Adhesion of *Staphylococcus epidermidis* and *Staphylococcus aureus* to intravascular cannulae

Ghadah Abusalim[1], Sulaiman Ali Alharbi[2], Hesham Khalil[3], Milton Wainwright[2,4] and Mohammad A. Khiyami [5]

[1]Department of laboratory Medicine, College of Applied Medical Sciences, Salman bin Abdluaziz University
[2]Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh-114 51, Saudi Arabia
[3]Department of Oral and Maxillofacial Surgery, College of Dentistry, King Saud University, Saudi Arabia.
[2,4]Department of Molecular Biology and Biotechnology, University of Sheffield, UK
[5] King Abdulaziz City for Science and Technology, P.O. Box 6086, Riyadh 11442, Saudi Arabia
E-mail: sharbi@ksu.edu.sa

Abstract: The use of implantable foreign devices in medicine has recently increased dramatically. Intravascular cannulae and catheters are used to administer fluids, medications, parenteral nutrition, and blood products in order to monitor hemodynamic status and also to provide hemodialysis. The early and late failure of inserted or implanted devices is largely the result of bacterial infection and may lead to the disruption of integration between the device and the tissues which surround it. *Staphylococcus aureus* and *Staphylococcus epidermidis* are widely considered to be the most common organisms causing device- related infection. Our study showed that *S. aureus* and *S. epidermidis* adhered to intravascular cannulae made up of PTFE, SPTFE and vialon. Adhesion of *S. epidermidis* and *S. aureus* to intravascular cannulae varied significantly depending upon the type of material used and the presence of coating materials. Both bacteria adhered less to PTFE followed by Vialon and SPTFE and the adhesion capacity of *S. aureus* and *S. epidermidis* increased over time. Coating intravascular cannulae with human serum albumin inhibited the adhesion of *S. aureus* and *S. epidermidis* to these cannulae, and pretreatment of cannulae with fibronectin inhibited the adhesion of *S. epidermidis* but increased the adhesion of *S. aureus* to all types of cannulae. Pretreatment of cannulae surface with potassium chloride or calcium chloride increased the adhesion of *S. aureus* and *S. epidermidis* to cannulae, suggesting a role for electrostatic forces in the mechanism of such adhesion. This study will hopefully clarify the mechanism of adhesion and provide possible means of preventing such adhesion either by the use of better material coatings or by interfering with the process of adhesion by targeting bacterial structures responsible for it. Currently we recommend the use of PTFE cannulae as they exhibit a lower bacterial adhesion capacity compared to the other tested cannulae.

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1. Introduction

The application of implantable foreign devices in medicine has recently increased dramatically, and devices such as grafts, artificial joints, and dental implants have been approved for use based upon their host tissue biocompatibility and functional characteristics. Implanted device-related infections largely follow from bacterial adhesion to biomaterials, and it has been found, both *in vitro* as well as *in vivo* studies, that susceptibility to bacterial infection is significantly increased in the presence of foreign body devices; the reasons why such infections occur are however, still not fully understood (Vacheethasanee et al., 1998). Intravascular cannulae and catheters are used to administer fluids, medications, parenteral nutrition, and blood products so as to monitor hemodynamic status and to provide hemodialysis. The presences of commensal microorganisms in the vicinity of these devices subject them to colonization by such bacteria. Such microorganisms originate either from a) the patient's skin, b) at the cannula or catheter insertion site, c) from a contaminated devices or d) from the hematogenous seeding of these bacteria. After colonization, bacteria tend to form biofilms which leads to complications in the management of device-related infections (Rupp and Fey, 2001). This study involved investigated of the adhesion of common skin commensals, namely *Staphylococcus epidermidis* and *Staphylococcus aureus,* to the surface of the most commonly used intravascular cannulae and catheters. The affect of factors such as time, plasma proteins coating and electrostatic forces, all of which influence adhesion, were also investigated. Bacterial adhesion to a device may necessitate its removal, a very costly intervention which may increases the risk of morbidity and mortality. As a result, it is important to understand the nature of the bacterial surface structures which mediates bacterial attachment, since this will influence the eventual choice of materials to be used.

2. Material and Methods

Three different types of commonly used intravascular cannulae were used in this study. Polytetrafluoroethylene (PTFE), siliconized polytetra-fluoroethylene (SPTFE) and Vialon cannulae. PTFE is a fluorocarbon solid, high-molecular-weight compound consisting of carbon and fluorine and is hydrophobic. SPTFE offers high resistance to kinking and has good compatibility. Vialon is a unique biomaterial developed over a decade ago especially for intravascular access; it causes less pressure on the vessel walls and as a result, less vessel penetration. The cannulae were cut into 2 centimeters length, heated at both ends (in order to close the caunulae) and then sterilized *Staphylococcus epidermidis* and *S. aureus* strains were obtained from the Alhammadi hospital, Riyadh, Saudi Arabia.

The strains used were isolated from a bacteremia caused by catheter related infections. *S. epidermidis* and *S. aureus* were grown in BHI medium at 37º C for 15-18 hours and the number of bacteria per milliliter was calculated using a dilution curve equation for each organism. Cultures were centrifuged at 5000 rpm for 15 minutes. The supernatant was then discarded and the cells were resuspended into BPS to obtain 10^8 bacteria/ml.*Stapylococcus epidermidis* and *S. aureus* were grown in BHI medium at 37º C for 15-18 hours and the number of bacteria per milliliter was calculated using a dilution curve equation for each organism. Cultures were centrifuged at 5000 rpm for 15 minutes. The supernatant was then discarded and the cells were resuspended into BPS to obtain 10^8 bacteria/ml. Samples of sterile intravascular cannula (2cms in length) were incubated with either *S. epidermidis* or *S. aureus* sterile phosphate buffered saline (PBS,4ml) (Sigma, UK). The number of bacteria added was adjusted according to the bacterial dilution curve done using the spectrophotometer at wave length 650 nm. A total of 1 x 10^8 cell/ml was used in this experiment. Incubation was at 37°C for 3, 8, 12 and 24 hours.

After incubation, non-adhered bacteria were removed by washing on three occasions with PBS. As a control, the liquid from all washing steps was examined for bacterial colonies by plating on nutrient agar. To remove the adherent bacteria, the samples were resuspended into 4 milliliters of sterile PBS containing Tween 20 and vortexed for three minutes. The number of bacteria adhered to the cannula was determined by serial dilution and plating on nutrient agar. After removal of adhered bacteria the cannulae were gently rolled onto the surface of nutrient agar plates to enumerate any remaining adhered bacteria. The experiments were repeated in triplicate. The viability of *S. epidermidis* and *S. aureus* in the presence of different cannulae was also tested.

Two centimeter pieces of intravascular cannula were incubated(for 2h) with 1 mg/ml human serum albumin (HSA) or 0.1 mg/ ml fibronectin (Fn) (Sigma, UK) in 4 ml tubes. The proteins were obtained from the laboratories of King Khalid University Hospital, King Saud University, Saudi Arabia. The cannula were washed three times with PBS then resuspended into 4 ml of sterile PBS and 10^8 bacteria were added. The tubes were incubated at 37ºC. Cannulae were washed 3 times with PBS to remove unattached bacteria.

After incubation, non-adhered bacteria were removed by washing with PBS three times. As a control, the liquid from all washing steps was examined for bacterial colonies by plating onto the surface of nutrient agar plates. To remove the adherent bacteria, the samples were resuspended into sterile PBS (4mls) containing Tween 20 and vortexed for three minutes. The number of bacteria adhering to each cannula was determined by serial dilution and plating on nutrient agar. After removal of adhered bacteria, the cannulae were gently rolled on nutrient agar plates to enumerate any remaining adhered bacteria. The experiments were repeated in triplicate.

On order to examine the role of electrostatic forces in the mechanism of adhesion of *S. epidermidis* and *S. aureus*, the intravascular cannulae were suspended for 2 hours at room temperature in 1 mM calcium chloride ($CaCl_2$). The cannulae were then washed three times with phosphate buffer saline then the adhesion assay was carried out as described above.

3. Results

Incubation numbers *S. aureus* were significantly higher in the presence of polytetrafluoroethylene (PTFE), siliconized polytetra-fluoroethylene (SPTFE) and Vialon than in the controls were no cannulae were present (Figure1).

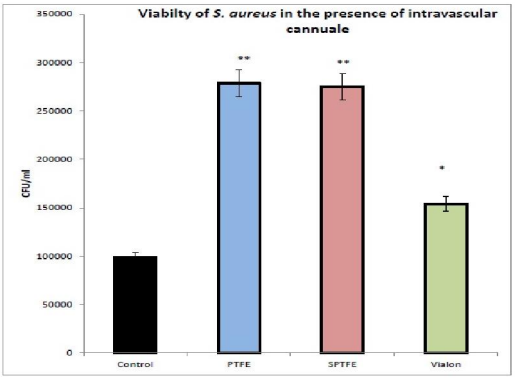

Figure 1: Growth of. *S. aureus* in the presence of intravascular cannuale (24 h incubation period). Polytetrafluoroethylene (PTFE), siliconized polytetra-fluoroethylene (SPTFE). Control represents the growth in the absence of cannulae. * P value < 0.05; ** P value < 0.01.

The numbers were highly significant in the case of SPTFE and PTFE. However, in the case of *S. epidermidis* the numbers of bacteria were significantly reduced (P value < 0.05) in the presence of the Vialon cannula after incubation for 24 hours. The growth of *S. epidermidis* in the presence of SPTFE and PTFE was not significantly affected. Adhesion of *S. aureus* and *S. epidermidis* to different intravascular cannulae was examined over an incubation period of 3 hours, (Figure 2.).

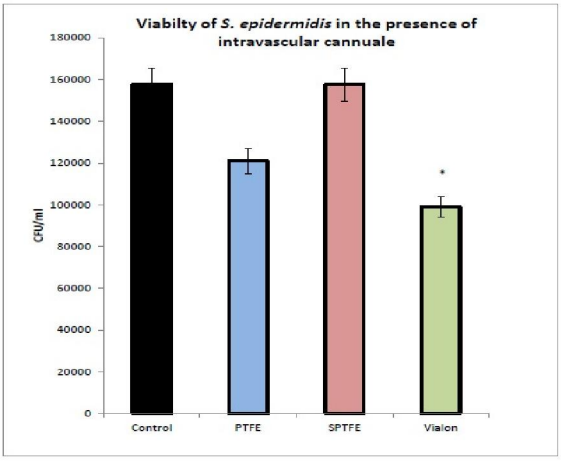

Figure 2: Growth of. *S. epidermidis* in the presence of intravascular cannuale (24 h. incubation period). Polytetrafluoroethylene (PTFE), siliconized polytetra-fluoroethylene (SPTFE). Control represents the growth in the absence of cannulae; * P value < 0.05.

Both *S. aureus* and *S. epidermidis* adhered to all the three intravascular cannulae, i.e. polytetrafluoroethylene (PTFE), siliconized polytetra-fluoroethylene (SPTFE) and Vialon. The capacity of these bacteria to adhere to the three types of cannulae varied. Adhesion of *S. aureus* and *S. epidermidis* were significantly higher (P value < 0.05) in case of SPTFE followed by Vialon and PTFE. Although Figure 3 shows higher capacity *of S. aureus* to adhere to intravascular cannulae than *S. epidermidis*, the adhesion is insignificant. There was a significant difference in adhesion to PTFE compared to SPTFE and Vialon, while no significant difference in adhesion was seen between SPTFE and Vialon.

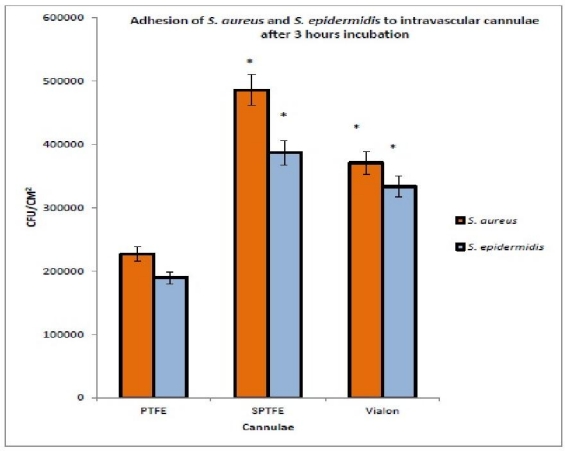

Figure 3. Adhesion of *S. epidermidis and S. aureus* to intravascular cannuale (3 h. incubation period). Polytetrafluoroethylene (PTFE), siliconized polytetra-fluoroethylene (SPTFE) and Vialon; * P value < 0.05.

Adhesion of *S. aureus* and *S. epidermidis* was examined after 8, 12 and 24 hours incubation with different intravascular cannulae. Both *S. epidermidis* and *S. aureus* showed increase in adhesion capacity with time. When compared to the three hours incubation, adhesion of *S. aureus* and *S. epidermidis* to PTFE cannula significantly increased by 65 and 80 % respectively after 8 hours incubation with cannulae (Figure 4).

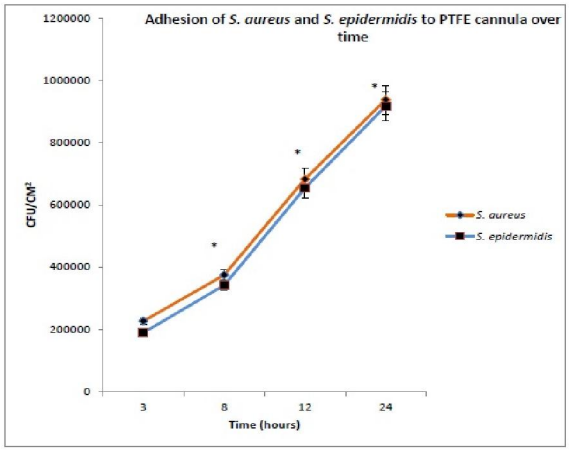

Figure 4. Adhesion of *S. aureus* and *S. epidermidis* to intravascular Polytetra-fluoroethylene (PTFE) cannula at 3, 8, 12 and 24 h; * indicates P value < 0.05.

Adhesion of both bacteria to SPTFE and vialon was also increased. *S. aureus* adhesion to SPTFE increased by 30 % and to Vialon by 33%, (Figure 5), while *S. epidermidis* adhesion to SPTFE and Vialon increased by 34%, (Figure 6).

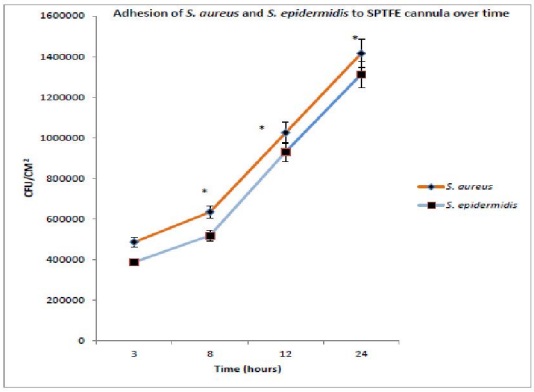

Figure 5: Adhesion of *S. aureus* and *S. epidermidis* to intravascular Siliconized polytetra-fluoroethylene (SPTFE) cannula at 3, 8, 12 and 24 h.;* indicates P value < 0.05.

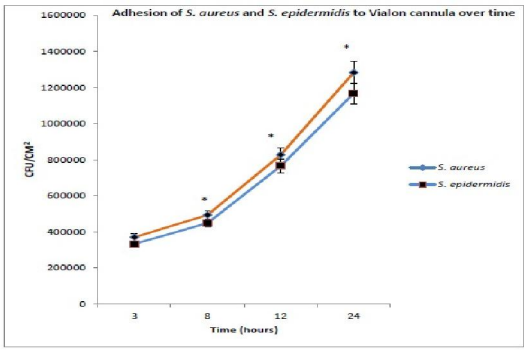

Figure 6: Adhesion of *S. aureus* and *S. epidermidis* to the intravascular Vialon cannula at 3, 8, 12 and 24 h; * indicates P value < 0.05.

Coating intravascular cannulae surfaces with human serum albumin resulted in a significant decrease in the adhesion of *S. aureus* to SPTFE cannula. The reduction percentage was 37% when compared to the control (Figure 7).

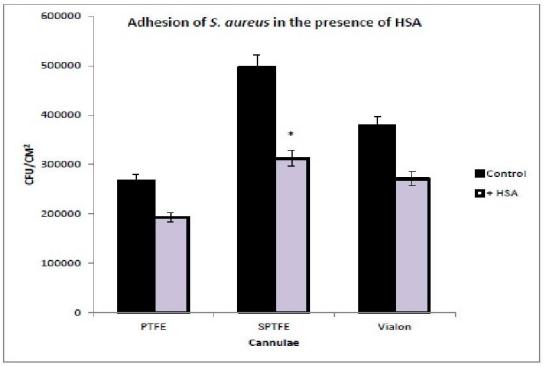

Figure 7: Adhesion of *S. aureus* to human serum albumin (HSA) coated intravascular cannuale at 3 hours incubation period. Polytetrafluoroethylene (PTFE), siliconized polytetra-fluoroethylene (SPTFE). The control represents bacterial adhesion in the absence of HAS; * indicates P value <0.05.

This decrease was found to be statistically different according to the T test (P < 0.001). Although there was slight reduction in adhesion of *S. aureus* to PTFE and Vilaon cannulae, the reduction in adhesion of *S. aureus* to both albumin coated PTFE and vialon was not statistically significant. In contrast, coating cannulae with fibronectin (Fn) significantly enhanced the adhesion of *S. aureus* to all of the three types of cannulae. Adhesion of *S. aureus* to PTFE, SPTFE and Vialon was increased by 56, 41 and 26% respectively, (Figure 9).

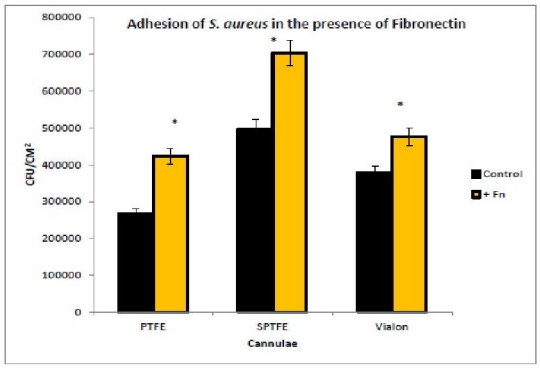

Figure 9: Adhesion of *S. aureus* to fibronectin (Fn) coated intravascular cannuale at 3 hours incubation period. Polytetrafluoroethylene (PTFE), siliconized polytetrafluoroethylene (SPTFE). The control represents bacterial adhesion in the absence of Fn; * indicates P value < 0.05.

In contrast both HSA and Fn decreased the adhesion of *S. epidermidis* to all cannulae. Coating cannulae with HSA decreased the adhesion of *S. epidermidis* to PTFE, SPTFE and vialon by 41, 30 and 26% respectively, while fibronectin reduced *S. epidermidis* adhesion to PTFE, SPTFE and Vialon by 43, 36 and 32 % respectively (Figures 8 and 10).

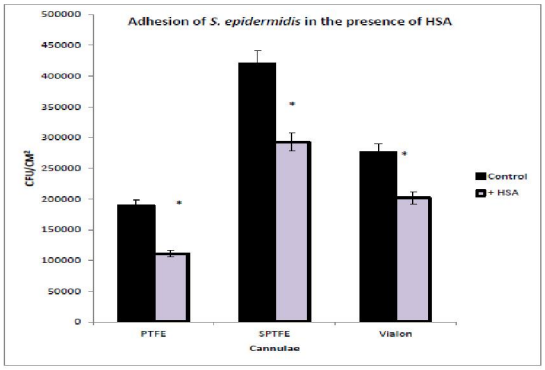

Figure 8: Adhesion of *S. epidermidis* to human serum albumin (HSA) coated intravascular cannuale (3 h. incubation period). Polytetrafluoroethylene (PTFE), siliconized polytetra-fluoroethylene (SPTFE). The control represents bacterial adhesion in the absence of HAS; * indicates P value <0.05.

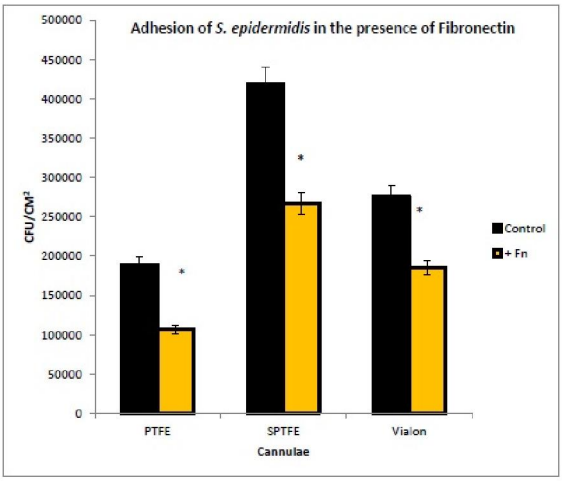

Figure 10: Adhesion of *S. epidermidis* to fibronectin (Fn) coated intravascular cannuale at 3 hours incubation period. Polytetrafluoroethylene (PTFE), siliconized polytetrafluoroethylene (SPTFE). The control represents bacterial adhesion in the absence of Fn; * indicates P value < 0.05.

Pretreatment of cannulae with calcium chloride ($CaCl_2$) was carried out to examine the role of electrostatic forces in the mechanism of *S. aureus* and *S. epidermidis* adhesion. Figures 11 and 12 show that pretreatment of PTFE, SPTFE and Vialon with $CaCl_2$, both of which are positively charged, caused a significant increase in *S. aureus* adhesion to these cannulae. Adhesion of *S. aureus* to PTFE, SPTFE and Vialon increased by 65, 37 and 32% respectively; increases which were statistically different (P < 0.05).

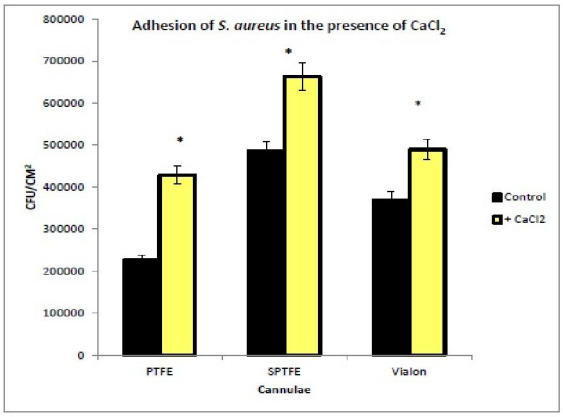

Figure 11: Adhesion of *S. aureus* to calcium chloride (CaCl2) coated intravascular cannuale at 3 hours incubation period. Polytetrafluoroethylene (PTFE) siliconized Polytetrafluoroethylene (SPTFE). The control represents bacterial adhesion in the absence of CaCl2; * indicates P value < 0.05.

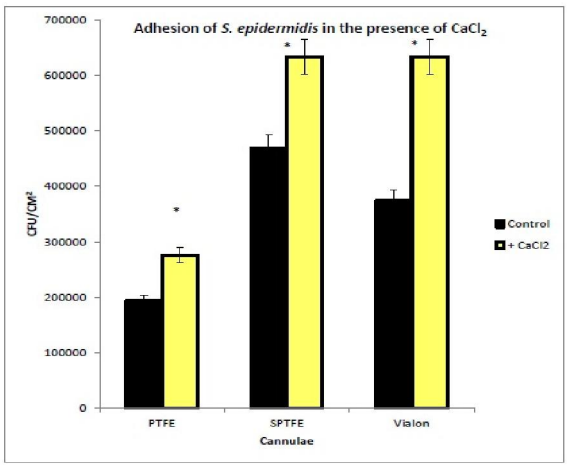

Figure 12: Adhesion of *S. epidermidis* to calcium chloride (CaCl2) coated intravascular cannuale at 3 hours incubation period. Polytetrafluoroethylene (PTFE) siliconized Polytetrafluoroethylene (SPTFE). The control represents bacterial adhesion in the absence of CaCl2; * indicates P value < 0.05.

4. Discussions

Over the past years, a considerable amount of research effort has been devoted to studies of the natural process of bacterial adhesion to biomaterial surfaces, but many questions remain to be answered. Intravenous catheters are today an essential component of hospital-based medical practice, since they provide the necessary vascular access for fluids, drugs, blood products and total parenteral nutrition. Their use is not without risk however, and problems relating to their use include bacteremia, infections and septic thrombophlebitis.

A number of types of cannulae or catheters exsit,including peripheral intravascular catheters, central venous catheters (CVC), peripherally inserted central venous catheters (PICC lines, which are inserted into the veins of the upper arms under image intensifier control, or into the antecubital fossa), midline catheters (these are inserted at the same site as a PICC line, but are much shorter and as a result, do not enter the superior vena cava), tunnelled CVC, such as Hickman catheters and finally, ports which are completely implanted under the skin and accessed with a needle (the latter two types are primarily used in patients undergoing chemotherapy). *Staphylococcus aureus* is the commonest and most important pathogen which cause intravenous catheter-related bacteremia accounting for 62% episodes of peripheral intravenous catheter-associated bacteremia, and 27% of CVC-

associated bacteremia reported in tertiary referral hospitals in Australia (Collignon, 1994).

Infections associated with medical devices are presently the most important pathogenic consequence of bacterial infection. Such infections are recognized as a two-stage process, i.e. the initial bacterial attachment, followed by cell division within an extracellular matrix, both leading to the formation of biofilms. The process of bacterial attachment to cells and inanimate surfaces has been the subject of considerable investigation (Tenney et al., 1986), especially since bacterial infection is considered one of the main reasons for early and late failure of inserted, or implanted devices. Such failures are largely the result of the disruption of integration between the device and the surrounding tissues. Furthermore, as bacterial biofilms can complicate such infections (Katsikogianni and Missirlis, 2004).

The aim of our study was to determine the ability of two common skin commensal, *S. aureus* and *S. epidermidis* to adhere to the most frequently used intravascular cannulae. *Staphylococcus aureus* is a nonpathogenic skin bacteria with a cell wall composed of polypeptide and polysaccharide chains as well as techoic acid. *Staphylococcus aureus*, *S. epidermidis* and *Candida albicans* are regarded as the most common organisms in relation to device-related infection. *Staphyococcus epidermidis* produces slime that promotes its ability to survive on foreign bodies, while *S. aureus* produces a significant number of virulence factors which include proteins that interact with the coagulation system and with the extra-cellular matrix. All of these factors make this bacterium, uniquely suited to aid its survival within blood vessels. Foreign bodies, such as catheters are associated with neutrophil dysfunction, including less effective phagocytosis and less effective killing in the area adjacent to the catheter (Indorf et al., 1999). It is recommended that replacement of peripheral venous catheters takes place at least 72 h. after insertion, since subsequent to this point the incidence of thrombophlebitis and bacterial colonization increases exponentially (Maki and Ringer, 1991). We found that *S. aureus* and *S. epidermidis* can adhere well to polytetrafluoroethylene, siliconized polytetra fluoroethylene and Vialon. Generally *S. aureus* has a greater capacity to adhere to all types of the tested cannulae. When compared to SPTFE and Vialon, the PTFE cannula showed the least adhesion properties, while the most pronounced highest adhesion capacity of these bacteria was exhibited by SPTFE. Sousa *et al* have shown that *S. epidermidis* can adhere more actively to silicone than acrylic, a result which support our findings (Sousa et al., 2009). In our study, the adhesion of both *S. aureus* and *S. epidermidis* was seen to be enhanced over the period of 8, 12 and 24 hours. The *in vitro* survival of Staphylococci undergoing adherence to intravascular catheters in the absence of conventional nutrients has been described in the literature, and it has been suggested that under these circumstances microorganisms can utilize some of the components of the catheters as a source of nutrients for growth (Peters et al., 1982). It may also be possible that adherence to the surface of foreign objects may allow organisms to enter into a state of metabolic dormancy (Franson et al., 1986). In order check this hypothesis we tested the effect of cannulae components on bacterial growth by incubating *S. aureus* ad *S. epidermidis* for 24 hours in the presence of cannulae. *Staphylococcus aureus* showed increased viability in the presence of PTFE, SPTFE and the Vialon cannulae, while a significant inhibition of *S. epidermidis* growth occurred in the presence of Vialon; findings which may explain the higher capacity of *S. aureus* to adhere to all of the tested cannulae.

Some *in vitro* studies have shown that the adsorbtion of plasma to synthetic materials usually reduces the ability of bacteria to adhere to these materials (Linnes et al., 2012). *In vivo* however, materials frequently adsorb various tissue proteins and organized thrombus formation occurs. The interaction of a microorganism with an organized surface of this type may differ when it is presumably presented with a complex rather than simple surface.(CHECK) Pascual and his group found that pre-incubation of Teflon catheters in human serum caused a reduction of between 80 to 90% in the adhesion of *S. epidermidis* (Pascual et al., 1986). In the present study, human serum albumin was shown to cause a significant decrease in the adherence of the *S. aureus* and *S. epidermidis* to different intravascular cannulae surfaces. The mechanisms which might account for the increase of *S. aureus* and *S. epidermidis* binding in the presence of HSA are not immediately obvious, but may differ according to bacterial strains and species, notably in relation to variations in bacterial cell surface properties.

Fibronectin, which is known for its ability to mediate the surface adhesion of eukaryotic cells, has also been shown to bind bacteria, including *S. aureus*, a fibronectin clearly promotes *S. aureus* adhesion to substratum surfaces. It has been shown that *S. aureus* adhesion to coverslips can be enhanced 20 times by the addition of fibrnectin (Vaudaux et al., 1984) . Fibronectin has been shown to markedly promoted adherence of all *S. aureus* strains, but in only four out of 19 strains of *S.epidermidis.*

Although fibronectin can inhibit *S. epidermidis* adherence to cobalt chrome alloy, and by some 90% to the surface of poly- methylmethacrylate (PMMA) surface (Herrmann et al., 1988). In our study we found

that coating cannulae with fibronectin enhanced the adhesion of *S. aureus* to all types of cannulae but, in contrast, adhesion of *S. epidermidis* was inhibited in the presence of fibronectin. Other studies have also shown that the adhesion of *S. epidermidis* to biomaterials can be inhibited in the presence of fibronectin (Linnes et al., 2012).The adhesion of bacteria to inert substratum surfaces as well as to other microbial cell surfaces is often described in terms of specific interactions between localized, specific molecular groups and also in terms of specific forces including a separate class of fundamental interaction forces (van Oss, 1995). Electrostatic forces may play a role in the process of adhesion of *S. aureus* and *S. epidermidis* to intravascular cannulae, in the present study for example the addition of KCl or $CaCl_2$ enhanced the adhesion of both *S. aureus* and *S. epidermidis* to different intravascular cannulae.

5. Conclusion

In this study, the adhesion of *S. aureus* and *S. epidermidis* to intravascular cannulae made up of PTFE, SPTFE and Vialon was investigated. It was found that *S. aureus* and *S. epidermidis* adhere to all types of intravenous cannulae. Both bacteria adhered less to PTFE followed by Vialon and SPTFE. The adhesion capacity of *S. aureus* and *S. epidermidis* was increased over time. Coating intravascular cannulae with human serum albumin inhibited the adhesion of *S. aureus* and *S. epidermidis* to these cannulae. Pretreatment of cannulae with fibronectin inhibited the adhesion of *S. epidermidis* but increased the adhesion of *S. aureus* to all types of cannulae used in the study. Pretreatment of cannulae surface with potassium chloride or calcium chloride increased the adhesion of *S. aureus* and *S. epidermidis* to cannulae indicating the role of electrostatic force in the mechanism of such adhesion. Adhesion of *S. epidermidis* and *S. aureus* to intravascular cannulae varied significantly according to the type of material and the presence of coating materials. Additional investigation is needed to clarify the mechanism of adhesion and discovering a possible way to prevent such adhesion either by better material coatings, or by interfering with process of adhesion by targeting the bacterial structures which are responsible for it.

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Corresponding Author:
Dr. Sulaiman Ali Alharbi
Department of Botany and Microbiology
College of Science, Building Number: 05
King Saud University, P.O. Box -2455
Riyadh-11451, Kingdom of Saudi Arabia
E-mail: sharbi@ksu.edu.sa

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