered "silent" medical errors since they may be caused without operator personnel knowledge during placement, site care, or line manipulation. About 20% of central venous catheters (CVCs) are removed because of suspected infection actually prove to be infected, and the diagnosis is always retrospective (2).

In the ICU setting, the incidence of infection is often higher than in the less acute in-patient or ambulatory setting. In the ICU, central venous access might be needed for extended periods of time; patients can be colonized with hospital- acquired organisms; and the catheter can be manipulated multiple times per day for the administration of fluids, drugs, and blood products. Moreover, some catheters can be inserted in urgent situations, during which optimal attention to aseptic technique might not be feasible (3).

Several factors have been described in the adult population as playing a role in the occurrence of nosocomial CRBSI. These factors include prolonged catheterization, poor aseptic insertion technique, emergent catheter placement, size of catheter, number of lumens, type of catheter material, location of catheter and frequency of catheter manipulations (4). Other factors include presence of an infusion therapy team, use of sterile barrier precautions, type of insertion site dressing, and frequency of system entry (hospital factors). Patient- related factors have also been identified, which include age, granulocytopenia, immune suppression and severity of underlying disease (5).

The present study was carried out to determine whether the Gram stain-acridine orange
leukocyte cytospin (AOLC) test could offer accuracy comparable to other methods for the diagnosis of catheter-related bloodstream infection in order to avoid inconvenient, unnecessary and costly central venous catheter removal.

2. Samples:
This study included 36 patients with central venous catheterization admitted to intensive care units (ICUs) and dialysis department in Benha University Hospital and Benha Teaching Hospital. All patients with CVC were clinically suspected to have CR-BSI, as suggested by their physicians, due to the presence of clinical symptoms of bacteremia (fever, tachycardia, hypotension, neutrophilia, neutropenia…etc) in absence of any other possible source of infection except for the CVC. They were 24 males and 12 females with their ages ranging between 20 years and 68 years with the mean age 51.3 ± 14.9 years.

The samples were subjected to two major diagnostic strategies: the first implemented catheter sparing approach, while the other strategy required catheter removal.

Catheter sparing diagnostic methods included paired quantitative blood cultures, pour-plate technique and acridine orange leukocyte cytospin /Gram test (AOLC/G test), while methods that required device removal were semiquantitative catheter segment culture (roll- plate method) and quantitative catheter segment culture (tip-flush method).

**Paired quantitative blood cultures:**
Quantitative blood culture for both peripheral vein and CVC blood using lysis centrifugation techniques (Isolator blood culture system) (Oxoid Ltd, Wade Road, Basing Stock, Hants, UK) using isolator 10 tubes. For peripheral blood collection, 6-10 ml of the peripheral venous blood was added to ISOLATOR 10 tube. The tube was centrifuged at 3000 x g for 30 minutes. The supernatant fluid was withdrawn from ISOLATOR 10 tubes, and the tube contents were vigorously mixed to achieve a homogeneous emulsion. The concentrate was evenly inoculated onto the selected agar media (blood, chocolate and MacConkey) and dispensed in a straight line across the surface of the agar. Plates were placed aerobically at 37°C for 24 hours. Another 6-10 ml of blood was aspirated from CVC and was processed by the same manner as above. If the CVC was infected, the blood drawn through it usually shows a greater than 5-folds increase in the concentration of organisms compared with the blood drawn percutaneously from a peripheral vein.

**Pour-plate technique:**
Twenty mL from Muller Hinton agar (Pasteur-production, Paris, France) was melted and then left to cool to reach 45-50 °C & then added to it 1 ml from heparinized blood & then poured into sterile Petri dish & the plates were incubated at 37°C for 48 hours and colonies were counted. A cut off limit of 1000 CFU/ml was used to define positive cases.

**Acridine orange leukocyte cytospin/Gram stain test:**

**Principle:**
Acridine orange is a flurochromatic dye which binds to nucleic acids of bacteria and other cells. Under UV light, acridine orange stains RNA and single-stranded DNA orange; double-stranded DNA appears green. When buffered at pH 3.5-4.0, acridine orange differentially stains microorganism from cellular materials.

**Procedure:**
The Gram stain and ALOC test require two 50 µL samples of catheter blood (treated with EDTA K3E-EDTA K3). Each sample was placed into polystyrene tubes to which was added 1.2 ml formaline (10% by volume) saline (0.025 mol/L) solution, and the mixture was left for 2 minutes. 2.8 ml of 0.19 mol/L saline was then added to each tube followed by centrifugation at 352 x g for 5 minutes. The supernatant was decanted and the cellular deposit was homogenized by vortexing for 5 seconds & then transferred to a cytospin cupule that contained a microscope slide. The cellular suspension was centrifuged at 153 x g for 5 minutes in a cytocentrifuge (Shandon, Runcorn, UK). A monolayer of leucocytes and microorganisms was placed on each of two microscope slides, then left to dry & then fixed with 100% methanol for 1-2 minutes. Excess methanol was drained and smear was allowed to dry. The slide was flooded with acridine orange for 2 minutes. The slide was rinsed thoroughly with tap water and allowed to dry. Acridine orange- stained smears was rapidly screened using fluorescent microscopy at 100x to 400x magnification for the presence of microorganisms fluorescing bright orange against pale green to yellow background and smears were read definitively at 1000x magnification with an oil immersion objective.

**Interpretation:**
Bacteria & fungi uniformly stain bright orange, whereas human epithelial and inflammatory cells and background debris stain pale green to yellow. Nuclei of activated leukocytes stain yellow, orange or red due to increase RNA production. Erythrocytes either don't stain or appear pale green. The presence, quantity and morphology of microorganism and the presence of leukocytes were noted. A minimum of 100 high-power fields were examined and the presence of any micro-organisms within the cellular monolayer (on either slide) was considered a positive result.
Central venous catheter tip cultures:

**Tip-roll method (roll-plate technique):**

The roll-plate technique was used as the reference method. The roll-plate technique was performed by transferring each distal 3-4 cm of the catheter (its tip) on blood agar plate and was rolled back & forth across the surface at least 3 to 4 times. The plates were examined for growth after overnight incubation at 37°C. The threshold of colony-forming units (C.F.U) per plate is ≥15.

**The tip-flush:**

The catheter was placed back & rubbed with a cotton wool swab impregnated with 2.5% chlorhexidine along the outer surface of the catheter tip and allowed to dry. The catheter lumen was then flushed with a nutrient broth by introducing a sterile syringe into the proximal end of the catheter tip lumen. The endoluminal flushing was repeated five times using 1 ml of nutrient broth and then the broth was vortexed for 15 seconds, 10 μl and 100 μl were inoculated over the entire surface of two 5% blood agar plates using sterile loop. Following overnight aerobic incubation at 37°C colonies were enumerated and colony count per milliliter of broth was calculated. Significant counts were defined as more than 100 CFU/ml broth.

### 3. Results:

The results of different methods used in detection of CRBSI was illustrated in Tables (1 & 2). Taking the roll-plate technique as the reference method, it was noted that in the diagnosis of CRBSI there was fair agreement (66.7%) between the results of pour plate and roll plate techniques (kappa=0.226), there was moderate agreement between the results of paired quantitative blood culture (83.3%), tip flush method (83.3%) and AOLC/G (80.6%) and the reference method (kappa=0.429, 0.429, 0.40 respectively).

The diagnostic validity test was done for different techniques used for diagnosis of CRBSI taking roll plate as a reference method and the results was illustrated in Table (3). The diagnostic validity tests for AOLC revealed that the sensitivity and specificity of AOLC were 78.8% and 100% respectively.

There was no significant statistical difference between sex of the patients and the incidence of CRBSI (X²=0.41 & p>0.05). Also there was no significant statistical difference between the incidence of CRBSI and the different indication for applying CVCs (Corrected X²=5.46 & p>0.05) as illustrated in Table (4).

Regarding the duration of catheter insertion, there was high significant statistical difference between CRBSI and duration of catheter insertion (X²=7.44 & p<0.001) as it was 96.6% in catheters which kept in place for more than one week (32 out of 33) but it was only 33.3% in catheters which kept in place for less than one week (1 out of 3).

The incidence of CRBSI was higher when the catheter was inserted in the internal jugular vein (100%) (6 out of 6) than when inserted in the subclavian vein (83.4%) (27 out of 30) and this difference was of statistical significance (X²=5.04 - p=0.05).

It was found that there was no significant statistical difference as regarding the incidence of CRBSI and the different underlying diseases of the patients as illustrated in Table (5), however there was a highly significant statistical difference in the incidence of CRBSI with different location of insertion of CVCs (Corrected X²=10.47 & P<0.001) as it was (100%), (27 out of 27) when the catheter was inserted in dialysis room and it was (75%), (3 out of 4) when the catheter was inserted in ICU while it was only (60%), (3 out of 5) when the catheter was inserted in the operating room (the most sterile).

As regards the number of catheter lumens, there was no significant statistical difference between the incidence of CRBSI and the number of catheter lumens (Corrected X² = 0.312 & P > 0.05) in spite of higher frequencies of significant colonization occurred in those catheters with 3 lumens (100%), (3 out of 3) versus those with 2 lumens (90.4%) (19 out of 21) and catheters with one lumen (91.6%) (11 out of 12).

There was a significant statistical difference between the incidence of CRBSI and the administration of antimicrobial therapy (Corrected X²=5.04 - p<0.05) as the CRBSI was 100% in patients who didn’t receive antimicrobial therapy and it was (90%) in patients who received antimicrobial therapy.

There was a significant statistical difference between the incidence of CRBSI and the application of maximal sterile barriers precautions during catheter insertion (X²=5.04 - p<0.05) and also there was a significant statistical difference between the incidence of CRBSI and the application of daily care of the catheter as it was (100%) (27 out of 27) in patients not applying daily care of catheter while it was only (66.7%) (6 out of 9) in patients applying daily care of the catheter (Corrected X²=5.94 & P<0.05).

Regarding the type of infused fluids there was a significant statistical difference in the rate of incidence of CRBSI and the type of infused fluids through the catheter lumen as it was (71.4%) (5 out of 7) when the parentral fluid is the only infusate and it was (100%) (23 out of 23) when blood is added to the parentral fluid (Corrected X²=6.39 & p<0.05).
Fig (1) Positive results of Roll plate technique as the plate shows number of colonies > 15

Fig (2) Positive results of Tip flush method as the plate shows number of colonies >100

Fig (3) Positive results of Pour plate technique as the plate shows number of colonies >1000

Fig (4) Positive results of AOLC test as it shows short bacilli stained bright orange against green background.

Fig (5) Positive results of AOLC test as it shows cocci stained bright orange against green background.

Table (1): The results of different methods used in detection of CRBSI:

<table>
<thead>
<tr>
<th>Method</th>
<th>CRBSI</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Roll –plate method</td>
<td>33</td>
<td>91.6</td>
<td>3</td>
</tr>
<tr>
<td>Tip flush method</td>
<td>27</td>
<td>75</td>
<td>9</td>
</tr>
<tr>
<td>Pour plate method</td>
<td>21</td>
<td>58.3</td>
<td>15</td>
</tr>
<tr>
<td>Paired quantitative blood culture</td>
<td>27</td>
<td>75</td>
<td>9</td>
</tr>
<tr>
<td>Gram stain /AOLC method</td>
<td>26</td>
<td>72.2</td>
<td>10</td>
</tr>
</tbody>
</table>

Table (2): Comparison between the results of Roll plate technique and other methods used for diagnosis of CRBSI

<table>
<thead>
<tr>
<th>Method</th>
<th>Agreement</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pour plate method</td>
<td>66.7</td>
<td>0.226</td>
</tr>
<tr>
<td>Tip flush method</td>
<td>83.3</td>
<td>0.429</td>
</tr>
<tr>
<td>Paired quantitative blood culture</td>
<td>83.3</td>
<td>0.429</td>
</tr>
<tr>
<td>Gram stain /AOLC method</td>
<td>80.6</td>
<td>0.382</td>
</tr>
</tbody>
</table>

Value of K Strength of agreement
0.2 Poor
0.21-0.4 Fair
0.41-0.6 Moderate
0.61-0.8 Good
0.81-1.00 Very good
Table (3): Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positive and false negative results of different techniques used for diagnosis of CRBSI taking roll plate as a reference method:

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>False positive</th>
<th>False negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pour plate Method</td>
<td>63.6</td>
<td>100</td>
<td>100</td>
<td>20</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Tip flush Method</td>
<td>81.8</td>
<td>100</td>
<td>100</td>
<td>33.3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Paired quantitative blood culture</td>
<td>81.8</td>
<td>100</td>
<td>100</td>
<td>33.3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>AOLC</td>
<td>78.8</td>
<td>100</td>
<td>100</td>
<td>30</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

Table (4): Incidence of CRBSI with different indications for applying CVC.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NO</th>
<th>%</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
<td>%</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Monitoring fluid</td>
<td>2</td>
<td>5.5</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>No peripheral access</td>
<td>8</td>
<td>22.4</td>
<td>7</td>
<td>87.5</td>
</tr>
<tr>
<td>Monitoring CVP</td>
<td>2</td>
<td>5.5</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>24</td>
<td>66.6</td>
<td>23</td>
<td>95.8</td>
</tr>
</tbody>
</table>

Corrected $X^2=5.46$  $p>0.05$

Table (5): Incidence of CRBSI with different underlying diseases of the patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NO</th>
<th>%</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal failure</td>
<td>24</td>
<td>66.6</td>
<td>23</td>
<td>95.8</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>2</td>
<td>5.5</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Cerebrovascular stroke</td>
<td>2</td>
<td>5.5</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Surgical</td>
<td>8</td>
<td>22.4</td>
<td>7</td>
<td>87.5</td>
</tr>
</tbody>
</table>

$X^2=5.46$  $p>0.05$

4. Discussion:

The use of vascular catheters is essential for the care of critically and chronically ill cancer and haemodialysis patients (12). However, vascular catheter-related bloodstream infections (CRBSIs) have become a leading cause of health-care-associated bloodstream infections and are associated with substantial morbidity and mortality (13).

The clinical diagnosis of CRI is complex as the patient is often presented with fever without another obvious source of infection. It also lacks accuracy; as when infection is suspected, a confident diagnosis requires removal of the CVC, and up to 85% of those catheters removed on clinical grounds alone are subsequently proven to be sterile (14). Therefore, the clinician suspecting CR-BSI is faced with a difficult dilemma given that CVC removal will result in loss of venous access, while an infected catheter left in-situ may lead to overwhelming sepsis (15).

There is often a poor correlation between the clinical assessment and laboratory findings. One of the main problems in establishing a diagnosis of CRI has been the lack of gold standard definitions for contamination, colonization and infections (localized and systemic) associated with intravascular devices including CVCs (16).

As the coagulase negative staphylococci (CoNS) are the predominant causative microorganism associated with CRI, the interpretation of microbiological findings is often complex as positive blood or catheter segment cultures may represent catheter colonization, infection, or sample contamination (17).

Rapid and accurate diagnosis of CRI is therefore essential for providing both optimal patient care and management, and reducing additional healthcare costs related to anti-microbial therapy and extended hospitalization. A wide range of approaches were used to overcome this obstacle and to diagnose CRBSI. These techniques can be distinguished into two categories: 1) techniques requiring CVC removal; and 2) CVC-sparing methods (in-situ methods) (18). These methods are expensive, time-consuming, and depend on culture techniques that require 24 to 48 hours for in-vitro culture to confirm the diagnosis of CR-BSI (19; 20). Acridine orange leukocyte cytopsin (AOLC) test is an alternative to conventional microbiological techniques. The test has the advantage of reporting data within 30 minutes with high sensitivity and specificity (18).

The aim of the present study is to determine whether the Gram stain-acridine orange leucocyte
cytospin (AOLC) test could offer accuracy comparable to other methods for the diagnosis of catheter-related blood stream infection and to avoid inconvenient, unnecessary and costly central venous catheter removal.

The rate of the CVC related blood stream infection in the present study was 91.6% by the reference roll plate method. In agreement with our result Abdulla et al., (21) reported a rate of 92% for CRBSI and Friedman et al., (22) reported that the incidence of BSI was 88% and the study demonstrated that intravascular devices and urinary tract infection were the most common sources of BSI, however lower rate of detection was reported by Rao et al., (23) as they reported a colonization rate of 62.5% for the central venous catheters and Oncu et al., (24) who reported that 30.3% of the CVCs were colonized.

These differences in the incidence of CRBSI in different studies may be explained by: difference in the type of patients as most of patients in our study were haemodialysis patients who kept the CVCs for long period of time with increased the chance for the incidence of CRBSI.

In the present study as regarding techniques requiring catheter removal, semi-quantitative method described by Maki (25) (roll plate method) was used in order to detect any case of significant catheter infection, and was used as a reference method on which the rest of the studied techniques had been evaluated. By this method 33 catheters out of 36 catheters were positive (91.6%) for CRBSI and only 3 catheters were negative.

Regarding the another method requiring catheter removal, quantitative method described by Cleri et al., (26) (tip flush method) was used in our study and diagnosed 27 CRBSI cases (75%) out of 36 cases. Comparing the performance of the tip flush method with the reference method, the sensitivity was 81.8%, and the specificity was 100%. Our result was in agreement with Farr, (27) and Raad et al., (28) who reported specificity 100 % and 98% respectively of tip flush method. However the sensitivities of the previous studies were 92% and 93% respectively. Their higher sensitivities of tip flush in comparison with our study may be attributed to their use of catheter sonication which greatly increases the number of microorganisms that can be quantified and thus potentially increasing the sensitivity.

Other methods not requiring catheter removal like pour plate, paired quantitative blood cultures and AOLC /G were used in this study. Using a simple pour plate technique, we determined the microbial concentration in sample of blood collected via the hub of CVCs while they were in position. The pour plate technique was positive in 21 cases only (58.3%) out of 36 cases.

The sensitivity of the pour plate method in comparison with the reference method was 63.6%, and the specificity was 100%. This result was in agreement with Andreumont et al., (7) who demonstrated that the specificity of this test in their study on cancer patients was 99%, and Chan et al., (29) who reported that the sensitivity and the specificity of the pour plate technique were 77.8% & 100% respectively. The higher sensitivity in their study may be due to large number of patients (90 patients) in comparison with our study (36 patients).

In the present study the sensitivity and the specificity of the paired quantitative blood culture technique were 81.1% & 100% respectively. The results of our study is close to the result of a prospective cohort study by Catton and his colleagues which compared the accuracy of three techniques not requiring catheter removal (quantitative blood culture technique, differential time to positivity and endoluminal brushing) for diagnosis of CRI, quantitative blood cultures yielded in their study a sensitivity and a specificity of 84% and 97% respectively (30).

Safdar et al., (6) reported that quantitative blood culture has a specificity of 98% and concluded that paired quantitative blood cultures is the most accurate diagnostic method in patients with long-term CVCs. This result was in agreement with our study, but they reported a sensitivity of 87% which was slightly higher than of our result. This may be due to the use of empirical antimicrobial therapy which affects the sensitivity of our test as 83.3% of our patients were on antimicrobial therapy, while Safdar et al., (6) started their test before the empirical antimicrobial therapy was given.

The acridine orange leucocyte cytospin (AOLC) test is an alternative to conventional microbiological techniques. The test has the advantage of reporting data within 30 minutes with high sensitivity and specificity (31). In our study the acridine orange leucocyte cytospin test diagnosed 26 (72.2%) cases out of 36 cases with specificity of 100%. Similar results were obtained by Rushforth et al., (32) and Bong et al., (33) who reported that the AOLC test has a specificity of 100% in diagnosing CR-BSI.

However the sensitivity of AOLC was 78.8%. The sensitivity of our result was less than that of Rushforth et al., (32) and Bong et al., (33) who reported a sensitivity of 87% for AOLC test. The relatively high sensitivity recorded by Rushforth et al., (32) and Bong et al., (33) may be due to the use of AOLC test alone - not combined by Gram stain- with possibility of false positive results in AOLC test which may occur due to granules from disintegrating leukocytes that may be mistaken as cocci. Such a mis-interpretation may be
corrected if the Gram stain is added. These results were confirmed by Worthington and Elliott, (34) who reported that the Gram stain increases the specificity of the AOLC test and allows early identification of organisms which, in turn, allows for specific antibiotic therapy to be started at once, rather than relying on empirical broad-spectrum antibiotic therapy.

On analysis of risk factors for CR-BSI in the current work, it was found that a highly significant association between the duration of catheterization and the occurrence of CR-BSI. This result was in agreement with the results of Furfaro et al., (35) and Raad et al., (36) who found that the incidence of CR-BSI increased from the fourth day of catheterization onward. Brun-Buisson et al., (37) reported increase in incidence of CRBSI from 2.5% at 3 days to 12% at 6 days and 22% after 7 days or more days of use. Also, Dimick et al., (38) declared a highly significant association between the duration of catheterization and CR-BSI. In contrast, Gowardman and coworkers (39) found no significant association between CR-BSI and the duration of catheterization.

The question of which venous catheterization site is associated with the higher risk of infection remains controversial. In the current work, the incidence of CRBSI was 100% when the catheter was inserted in the internal jugular vein access, but it was 90% when the catheter was inserted in the subclavian vein access. So the subclavian vein is superior to the internal jugular vein although it was of no statistical significance. This result was in agreement with Zing et al.,(18) who preferred and recommended the subclavian vein as a site for catheter insertion as the jugular access is associated with significantly more CR-BSI than subclavian access, which is probably due to three factors favoring skin colonization: the proximity of the insertion site to the mouth and the oropharyngeal secretion; the higher density of local skin flora due to the higher local skin temperature; and the difficulties in maintaining occlusive dressings. However Gowardman and coworkers (39) reported that no significant association was found between the site of catheter insertion and the occurrence of CR-BSI. Also Lorente et al., (13) demonstrated that femoral venous access had a significant higher rate of CR-BSI than the peripheral and subclavian access. This is probably due to greater degree of bacterial colonization of the groin compared to the shoulder and neck.

In this study there was no significant statistical difference between the incidence of CRBSI and the number of lumens of the catheter (p>0.05).This result was in agreement with Gupta et al., (40) and Gowardman et al., (39) who found no association between the number of catheter lumens and the occurrence of CR-BSI. Yet, Brismar et al., (41) and McCarthy et al.,(42) demonstrated that the use of multiple-lumen catheters increases the risk of CR-BSI, and linked that to the increased risk of endoluminal contamination. Also, Templeton et al., (43) reported that each additional lumen increases the risk of CR-BSI.

In the present study, half of the patients were on antimicrobial treatment (83.3%). There was significant statistical difference between the incidence of CRBSI and the administration of antimicrobial therapy, as the incidence of CRBSI was 100% in patients who didn’t receive antimicrobial therapy and it was only 90% in patients who received antimicrobial therapy. In the study conducted by Oncu et al., (44), they suggested that the use of glycopeptides antibiotic during catheterization seem to have protective effect against catheter related infection, as the incidence of CRI was higher in patients who were not using glycopeptides antibiotic (24%) than patients who used glycopeptides antibiotic during catheterization (4.4%).

Our results were in disagreement with Wilcox et al.,(45) who found that the antibiotic treatment did not affect the result of CRBSI, and explained that by the fact that Staphylococcus epidermidis strains causing CRBSI is protected from the antibiotic effect by the slime which they produce. Also Ljungman et al., (46) reported that oral or parenteral antibacterial or antifungal drugs might not reduce the incidence of CRBSI among adults.

Several studies proved that maximal sterile barriers precautions (e.g. cap, mask, sterile gown, sterile gloves and large sterile drape) during the insertion of CVCs substantially reduce the incidence of CRBSI compared with standard precautions (e.g. sterile gloves and small drapes) (36 & 46). This was in agreement with the result of the present study in which the CVC colonization was higher in CVCs which were inserted without applying the maximal sterile barriers precautions compared with those CVCs inserted with applying the maximal sterile barriers precautions and such difference was statistically significant (X2=5.04 & P<0.05).

Conclusion:

From this study, it could be concluded that the Gram stain-AOLC test is a simple, rapid, sensitive and specific test that could be used as a first line screening test for the in situ diagnosis of CRBSI. This policy can prevent the unnecessary removal of uninfected catheters and significantly extends the life span of catheters and lowers the risks and costs of mechanical complications associated with new catheter placement. In positive cases, the results of the gram stain-AOLC test support the decision to remove the infected catheter and empirical antibiotic therapy could be initiated and blood culture is to be collected.
in order to isolate the organism and determine its antimicrobial susceptibility pattern.

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2/2/2013