Comparison of microbial adherence to antiseptic and antibiotic central venous catheters using a novel agar subcutaneous infection model

Trupti A. Gaonkar and Shanta M. Modak*

Introduction

Central venous catheters coated with antimicrobials have been shown to reduce catheter-related infection. To date, the most effective agents used to treat the catheters include either a combination of chlorhexidine and silver sulfadiazine or minocycline and rifampicin. The efficacy of antimicrobial catheters is normally screened using in vitro methods and animal models before clinical trials. A modified Kirby-Bauer zone of inhibition assay is often used to assess the antimicrobial spectrum and to determine the half-life of activity of catheters both in vitro and in vivo. The use of zone of inhibition testing for predicting the clinical efficacy of antimicrobial catheters has also been proposed.

Another in vitro method routinely used for evaluation of antimicrobial efficacy of catheters is the measurement of bacterial adherence on catheter surfaces. This involves the exposure of catheter segments to growing bacterial cultures or bacteria suspended in phosphate buffered saline. Determination of adherent bacteria is then done semi-quantitatively by rolling on agar plates or quantitatively using sonication methods.

Since the in vitro adherence methods involve the exposure of catheters to bacteria in liquid media, they may not accurately reflect the colonization of catheters in the subcutaneous area in vivo (in animal models or in patients). In vivo colonization of the outer surface of the catheter is the result of bacterial migration from the skin around the catheter and through the subcutaneous tract. Therefore, the efficacy of antimicrobial catheters is evaluated in animals before clinical trials by implanting them subcutaneously.

An agar subcutaneous infection model (agar model), which simulates the rat subcutaneous infection model (rat model), was developed to assess the ability of antimicrobial catheters to resist microbial colonization. The catheters were implanted in the agar and rat models and the insertion sites were infected immediately or on day 7, 14 or 21 post-implantation. The catheters implanted in the agar model were transferred to fresh media one day before infection on day 7, 14 or 21. The efficacy of chlorhexidine and silver sulfadiazine impregnated (CS) catheters, CS catheters with higher levels of chlorhexidine (CS+ catheters), minocycline-rifampicin (MR) catheters and silver catheters against Staphylococcus aureus and rifampicin-resistant Staphylococcus epidermidis RIF-r2 was compared in the agar and rat models. No significant difference in the adherence or the drug release was found between the in vitro and in vivo models. In both models, CS+ and MR catheters were effective against S. aureus even when infected on day 14, whereas CS catheters were colonized when challenged on day 7. CS+ catheters were effective against S. epidermidis RIF-r2, whereas MR catheters showed adherence when infected on day 7. CS+ catheters prevented colonization of all the organisms including, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Candida albicans in the agar model, whereas MR catheters were effective only against S. aureus and S. epidermidis strains. Silver catheters were ineffective against all the organisms. The agar model may be used to predict the in vivo efficacy of antimicrobial catheters against various pathogens.

Keywords: colonization, antimicrobials, silver sulfadiazine, chlorhexidine

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first step in screening antimicrobial catheters before final evaluation in animals.

In this study, we describe an agar subcutaneous infection model (agar model) that simulates the rat subcutaneous infection model (rat model) for evaluation of the efficacy of various antimicrobial catheters in resisting microbial colonization. Antiseptic catheters (chlorhexidine and silver sulfadiazine impregnated), antibiotic catheters (minocycline and rifampicin coated) and Vantex Oligon (silver) catheters were evaluated using this model and the rat model. The results of this study are presented here.

Materials and methods

Cultures

The following organisms were used: S. aureus ATCC 10390, S. epidermidis ATCC 35983, Enterobacter aerogenes ATCC 14053, Klebsiella pneumoniae ATCC 13883, Pseudomonas aeruginosa ATCC 27853, Candida albicans (F60552) and Escherichia coli isolates used in this study were from catheterized patients at the New York Presbyterian Medical Center, New York.

Two rifampicin-resistant S. epidermidis strains were used, one, a clinical isolate from Harlem Hospital, New York (RIF-r1, MIC > 500 mg/L) and the other, a susceptible strain made resistant in our laboratory by repeated in vitro exposure to rifampicin (RIF-r2, MIC > 500 mg/L). Both these strains were susceptible to minocycline (MIC=0.094–0.1 mg/L).

Culture media

Trypticase soy broth (TSB), trypticase soy agar (TSA), drug inactivating Dey/Engley agar (DE agar) and Sabouraud dextrose broth (SDB) were purchased from Fisher Scientific Co., GA, USA. Bacto-agar was purchased from Becton-Dickinson, MD, USA and bovine adult serum (BAS) and PBS were obtained from Sigma–Aldrich, St Louis, MO, USA. Whole milk UHT (Parmalat) was obtained from a local store.

Drug neutralizing broth (DNB) containing 3% TSB, 0.1% tryptone, 0.5% sodium thiosulphate, 0.1% protease peptone, 0.6% sodium oleate, 2% lecithin and 5% Tween-80 was prepared by deionized water.

Soft agar medium for agar model: A special soft agar medium comprising 1.0% Bacto-agar + 20% BAS + 0.5% whole milk + 0.03% TSB in PBS was constituted to simulate subcutaneous tissue. The agar and TSB were dissolved in PBS and autoclaved at 121°C for 20 min. After cooling to 50–55°C, the BAS and milk were added aseptically to the rest of the medium.

The soft agar medium used for testing C. albicans contained 0.5% galactose and 0.1 mM calcium chloride in addition to the above ingredients.

Catheters

Polyurethane 7 Fr triple lumen catheters (untreated as well as antiseptic impregnated) were obtained from Arrow International Inc., Reading, PA, USA. The antiseptic catheters were Arrowgard, impregnated with chlorhexidine and silver sulfadiazine on the outer surface only (CS), and Arrowgard+, which is the same as CS but impregnated with chlorhexidine on the luminal surface and with a higher concentration of chlorhexidine on the outer surface (CS+). Antibiotic Cook Spectrum catheters coated with minocycline and rifampicin (MR) were purchased from Cook Critical Care, Bloomington, IN, USA. Vantex Oligon catheters (silver) were purchased from Baxter Health Corporation, Irvine, CA, USA.

A 2 cm segment adjacent to the hub and a 5 cm segment from the tip of each catheter were cut off and the middle portion of the catheter was divided into 4 cm long segments (this was done to obtain segments with more or less uniform drug levels). These 4 cm segments were sealed at both ends by heat and were sterilized by ethylene oxide (ETO).

Preparation of microbial cultures

All the bacterial cultures were grown overnight at 37°C in TSB and diluted with PBS to a concentration of 10^6 cfu/mL. A 30 µL aliquot of this culture was used for inoculation of each catheter. C. albicans was prepared by inoculating 2 mL of a 48 h culture (grown in SDB with 9% galactose at 37°C) into 8 mL of the same medium in a 50 mL flask. This was then incubated with shaking at 100 rpm at 25°C for 24 h. The cells were harvested by centrifugation, the supernatant discarded and the cells washed and resuspended in PBS containing 0.5% galactose and 0.1 mM CaCl2 to give a cell density of 1–2 × 10^6 cfu/mL; 30 µL of this suspension was then used to inoculate each catheter.

Figure 1. Agar model (bacterial adherence in catheters).

Agar model

A novel agar subcutaneous infection model was developed (Figure 1). Soft agar medium (12 mL) was placed in culture tubes and allowed to solidify. Catheter segments were then pushed vertically into the centre of the medium in each tube with 0.5 cm of the catheter protruding out of the medium (insertion site). While inserting, the catheter displaces the medium to form a tract which gets resealed due to the semisolid consistency of the soft agar medium leaving no visible space between the catheter and the surrounding medium.

For inoculation, each catheter segment was pulled out (approximately 2 cm) from the tract and the catheter surface was inoculated with 10 µL of the microbial culture diluted to the desired density. After pushing the catheter back into the tract, 20 µL of the same culture was used to inoculate the catheter insertion site. The caps of all tubes were sealed with
parafilm to prevent desiccation and the tubes were incubated at 37°C. Microbial adherence was determined 7 days post-inoculation in both models.

For evaluation of initial efficacy, the catheters were inoculated immediately post-implantation (1st day of infection). However, for long-term efficacy, the inoculation was performed on day 7, 14 or 21 post-implantation (7th, 14th or 21st day of infection). The catheters from the latter groups were transferred to fresh medium 1 day before inoculation. This was done to simulate the drug clearance in vivo and to allow the drug released during the period between transfer to fresh media and inoculation to accumulate in the agar around the catheter.

In each experiment, five segments from each catheter group were tested and three such parallel experiments were carried out for each study (total of 15 segments in each group).

In vivo rat subcutaneous infection model (rat model): All animal studies were conducted under a protocol approved by the Institutional Animal Care and Use Committee of Columbia University. The protocol is in compliance with all federal and state regulations including the Animal Welfare Act. In each group, a total of five female Sprague–Dawley rats weighing between 250 and 300 g were used for each study. The rats were housed in individual cages. The rats were anaesthetized by administrating ketamine + xylazine intraperitoneally.

To determine the efficacy, three segments of catheters (each of 4 cm length) were implanted subcutaneously (3 cm apart) on the dorsal side in each rat allowing 0.5 cm to protrude out through the insertion site. For 1st day infection, the catheters were infected immediately after implantation and for long-term efficacy, the inoculation was performed at 7 or 14 days post-implantation. The catheter segment (about 2 cm) was pulled out from the tract and was inoculated with 10 μL of 1 × 10⁷ cfu/mL of S. aureus or S. epidermidis RIF-r2 and pushed back in leaving 0.5 cm protruding out of the tract. After closing the insertion site with sterile clips, it was inoculated with another 20 μL of the same culture. The catheters were removed from the animals 7 days post-infection and the bacterial adherence was determined using a modification of the method described previously.²

### Determination of microbial adherence to catheters

In all cases, a swab culture was taken from the insertion site of each catheter 7 days post-infection and subcultured on TSA. The catheter segments were then removed and blotted with tissue paper to remove the fluid on the catheter. In the agar model, all five catheter segments from one group were transferred to a 50 mL culture tube containing 25 mL (5 mL/segment) of saline. In the rat model, all three segments from each rat were transferred to 15 mL of saline. Each tube was capped and inverted three times. The segments were removed from the saline and touched against the surface of the tube to drip off the excess saline and then transferred to another test tube containing 25/15 mL of saline. This procedure was repeated a total of three times to remove the non-adherent bacteria.

After the final rinse, the catheters were blotted with tissue paper and trimmed to 2 cm lengths by cutting off 1 cm from each end. Each 2 cm segment was then placed in 4 mL of DNB in a culture tube and sonicated for 20 min in an ultrasonic water bath (Astrasan Sonifier, Farmingdale, NY, USA; 110 V, 50–60 Hz). This sonicate (0.5 mL) was subcultured on DE agar plates, which were then incubated at 37°C for 24–48 h, and colony counts were determined.

### Comparison of S. aureus adherence to catheters in the agar and rat models

Adherence of S. aureus on CS, CS+, MR and control catheters infected on day 1, day 7 and day 14 was evaluated in the agar and rat models as described above. Adherence on silver catheters infected on day 1 was compared in both models.

### Comparison of S. epidermidis RIF-r2 adherence to catheters in the agar and rat models

CS, CS+, MR, silver and control catheters were evaluated for their efficacy in preventing S. epidermidis RIF-r2 adherence in the agar and rat models when challenged on day 1 or day 7.

### Comparison of drug retention in catheters implanted in the agar and rat models

CS and CS+ catheters (two sets each) were implanted in the agar model as well as in rats (15 catheters in each group) without any bacterial challenge. One of the sets from each model was explanted on day 7 and the other on day 14. The residual chlorhexidine levels in these segments were determined. Initial levels of chlorhexidine in CS and CS+ catheters used for implantation were determined by using segments from the pooled catheter segments. The chlorhexidine levels were determined using modification of the method described previously.³ A segment (1 cm length) from each catheter (15 segments from each group) was extracted with 2 mL of dichloromethane for 20 min with intermittent vortexing. To this was added, 4 mL of 50% reagent alcohol and extraction was carried out for 20 min. The two layers were separated by centrifugation and the top layer was read spectrophotometrically at 259 nm to determine the chlorhexidine concentration.

### Evaluation of the initial efficacy of catheters against various organisms in the agar model

Initial efficacy of CS, CS+, MR, silver and control catheters against S. epidermidis, S. epidermidis RIF-r1, E. coli, K. pneumoniae, E. aerogenes, P. aeruginosa and C. albicans was evaluated by inoculating immediately after implantation on day 1. The adherence was determined 7 days post-inoculation.

### Evaluation of long-term efficacy of catheters against S. epidermidis and E. coli in the agar model

The long-term efficacy of CS, CS+, MR and control catheters against adherence of S. epidermidis and E. coli was evaluated by inoculating the catheters on the 7th, 14th or 21st day post-implantation. In all the groups, the catheters were transferred to fresh medium 24 h before inoculation.

### Data analysis

Data analysis was carried out using BMDP software (University of California, Berkeley, CA, USA). Group differences in the bacterial colonization and drug levels in test and control catheters between the agar and rat models were determined using an analysis of variance with multiple comparisons carried out using the Bonferroni method. Results were expressed as median and range.

Differences in adherence of various organisms among catheter groups were analysed by means of the non-parametric Kruskal–Wallis test with multiple comparisons. Pair-wise differences were analysed by the non-parametric Mann–Whitney rank-sum test. A P value of <0.05 was considered to be statistically significant.

### Results

Comparison of S. aureus adherence to catheters in the agar and rat models

In both the agar and rat models, adherence of S. aureus to CS+ and MR catheters (0–10 cfu/cm) was significantly lower than in control catheters (1.9 × 10⁴–4.0 × 10⁴ cfu/cm) when infected on day 1, 7 or 14.
(P < 0.05). CS catheters had no adherence when challenged on day 1 (P > 0.05). However when challenged on day 7, CS catheters had an adherence of 1.6 × 10^3–5.2 × 10^3 cfu/cm (P = not significant). Silver catheters showed an adherence of 2.8 × 10^3–4.0 × 10^3 cfu/cm in the agar and rat models, which was not significantly different from that in control catheters.

No significant difference was seen in the adherence pattern in all the groups between the agar and rat models (Table 1).

**Comparison of S. epidermidis RIF-r2 adherence in the agar and rat models**

The mean colonization of *S. epidermidis* RIF-r2 on the CS, CS+ and MR catheters when infected immediately after implantation was 0–36 cfu/cm in the agar and rat models, whereas control catheters had 4.0 × 10^3–5.7 × 10^3 cfu/cm (P > 0.05). No significant difference was seen between the adherence to silver catheters (5.1 × 10^3–7.4 × 10^3 cfu/cm) and control catheters. The adherence of this organism on CS (1.4 × 10^3–1.5 × 10^4 cfu/cm) and MR (3.8 × 10^3–4.3 × 10^4 cfu/cm) catheters on the 7th day of infection was also not significantly different from the control group (5.5 × 10^3–6.0 × 10^3 cfu/cm), whereas CS+ catheters (0–8 cfu/cm) prevented adherence significantly (P < 0.05) (Table 2).

There was no significant difference in the adherence of *S. epidermidis* RIF-r2 to various catheter groups between the agar and rat models.

**Drug retention in catheters implanted in the agar and rat models**

The median drug levels for CS and CS+ catheters before implantation were 137 (131–150) µg/cm and 572 (531–618) µg/cm, respectively. On the 7th day post-implantation, the median drug levels in CS catheters were 32 (25–36) µg/cm and 24 (18–30) µg/cm in the agar and rat models, respectively. For CS+ catheters, these were 277 (255–308) µg/cm and 264 (234–283) µg/cm, respectively.

On the 14th day, drug levels in CS+ catheters implanted in agar and rat models were 248 (222–268) µg/cm and 232 (206–264) µg/cm, respectively (Figure 2).

There was no significant difference in drug levels of CS and CS+ catheters implanted in agar and rat models at 7 and 14 days post-implantation.

**Evaluation of the initial efficacy of catheters against various organisms in the agar model**

When tested for initial efficacy against *S. epidermidis* (ATCC strain) and rifampicin-resistant *S. epidermidis* RIF-r1; CS, CS+ and MR catheters had adherence of 0–4 cfu/cm, whereas control catheters had 1.7 × 10^3–8.0 × 10^3 cfu/cm (P < 0.05) (Table 3). In the case of *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *C. albicans*, the adherence to CS (0–3.6 × 10^4 cfu/cm) and CS+ (0–4 cfu/cm) catheters was significantly lower than control catheters (4.0 × 10^4–1.6 × 10^5 cfu/cm, P < 0.05) except for adherence of *P. aeruginosa* to CS catheters (7.5 × 10^4 cfu/cm), which showed no significant difference from the control group.

The adherence of these organisms to the MR (1.0 × 10^3–6.2 × 10^3 cfu/cm) and silver (3.8 × 10^2–2.0 × 10^4 cfu/cm) catheters was not significantly different from that of the control group (Table 3).
Microbial adherence to venous catheters

Evaluation of long-term efficacy of catheters against S. epidermidis and E. coli in the agar model

The long-term efficacy (infection on the 7th, 14th or 21st day of infection) of CS, CS+ and MR catheters against the adherence of S. epidermidis and E. coli is shown in Table 4.

The mean adherence of S. epidermidis to CS+ and MR catheters on the 7th, 14th and 21st day of infection was 0 cfu/cm, whereas control catheters showed adherence of 3.5 × 10^3–6.2 × 10^3 cfu/cm (P<0.05). The adherence of S. epidermidis on CS catheters when infected on the 7th or 14th day (9.0 × 10^2–2.8 × 10^2 cfu/cm) was not significantly different from that in the control group (Table 4).

CS+ catheters had adherence of 0–16 cfu/cm against E. coli on the 7th or 14th day of infection compared with control values of 2.8 × 10^3–6.4 × 10^3 cfu/cm (P<0.05). CS (2.4 × 10^3 cfu/cm) and MR (3.5 × 10^3 cfu/cm) catheters showed adherences that were not significantly different compared with control catheters.

Discussion

This study describes the development of an in vitro subcutaneous infection model using a special agar medium (agar model) for evaluation of antimicrobial catheters. The agar model simulates the animal model in the following way. When the catheters are infected, the bacteria migrate from the insertion site along the outer surface of the catheter to the distal end into the medium similar to the migration from skin to the subcutaneous tract in vivo. To simulate drug clearance in vivo, the catheters in the agar model were transferred to fresh agar medium 24 h before inoculation.

The drug retention and bacterial adherence to CS and CS+ catheters in the agar and rat models were found to be similar at all time periods (Tables 1 and 2 and Figure 2). During the standardization of the agar model, when CS+ catheters were infected with S. aureus immediately after transfer to fresh media on day 7, the adherence pattern did not match with that in rats. The adherence was significantly higher compared with that in which the catheters were transferred to fresh media 24 h before the 7th day of infection. In fact in the latter experiment CS+ catheters showed no adherence, which was similar to that found in the rat model. It appears that the drug released from the catheter in the agar and rat models between the 6th and the 7th day (25–30 µg/cm; data not shown) accumulates in the agar/tissue around the catheter and also plays a role in the prevention of catheter colonization.

In both the models, MR catheters were less effective than CS+ catheters against the RIF-r2 strain when challenged on the 7th day.

<table>
<thead>
<tr>
<th>Adherence [cfu/cm; median (range)]</th>
<th>agar model</th>
<th>rat model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.7 × 10^3 (3.4 × 10^3–8.0 × 10^3)</td>
<td>4.0 × 10^3 (2.2 × 10^3–7.5 × 10^3)</td>
</tr>
<tr>
<td>CS</td>
<td>0 (0–10)*</td>
<td>10 (0–16)*</td>
</tr>
<tr>
<td>CS+</td>
<td>0*</td>
<td>0 (0–12)*</td>
</tr>
<tr>
<td>MR</td>
<td>24 (12–60)*</td>
<td>36 (10–72)*</td>
</tr>
<tr>
<td>Silver</td>
<td>7.4 × 10^3 (4.2 × 10^3–9.9 × 10^3)*</td>
<td>5.1 × 10^3 (3.0 × 10^3–9.6 × 10^3)*</td>
</tr>
<tr>
<td>Silver</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
However, when infected immediately after implantation, MR catheters did prove effective. The ineffectiveness on day 7 may be due to the rapid rate of release of minocycline relative to rifampicin from the MR catheter. This disproportionate drug release has been shown to alter the ratio of minocycline to rifampicin in the catheter from 1:1 on day 1 to 1:6.5 on day 7 resulting in an insufficient amount of minocycline to be effective against \textit{S. epidermidis} RIF-r2 strain.18

In the agar model, MR and CS+ catheters were effective against \textit{S. aureus} and \textit{S. epidermidis} for more than 14 days, whereas CS catheters showed adherence after the 7th day. Similar results have been reported with CS catheters in clinical studies where these catheters had higher colonization after 7 days.11

Animal models have been described for evaluating adherence of \textit{S. aureus} or \textit{S. epidermidis} to catheters.1,2,4,12,16,17,30,31 However, no models are available for evaluating the adherence of other organisms. Since agar and rat models showed a similar pattern of drug retention and efficacy against \textit{S. aureus} and \textit{S. epidermidis} RIF-r2, the agar model was used to determine the efficacy of CS, CS+, MR and silver catheters against other organisms. Adherence of \textit{E. coli}, \textit{E. aerogenes}, \textit{K. pneumoniae}, \textit{P. aeruginosa} and \textit{C. albicans} to CS+ catheters was significantly lower compared with control, MR and silver catheters when they were inoculated immediately after implant. In fact MR and silver catheters were not effective against these organisms. Adherence of \textit{S. epidermidis} and \textit{E. coli} in the agar model

\begin{table}
\centering
\caption{Microbial adherence on CS, CS+, MR and silver catheters in the agar model (challenged immediately after implantation)}
\begin{tabular}{llllll}
\hline
Organism & control & CS & CS+ & MR & silver \\
\hline
\textit{S. epidermidis} & $1.7 \times 10^3$ & 0* & 0* & 0* & 2.6 $\times 10^3$ \textsuperscript{†} \\
& $(1.0 \times 10^2–3.8 \times 10^3)$ & $(0–4)$ & $(0–16)$ & $4^*$ & $(1.2 \times 10^2–5.0 \times 10^3)$ \textsuperscript{†} \\
\textit{S. epidermidis} RIF-r1 & $8.0 \times 10^3$ & 0* & 0* & 4* & $2.5 \times 10^3$ \textsuperscript{†} \\
& $(5.0 \times 10^2–1.2 \times 10^4)$ & $(–)$ & $(–)$ & $(0–4)$ & $(1.5 \times 10^2–5.1 \times 10^3)$ \textsuperscript{†} \\
\textit{E. aerogenes} & $4.0 \times 10^5$ & 0* & 0* & $1.8 \times 10^3$ \textsuperscript{†} & $4.0 \times 10^3$ \textsuperscript{†} \\
& $(2.1 \times 10^3–6.4 \times 10^3)$ & $(0–12)$ & $(–)$ & $(8.4 \times 10^2–5.2 \times 10^3)$ & $(1.6 \times 10^2–6.2 \times 10^3)$ \textsuperscript{†} \\
\textit{E. coli} & $2.6 \times 10^6$ & 0* & 0* & $2.8 \times 10^3$ \textsuperscript{†} & $7.2 \times 10^3$ \textsuperscript{†} \\
& $(1.2 \times 10^4–4.6 \times 10^4)$ & $(–)$ & $(–)$ & $(9.0 \times 10^2–4.0 \times 10^3)$ & $(4.0 \times 10^2–9.4 \times 10^3)$ \textsuperscript{†} \\
\textit{K. pneumoniae} & $1.6 \times 10^6$ & $3.6 \times 10^4$ & 0* & $6.2 \times 10^3$ \textsuperscript{†} & $2.0 \times 10^4$ \textsuperscript{†} \\
& $(9.3 \times 10^3–2.4 \times 10^4)$ & $(1.5 \times 10^6–5.3 \times 10^5)$ & $(0–8)$ & $(4.0 \times 10^2–1.2 \times 10^4)$ & $(1.0 \times 10^2–3.6 \times 10^4)$ \\
\textit{P. aeruginosa} & $3.8 \times 10^4$ & $7.5 \times 10^3$ & $4^*$ & $1.0 \times 10^3$ \textsuperscript{†} & $3.8 \times 10^3$ \textsuperscript{†} \\
& $(1.1 \times 10^3–4.9 \times 10^3)$ & $(3.9 \times 10^5–9.8 \times 10^4)$ & $(0–64)$ & $(5.2 \times 10^2–2.6 \times 10^3)$ & $(1.4 \times 10^2–6.0 \times 10^3)$ \\
\textit{C. albicans} & $4.0 \times 10^6$ & 0* & 0* & $2.0 \times 10^3$ \textsuperscript{†} & $4.4 \times 10^3$ \textsuperscript{†} \\
& $(2.4 \times 10^2–8.0 \times 10^3)$ & $(–)$ & $(–)$ & $(1.0 \times 10^3–4.0 \times 10^3)$ & $(3.2 \times 10^2–7.1 \times 10^3)$ \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Long-term efficacy of catheters against \textit{S. epidermidis} and \textit{E. coli} in the agar model}
\begin{tabular}{llll}
\hline
Organism & control & CS & CS+ & MR \\
\hline
\textit{S. epidermidis} & $3.5 \times 10^3$ & $9.0 \times 10^2$ \textsuperscript{†} & 0* & 0* \\
& $(2.0 \times 10^3–6.5 \times 10^3)$ & $(6.0 \times 10^2–1.3 \times 10^3)$ & $(0–16)$ & $(–)$ \\
& $3.8 \times 10^3$ & $2.8 \times 10^3$ \textsuperscript{†} & 0* & 0* \\
& $(2.2 \times 10^3–5.2 \times 10^3)$ & $(1.0 \times 10^3–4.6 \times 10^3)$ & $(0–20)$ & $(–)$ \\
& $6.2 \times 10^3$ & ND & 0* & 0* \\
& $(4.0 \times 10^3–9.2 \times 10^3)$ & $(–)$ & $(0–56)$ & $(–)$ \\
\textit{E. coli} & $2.8 \times 10^3$ & $2.4 \times 10^3$ \textsuperscript{†} & 0* & $3.5 \times 10^3$ \textsuperscript{†} \\
& $(1.1 \times 10^3–4.9 \times 10^3)$ & $(8.2 \times 10^2–4.8 \times 10^3)$ & $(–)$ & $(1.8 \times 10^3–5.8 \times 10^3)$ \\
& $6.4 \times 10^3$ & ND & 16* & ND \\
& $(3.8 \times 10^3–8.8 \times 10^3)$ & $(–)$ & $(0–58)$ & $(–)$ \\
\hline
\end{tabular}
\end{table}

Number of catheters tested in each group = 15. Comparison between test (CS, CS+, MR and silver) and control groups: *P < 0.05; †P = not significant. ND, not done.

Number of catheters tested in each group = 15. Comparison between test (CS, CS+, MR and silver) and control groups: *P < 0.05; †P = not significant. ND, not done.
In another in vitro study by Yorganci et al., the efficacy of MR catheters in preventing the adherence of K. pneumoniae was evaluated by exposing them to PBS cultures for 30 min. In this setting, these catheters were ineffective. However, when the exposed catheters were further transferred to a culture-free medium for 24 h and the adherence was determined, significantly lower colonization was detected on the catheter surface. In a similar study with S. aureus and S. epidermidis by these authors, CS+ catheters showed superior efficacy to MR catheters against these organisms. These results do not appear to match that found in the animal model described in our study. In our agar model, both CS+ and MR catheters were equally effective as that found in the animal model. Darouiche et al. in their clinical study showed a lower rate of colonization of K. pneumonia and P. aeruginosa in MR catheters compared with that in CS catheters and no significant difference in the case of yeast (3% and 2%, respectively).

In the agar model, both CS and MR catheters had adherence of K. pneumonia and P. aeruginosa 7 days post-infection. However, MR catheters showed higher adherence of these organisms. The reason for the difference in these results between the clinical studies and agar model is not clear. It is possible that in the clinical study, the infection originated a few days after implantation from the luminal surfaces of CS catheters which were not impregnated with the drug. Higher colonization of these organisms as well as C. albicans in MR catheters in the agar model may be due to the exposure of the outer surface of the catheter to higher and continuous bacterial challenge from the insertion site, a condition that can occur in vivo. It appears from our study that CS+ catheters, which are impregnated both on the luminal and outer surface, may show long-term and broad-spectrum activity in the clinical setting. The inefficacy of silver catheters in the agar and rat models may be attributed to either the binding of silver released from the catheter to the proteins and chlorides in the media/tissue or lack of release of an effective amount of silver from the catheter.

In conclusion, we have developed an agar model, which simulates the rat subcutaneous infection model for evaluating the initial and long-term efficacy of antimicrobial catheters in preventing colonization by various microorganisms. Evaluation of the efficacy of antibiotic- and silver-coated catheters using this method showed that the antiseptic catheters, especially CS+, were effective against all the organisms including rifampicin-resistant S. epidermidis, whereas the antibiotic catheters were effective only against S. aureus and S. epidermidis. Silver catheters were ineffective against all the organisms tested. The agar model may be valuable in predicting the in vivo efficacy of various antimicrobial catheters against different organisms. Although this study was conducted with antimicrobial polyurethane catheters, this model can be applied to antimicrobial catheters made from other substrates such as silicone. This in vitro method may prove a viable alternative to more cumbersome studies with animal models.

Acknowledgements

We thank Suhas Tambe for his assistance in our initial experiments.

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