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Efficacy of trypsin in enhancing assessment of bacterial colonisation of vascular catheters*

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SUMMARY

Since the number of organisms isolated from a medical device is crucial in assessing the likelihood of device-associated infection, we examined whether incubation of catheters in trypsin before sonication can increase the yield of superficially colonised vascular catheters *in vitro* and those removed from patients. Polyurethane and silicone catheters were individually colonised *in vitro* with individual clinical isolates including *Staphylococcus aureus* and *Escherichia coli*. Equal numbers of 1 cm segments of colonised catheters were then individually incubated either in a trypsin-containing solution or a control solution without trypsin. Each solution containing the segment was then sonicated and cultured quantitatively. In the clinical arm, indwelling catheters removed from patients were also cut into 1 cm segments that were equally suspended in the trypsin-containing or control solution and then sonicated and cultured quantitatively. Trypsin-based sonication enhanced the detection of *S. aureus* on colonised polyurethane and silicone catheters *in vitro* by 14- and 30-fold, respectively ($P = 0.03$ and $P = 0.04$), and the detection of *E. coli* on colonised polyurethane and silicone catheters by 3- and 6-fold, respectively ($P = 0.04$ and $P = 0.05$). Compared with sonication alone, trypsin followed by sonication resulted in 10% increase in the detectability of significant colonisation of indwelling catheters removed from patients and 11% increase in the mean colony counts of colonising organisms ($P = 0.04$). Exposure of catheters to trypsin before sonication improves the sensitivity of sonication and enhances the accuracy of assessing significant catheter colonisation.

Keywords

Bacteria; Biofilm; Catheter; Infection; Trypsin

Introduction

A majority of all nosocomial bloodstream infections (BSIs) are associated with vascular access lines.[1,2] More than 250 000 episodes of catheter-associated BSI occur each year in the USA

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leading to substantial morbidity and mortality.[1–3] A number of techniques have been developed to assess the colonisation of indwelling vascular catheters including flushing the catheter lumen with broth, centrifugation, vortexing, broth culturing, roll-plating, and sonication.[4–9] It is reported that the risk of catheter-related BSI and local catheter-related infection is directly associated with the number of bacterial colonies isolated from the catheter.[9,10] Significant colonisation that can be associated with clinical infection is defined as growth of ≥15 colony-forming units (cfu) by using the semiquantitative method of roll-plating or ≥1000 cfu by using the quantitative sonication method.[3,6,8,11]

In the predominantly used method of semiquantitative roll-plating, the surface of the explanted device is rolled over a nutrient agar. Although this method may partially retrieve organisms that adhere to the external surface of a catheter, it fails to detect organisms bound to the internal catheter surface. Additionally, the shape, polymeric nature, flexibility, and size of the catheter may further hinder use of the roll-plate technique. Although the sonication method more accurately reflects the number of organisms colonising a catheter, sonication alone is also reportedly inadequate for removing bacteria from the colonising surfaces since it may only retrieve organisms that can easily detach from the catheter surface into the culturing solution, leaving many organisms unaccounted for.[9,12]

Since pathogenesis of device-related infection is related to the universal formation of a matrix called biofilm that nests the pathogens shortly after implantation of the device, many colonising organisms become tightly anchored within this biofilm matrix, which externally and internally surrounds the colonised catheter.[13] Traditional physical methods used to assess catheter colonisation, including sonication alone, are usually unable to effectively disrupt this biofilm and so to release all the organisms within this network. A combination of physical and chemical means that can disrupt this biofilm that traps the organisms may yield more accurate and earlier diagnosis of significant catheter colonisation and catheter-related infection.[12] In this study, we compared the efficacy of trypsin, a digestive protein that has been used for detachment of anchored cells, followed by sonication for enumerating organisms isolated from catheters exposed to bacteria in vitro as well as those removed from patients.

Methods

In vitro study

To colonise devices, 7 French polyurethane catheters (Cook Inc., Bloomington, IN, USA) and 14 French silicone catheters (Rüsch Inc., Duluth, GA, USA) were individually immersed in 10^3 cfu/mL bacterial suspensions in trypticase soy broth (TSB) of clinical isolates of *Staphylococcus aureus* or *Escherichia coli* that had previously caused clinical infection, and incubated at 37°C on a tube rocker for 8 or 6 h, respectively. After removal from the bacterial suspension, each catheter was serially dipped in three tubes containing 50 mL of normal saline and purged with 5 mL of normal saline in each lumen to remove planktonic micro-organisms from the catheter surfaces. Catheters were then cut into 1 cm segments and individually incubated in 1 mL of Hanks' balanced salt solution (HBSS) with Phenol Red or 1 mL of 10× porcine trypsin with Phenol Red at 37°C for 1 h. Each solution containing the catheter segment was then sonicated in a water bath sonicator with 100 W output for 5 min and then vortexed for 30 s. One-hundred-microlitre aliquots of each sonicated suspension and subsequent serial dilutions were then cultured quantitatively on trypticase soy agar (TSA) with sheep blood and colony counts were determined after 48 h. We repeated the experiment three times with 4 cm segments of catheter for a total of 12 observations per culture group for each micro-organism.

Clinical study

The clinical arm of this study that examined the efficacy of trypsin treatment of catheters followed by sonication in assessing colonisation of vascular catheters was approved by the Institutional Review Board (IRB) at Baylor College of Medicine and by the Research and Development (R&D) Committee at the Michael E. DeBakey Veterans Affairs Medical Center (MEDVAMC) in Houston, Texas, USA prior to the initiation of the study. We collected vascular catheters that were ordered for removal by the patients' primary care physicians due to one or more of the following reasons: (i) completion of intravenous therapy; (ii) positive blood culture; (iii) suspected catheter-related infection; or (iv) mechanical malfunction or obstruction of the catheter. Each catheter was retrieved and transported to the laboratory in a sterile fashion. In the laboratory, an 8 cm distal section of the removed catheter was cut into eight 1 cm segments starting from the tip. If the tip of the catheter was sent by the caring physician to the hospital's microbiology laboratory for culture using the roll-plate technique, we used a more proximal 8 cm section of the catheter for our experiment. Catheter segments were divided into either the trypsin or control group that alternated from the tip toward the hub of the catheter. Four alternative 1 cm segments were individually placed in four 1 mL HBSS with Phenol Red. The other four 1 cm segments were individually placed in four 1 mL 10× porcine trypsin solutions with Phenol Red. The solutions were then incubated at 37°C for 1 h. All solutions were subsequently sonicated for 5 min and vortexed for 30 s. The solutions from each group (trypsin and control) were pooled and vortexed for about 10 s. One-hundred-microlitre aliquots of each suspension and subsequent serial dilutions were then quantitatively cultured on to TSA plates with sheep blood. Colony counts were recorded after 48 h.

Statistical analysis

We used Stata software version 8.2 (Stata Corp., College Station, TX, USA) to conduct the statistical analyses. Student's *t*-test was used to compare the mean colony counts between the tested groups (trypsin treatment/sonication vs sonication alone of catheter segments). $P \leq 0.05$ was accepted as significantly different.

Results

In vitro study

Compared with sonication alone (control), the use of trypsin followed by sonication yielded 14- and 30-fold higher colony counts of *S. aureus* from polyurethane and silicone catheters, respectively [$1.3 \times 10^4 \pm 3.3 \times 10^3$ (SE) vs $9.4 \times 10^2 \pm 3.0 \times 10^2$ cfu/cm, $P = 0.03$; and $1.6 \times 10^5 \pm 4.7 \times 10^4$ vs $5.5 \times 10^3 \pm 1.3 \times 10^3$ cfu/cm, $P = 0.04$]. In addition, trypsin followed by sonication enhanced the number of colony counts of *E. coli* retrieved from polyurethane and silicone catheters by 3- and 6-fold, respectively, compared with sonication alone ($8.1 \times 10^5 \pm 2.2 \times 10^5$ vs $2.9 \times 10^5 \pm 6.5 \times 10^4$ cfu/cm, $P = 0.04$; an $5.5 \times 10^3 \pm 1.4 \times 10^3$ cfu/cm vs $8.8 \times 10^2 \pm 2.2 \times 10^2$ cfu/cm, $P = 0.05$). Figures 1 and 2 show the mean colony counts retrieved from polyurethane and silicone catheters, respectively, according to the culture method.

Clinical study

Since the objective of the study was to compare the two culture techniques, we only included 40 catheters that yielded positive cultures by either method or both in this study. All 40 catheters yielded bacterial growth by using the trypsin/sonication technique, but only 34 (85%) catheters yielded positive culture results using the sonication method. Sixty-three percent (25/40) of catheters demonstrated significant colonisation (defined as growth of ≥1000 cfu) when using trypsin followed by sonication method, but only 53% (21/40) of catheters exhibited significant colonisation using the sonication method alone.[3,11] In addition, the mean colony count from cultured catheters was significantly higher ($5.2 \times 10^5 \pm 2.8 \times 10^5$ cfu) using the trypsin/

sonication method vs sonication method alone [$8.9 \times 10^4 \pm 4.8 \times 10^4$ cfu ($P = 0.04$, one-tailed Student's *t*-test)] (Figure 3).

Discussion

Catheter-associated BSI, the most common serious complication of intravascular devices, can be fatal and expensive to manage. However, there is no consensus on a standard and accurate method to diagnose the infections associated with vascular devices.[14] Since determination of the number of cfu of organisms colonising the catheter is crucial in establishing possible device-associated infection, underestimation of the number of bacterial colonies on the catheter may underdiagnose clinical infection.[9,10] Accordingly, undiagnosed clinical infections could be mismanaged, thereby leading to higher morbidity and mortality as well as increased overall cost of patient care.

Currently, the two widely used diagnostic techniques to assess catheter colonisation include roll-plating of the catheter segment on nutrient agar and sonication of the catheter segment followed by plating of the sonication fluid. Although the roll-plate method is the more prevalent technique, the shape of the catheter, its stiffness, and/or its inflexibility may prevent the catheter segment from completely coming in contact with the surface of the agar during roll-plating, thereby reducing the number of detectable bacterial colonies on the device surface.[15] For the same reasons, catheters with protuberances and/or surface indentations are not suitable for roll-plating.[16] Since prolonged placement of a catheter is usually associated with increased catheter hub manipulation and, therefore, higher likelihood of colonisation of the internal surface, the roll-plate method can be even less sensitive for culturing long term catheters. Although the sonication method may potentially be more sensitive than the roll-plate method, it may still underestimate the number of bacterial colonies that could be removed from the colonised catheter surface. Since the biofilm may shield the pathogens from conventional physical detachment methods, sonication alone might fail to detect some of the strongly attached biofilm-embedded organisms.[12]

A combination of physical (sonication) and chemical (trypsin) techniques could be more suitable in detaching pathogens from the biofilm matrix into the surrounding media and can enhance the culture yield of colonising organisms. Cell surface proteins may play an important role in bacterial attachment to medical devices and may contribute to the formation of biofilm.[17] Their concentration has been reported to be directly correlated with the adhesion density.18 Therefore, removal of cell surface proteins may contribute to the detachment of bacterial cells anchored to a device.17 Trypsin, a pancreas-derived enzyme, is commonly used in cell culturing for the dissociation of anchorage-dependent eukaryotic cells by breaking down the glycoprotein medium joining the cells to the surface of the culturing container.19 In addition, trypsin has been used for dissociation of thermophilic bacilli and thermophilic streptococci from stainless steel.[17,20] As a result, trypsin as a proteolytic enzymatic agent followed by sonication technique may be used to detach sessile micro-organisms anchored within the biofilm network. The results from the clinical study show that the use of trypsin followed by sonication increases the likelihood of detecting clinically relevant catheter colonisation by 10% and significantly increases the average colony counts from cultured *ex in-vivo* catheters as compared to sonication alone. Likewise, the *in vitro* study demonstrated that exposing colonised catheters to trypsin followed by sonication magnifies the number of detected organisms up to 30-fold compared with sonication alone.

This study indicates that trypsin followed by sonication is a more effective means than sonication alone to dissociate more organisms from the biofilm matrix of colonised catheters, thereby improving the assessment of catheter colonisation and possible catheter-associated BSI. Although this technique requires an additional processing step compared with sonication

alone, it may become a valuable tool in clinical microbiology laboratories where the inaccuracy of colonisation assessment may underdiagnose clinical infection. However, larger clinical studies are warranted to further assess the clinical implications of this novel trypsin–sonication culture method.

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Conflict of interest statement

None declared.

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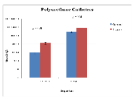

Figure 1.
Mean colony counts retrieved from polyurethane catheters infected in vitro. Dark grey bars: control (sonication alone); light grey bars: sonication plus trypsin.

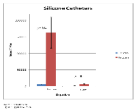

Figure 2.
Mean colony counts retrieved from silicone catheters infected *in vitro*. Dark grey bars: control (sonication alone); light grey bars: trypsin plus sonication.

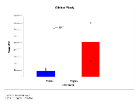

Figure 3.
Mean colony counts from removed catheters during clinical study. Dark grey bars: control (sonication alone); light grey bars: trypsin plus sonication.