Anti-adherence activity and antimicrobial durability of anti-infective-coated catheters against multidrug-resistant bacteria


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Received 21 December 2007; returned 21 March 2008; revised 4 June 2008; accepted 16 June 2008

Introduction

Central venous catheters (CVCs) have become essential devices used in the medical care of critically ill, haemodialysis and cancer patients. However, such devices have become the leading source of bloodstream infections in these patient populations. It is estimated that in the USA alone, over 5 million CVCs are inserted annually. In intensive care units (ICUs) of the USA, ~50% of the patients have CVCs, accounting for about 15 million CVC days/year. CVCs are responsible for over 400 000 cases of nosocomial bacteraemia annually in the USA. In ICUs alone, it has been estimated that approximately 80 000 catheter-related bloodstream infections (CRBSIs) occur annually, with an estimated cost that ranges between $296 million and $2.3 billion and attributable mortalities of 4% to 20%, resulting in approximately 10 000 to 20 000 deaths annually. These facts clearly dictated the need for innovative strategies to prevent CRBSIs.

Antimicrobial CVCs represent novel technological innovations in that the catheter surfaces are coated with antimicrobial agents, rendering them more resistant to colonization by microorganisms. In a previous study, we compared the in vitro activity of antimicrobial CVCs against fungi (Candida species), methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa. These anti-infective CVCs were shown to be clinically efficacious in preventing colonization and/or CRBSI.

Research has shown that vancomycin-resistant S. aureus (VRSA) is associated with catheter infections, and Stenotrophomonas maltophilia, Acinetobacter baumannii/ calcoaceticus and Enterobacter agglomerans are among the

Objectives: To investigate the anti-adherence and antimicrobial durability of anti-infective catheters against multidrug-resistant (MDR) Staphylococcus aureus (resistant to vancomycin, rifampicin and methicillin) and MDR Gram-negative bacteria (Stenotrophomonas maltophilia, Acinetobacter baumannii/calcoaceticus and Enterobacter agglomerans) that are often associated with catheter-related bloodstream infections (CRBSIs).

Methods: Catheters impregnated with minocycline and rifampicin (M/R) or with silver-platinum and carbon (SPC) or with chlorhexidine and silver sulfadiazine (CHX/SS) were compared with non-coated catheters. Adherence of organisms was tested by using an established biofilm colonization model. All isolates were rifampicin-resistant. Antimicrobial durability was tested by soaking 1 cm segments of the catheter in serum and determining zones of inhibition against the tested organisms at weekly intervals.

Results: The M/R catheters showed significantly superior anti-adherence activity and more prolonged antimicrobial durability when compared with CHX/SS-central venous catheter (CVC), SPC-CVC and uncoated control catheters against MDR and vancomycin-resistant S. aureus (MDR VRSA) (all P values < 0.02), MDR S. maltophilia (all P values < 0.005) and MDR A. baumannii/calcoaceticus (all P values < 0.002), respectively. M/R-CVC and CHX/SS-CVC had comparable anti-adherence and antimicrobial durability against MDR E. agglomerans, and these two were superior to SPC-CVC and the uncoated control catheters (all P values < 0.001).

Conclusions: M/R-CVC demonstrated superior anti-adherence activity and more prolonged antimicrobial durability when compared with other approved anti-infective catheters against MDR VRSA and/or MDR Gram-negative bacteria that are often associated with CRBSIs. This finding could explain their efficacy and better performance in clinical studies.

Keywords: coated catheters, catheter infections, VRSA, Acinetobacter, Stenotrophomonas maltophilia
leading causes of catheter-related Gram-negative bacteraemia.\(^7\)
Therefore, in this current study, we compare the activity of anti-
microbial CVCs against pan-resistant bacteria associated with
CRBSI in high-risk patients, such as multidrug-resistant (MDR)
VRSA, MDR \(E.\) agglomerans and MDR \(S.\) maltophilia and \(A.^
baumannii/calcocaeiticus.\)

Materials and methods
The anti-infective catheters investigated were impregnated
with minocycline and rifampicin antibiotics (M/R-CVC Spectrum, Cook
Medical, Bloomington, IN, USA) or with silver-platinum and carbon
(SPC-CVC Oligon, Edwards Life Science, Irvine, CA, USA) or with
chlorhexidine and silver sulfadiazine (CHX/SS-CVC, Arrow Gard
Plus, Arrow International, Reading, PA, USA). These catheters were
compared with non-coated catheters. Adherence of organisms was
tested by using an established biofilm colonization model.

Adherence testing
We evaluated bacterial adherence to the surfaces of uncoated CVCs,
M/R-CVC, CHX/SS-CVC and SPC-CVC. All tested polyurethane cathe-
sters had a diameter of 2 mm. Four 5 mm long segments of
each catheter type were tested per organism. The method for testing
adherence has been described previously.\(^2\) Sterile CVC segments
were placed into sterile 24-well tissue culture plates (Corning
Costar, Corning, NY, USA) containing 1 mL of plasma to enhance
the formation and binding of blood proteins and biofilm to the sur-
faces of the catheter segments. The plates were then placed in the
incubator for 24 h at 37°C. The plasma was removed from the wells,
with the catheter segments left inside, replaced with 1 mL of
Mueller–Hinton broth that had been inoculated with \(5.5 \times 10^7\)

cfu/mL of microorganisms and incubated at 37°C for 24 h. All organisms
used were rifampicin-resistant (MIC \(\geq 8.0\) mg/L) and included MDR
VRSA (resistant to vancomycin, rifampicin and methicillin) as
well as MDR Gram-negative bacteria (\(S.\) maltophilia, \(A.\) baumannii/
calcocaeiticus and \(E.\) agglomerans), all of which were clinical iso-
lates that had previously caused CRBSIs in cancer patients. However,
all of the MDR organisms tested were minocycline-susceptible
(MIC \(= 1.0\) mg/L). The inoculum was then removed and segments
were washed in 0.9% sterile saline with shaking for 30 min to
remove any planktonic organism. After washing, segments were
sonicated for 15 min in 5 mL of 0.9% sterile saline and then plated
on trypticase soy agar +5% sheep blood (BD, Sparks, MD, USA).
Plates were incubated, inverted at 37°C for 24 h and then counted
for colony growth. A colony count of 100 cfu was recorded for any
growth \(\geq 100\) cfu, and dilution factors (50) were taken into account
to calculate the final cfu/segment. The upper limit of our detection
was 5000 cfu/mL.

Zones of inhibition and antimicrobial durability
We used a modified Kirby Bauer method to evaluate baseline anti-
microbial activities of catheter segments, as described previously.\(^3\)
Duplicates of catheter segments were vertically embedded in Mueller–
Hinton agar plates (BD) coated with one of the organisms that had previ-
ously caused CRBSI in cancer patients. The plates were incubated
overnight at 37°C, and the zones of inhibition produced around
catheter segments were measured and recorded as the diameters,
in millimetres, across the centres of the embedded catheter segments.

Furthermore, the antimicrobial durability of catheter segments
was also assessed over time by weekly testing for zones of
inhibition produced by segments after they were soaked in serum.
The catheter segments were placed in sterile 50 mL polystyrene
tubes (Falcon, Franklin, NJ, USA) containing 10 mL of sterile
serum as a suitable biological body fluid and were incubated at
37°C. The 10 mL volume was used to ensure the complete immer-
sion of all the segments placed in the tube. At weekly intervals, the
serum was changed, and two segments per catheter type were tested
to determine antimicrobial durability after the segments had been
immersed in serum. Zones of inhibition were determined using the
modified Kirby–Bauer method against the same organisms men-
tioned earlier.

Statistical methods
For each bacterium strain, the number of viable organisms adhering to
the catheter segments measured by cfu was compared by the
Kruskal–Wallis test \((P\) value less than 0.05 was considered statisti-
cally significant). If a significant result was detected for the test, we
used Wilcoxon rank sum tests for pairwise comparisons. The \(\alpha\) levels
of the post hoc pairwise comparisons were adjusted using a sequential
Bonferroni adjustment to control type 1 error. The durabilities of
various catheters against the organism measured by the diameter of
the zone of inhibition were compared by two-way non-parametric
analysis of variance (ANOVA) (using ANOVA in conjunction with
rank transformation). If the catheter effect on durability was signifi-
cant, multiple comparisons of catheter effect were performed, and the
\(\alpha\) levels of the post hoc pairwise comparisons were also adjusted
using a sequential Bonferroni adjustment. All analyses were per-
formed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results
Adherence testing
Table 1 shows the microbial adherence of various organisms
(MDR VRSA, MDR \(A.\) baumannii/calcocaeiticus, MDR \(E.\) agglomerans and MDR \(S.\) maltophilia) to the surfaces of all
anti-infective catheter segments tested in comparison with the
adherence to control uncoated catheters. As shown in Table 1,
M/R-CVC was the only anti-infective CVC that significantly
decreased the adherence of all resistant bacteria tested when com-
pared with control uncoated catheters.

Furthermore, M/R-CVC did significantly decrease the adher-
ence of MDR VRSA compared with SPC-CVC \((P = 0.001)\) and
CHX/SS-CVC \((P = 0.02)\). Similarly, M/R-CVC did significantly
decrease the adherence of MDR \(A.\) baumannii/calcocaeiticus
when compared with CHX/SS-CVC \((P = 0.002)\), SPC-CVC
\((P = 0.002)\) and the control uncoated catheter segments \((P =
0.002)\). With respect to \(E.\) agglomerans, M/R-CVC and CHX/
SS-CVC were equivalent in being significantly more efficacious
than SPC-CVC \((P < 0.001)\) and the uncoated control cata-
ther segments in preventing the adherence of this organism
\((both\ P < 0.001)\). In addition, M/R-CVC was significantly more
efficacious than all other catheters, including CHX/SS-CVC, SPC-
CVC and the uncoated catheter in preventing the adherence of
MDR \(S.\) maltophilia \((all\ P\ values < 0.005)\).

Antimicrobial durability
As shown in Figure 1(a), M/R-CVC had a significantly longer
antimicrobial durability against VRSA when compared with
Table 1. Adherence of bacteria to polyurethane CVC surfaces

<table>
<thead>
<tr>
<th>Device</th>
<th>Acinetobacter</th>
<th>E. agglomerans</th>
<th>S. maltophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control CVC</td>
<td>5000</td>
<td>5000</td>
<td>5000</td>
</tr>
<tr>
<td>M/R-CVC</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CHX/SS-CVC</td>
<td>525</td>
<td>1556</td>
<td>0</td>
</tr>
<tr>
<td>SPC-CVC</td>
<td>5000</td>
<td>5000</td>
<td>5000</td>
</tr>
</tbody>
</table>

P values indicate results of comparisons between control CVC and each of the other types of CVCs (Wilcoxon rank sum test). NS, statistically non-significant.

Discussion

M/R-CVC showed significantly improved antimicrobial adherence activity and more prolonged antimicrobial durability when compared with CHX/SS-CVC, SPC-CVC and uncoated control catheters against MDR VRSA, MDR S. maltophilia and MDR A. baumannii/calcoaceticus. In contrast, both CHX/SS-CVC and M/R-CVC had comparable antimicrobial adherence and durability against MDR E. agglomerans, but were superior to SPC-CVC and uncoated control catheters (all P values < 0.001).

The data from this current study support those from a large number of clinical trials testing various anti-infective catheters. M/R-coated CVC followed by CHX/SS-coated CVC were associated with a significant reduction in CRBSI in the largest number of clinical trials.1,4,5

In a multicentre prospective randomized trial by Darouiche et al.,8 comparing these two anti-infective CVCs, it was demonstrated that among indwelling catheters that remained in place for more than 7 days, the frequency of CRBSI in the CHX/SS-CVC group was 6.4%, whereas in the M/R-CVC group was only 0.7% (P = 0.01). Hence, the significantly more prolonged antimicrobial durability of M/R-CVC (as demonstrated in the current study) could have contributed in a major way to its superiority over the first-generation CHX/SS-CVC, which is known to have antimicrobial durability equal to or shorter than the second-generation CHX/SS-CVC. Furthermore, data from this study showing superior and more prolonged antimicrobial durability of M/R-CVC over other anti-infective catheters, including CHX/SS-CVC, against MDR VRSA and other MDR Gram-negative bacilli (A. baumannii/calcoaceticus and S. maltophilia) could explain the efficacy of M/R-CVC in high-risk patients requiring prolonged catheterization.1,5,8 In addition to the prolonged durability of M/R-CVC, their unique activity in preventing the adherence of pan-resistant bacteria could explain their high efficacy in clinical trials.5,8

Our data have also shown that M/R-CVC and CHX/SS-CVC were significantly superior to SPC-CVC in terms of antimicrobial durability and anti-adherence activity directed against MDR VRSA and MDR Gram-negative bacilli. This could explain the success of these two types of anti-infective catheters in clinical trials and the limitation of SPC-CVC in clinical testing. More recently, two prospective randomized controlled trials failed to
show any benefit of the novel SPC-CVC in reducing CRBSI, and Fraenkel et al. have shown that M/R-coated CVCs are associated with a significantly lower risk of microbial colonization when compared with SPC-CVC.

All the MDR organisms tested in our study were rifampicin-resistant. Although Sampath et al. showed previously that rifampicin-resistant Staphylococcus epidermidis is initially inhibited by M/R- and CHX-SS-CVC, this inhibition was not maintained beyond 7 days for M/R-CVC. In contrast, our current study showed that M/R-CVC maintained antimicrobial activity against all of the MDR rifampicin-resistant pathogenic organisms (including MDR VRSA and MDR Gram-negatives) for a period that exceeded 21 days (Figure 1a–d). Furthermore, the antimicrobial durability of M/R-CVC was significantly more prolonged than the other CVCs against MDR S. maltophilia. M/R-CVC had a significantly more prolonged durability than the other CVCs.

In conclusion, the ability of microbial organisms to adhere to the surface of anti-infective catheters by testing such catheters in a biofilm colonization model and the antimicrobial durability of catheters in serum could explain their clinical performance. M/R-CVC were associated with more prolonged antimicrobial durability and higher efficacy in decreasing the microbial adherence of MDR VRSA as well as MDR S. maltophilia and A. baumannii/calcoaceticus when compared with CHX/SS-CVC and SPC-CVC. The CHX/SS-CVC had activity equivalent to that of the M/R-CVC in preventing the adherence of and maintaining antimicrobial durability against MDR E. agglomerans. Future studies should focus on improving the antimicrobial durability of the antiseptic chlorhexidine-based CVC and broadening the spectrum of the M/R-CVC to include an antifungal agent.

Acknowledgements

We are grateful to Mr Xiang Fang for extensively reviewing and editing the manuscript. We are also thankful to Ms Laura Claburn for her assistance in preparing this manuscript.

Funding

This original research was funded by the Infectious Disease Innovative Catheter Research Fund—Department of Infectious
Diseases, Infection Control and Employee Health. No other funding was received for this research.

Transparency declarations
I. R. is a co-inventor of two patents associated with devices coated with minocycline and rifampicin. These patents are the property of The University of Texas M. D. Anderson Cancer Center and Baylor College of Medicine. Both patents were licensed to Cook Critical Care, American Medical Systems, Biomet and TyRx with royalty rights to the institutions and inventors involved. In addition, I. R. is a co-inventor of patents associated with minocycline and EDTA catheter flush solution. These patents are the property of The University of Texas M. D. Anderson Cancer Center, Baylor College of Medicine and Wake Forest University. At times over the last 16 years, I. R. has received research grant support through M. D. Anderson Cancer Center from companies that produce CVCs or products that relate to CVCs. These include: Kimberly Clark Corporation, Becton Dickinson, Abbott, Implemed Co., Immunomedics and Cook. Other authors: none to declare.

References