Micron 78 (2015) 33–39

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Risk factors and biofilm detection on central venous catheters of patients attended at tertiary hospital

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ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form 1 July 2015
Accepted 1 July 2015
Available online 9 July 2015

Keywords:
Biofilm
Central venous catheter (CVC)
Roll plate method
Sonication
Scanning electron microscopy (SEM)
Catheter-related infections (CRI)

ABSTRACT

Aim: To determinate the significance of risk factors with the presence of biofilm on catheters of patients attended at tertiary hospital cares.
Material and methods: A total of 126 patients were included, data collection by observing the handling of the CVC, clinical history and microbiological isolation methods of CVCs tips (Roll-plate, sonication and scanning electron microscopy) were evaluated.
Results: Certain factors, such as the lack of proper hand washing, the use of primary barriers and preparing medications in the same hospital service, showed an important relationship between biofilm formation in CVCs. The sonication method presented that most of the samples had isolation of multispecies 29 samples (64%); in contrast with the roll-plate method, just one sample (3%) was isolated.
Conclusions: The importance of the strict aseptic techniques of insertion and of the handlings of CVC was highlighted, the failure of both techniques was related to the biofilm formation and was evidenced using the scanning electron microscopy. Since this tool is not available in most hospitals, we present the correlation of those evidences with other standard microbiological methods and risk factors, which are necessary for the sensible detection of the different steps of the biofilm formation on CVC and their correct interpretation with clinical evidences.

1. Introduction

Vascular access by use of intravenous catheters has a great importance and it is an essential element of modern medical care. Central venous catheter (CVC) is considered one of the preferred devices, especially in patients with critical illnesses, cancer or without peripheral venous access sites, as well as patients who require multiple intravenous infusions, parenteral nutrition or frequent blood sampling (Chernetsky Tejedor et al., 2012). CVC provide necessary vascular access; however, these devices are highly related to complications, early complications related to CVC placement include bleeding, cardiac arrhythmia, malposition, air embolism, pneumothorax and, rarely, injury to vessels or nerves. Late complications include infection, thrombosis and catheter malfunctions. The relationship between CVC and infections is considered the leading cause of morbidity and the second cause of mortality among patients who carry it (Schiffer et al., 2013), which requires immediate removal of the catheter and the loss of an indispensable tool for the treatment of the patient (Frasca et al., 2010). The origin of the infection complications related to CVC are highly associated with intravascular therapy which can be caused by different contamination sources. It may be given by defects in containers ranging solutions intended for intravenous administration, defects in the air filters infusion therapy device during catheter manipulation, administration of contaminated solutions, multi-lumen catheters and the most common is by the catheter installation, where on the first place, the body is a potential surface of bacterial adhesion and central venous catheters represent a passageway

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http://dx.doi.org/10.1016/j.micron.2015.07.001
0968-4328/

for microorganisms between the environment and the body. Therefore, microorganisms that are part of the normal biota in skin easily enter through the insertion site and get directly into the bloodstream causing these infections (Rolighed Thomsen et al., 2011a). The complicated process of colonization begins with the microorganism adhesion resulting in biofilm formation (Borges et al., 2014). Biofilms are an aggregate of microbial cells adherent to a living or nonliving surface, embedded within a matrix of extracellular polymeric substances (EPS) of microbial origin. Biofilm EPS is an amalgam of extracellular macromolecules including nucleic acids, proteins, polysaccharides and lipids (Flemming and Wingender, 2010). Within the biofilm microbial cells are physiologically distinct from planktonic or single, free-floating cells of the same organism; however nowadays this crucial distinction is not a simple determination that can be evaluated by the tests and examinations usually employed in medical diagnostic work-ups. Classically, bacteria exhibit recalcitrance to antibiotics when they are in biofilms (Hall-Stoodley et al., 2012a).

The evaluation for the diagnostic of biofilm-associated infections is even more challenging, because the infectious agents are often unknown, and pathologically significant biofilm infections need to be distinguished from microbial colonization with non-pathogenic organisms (Hall-Stoodley et al., 2012a). On the other hand, clinical criteria, like fever and chills that are often associated with catheter-related infection (CRI), are not specific and local catheter inflammation and phlebitis could exist in the absence of CRI or even a local infection, as it has been reported with peripherally inserted central catheters (Lorente et al., 2014). Therefore microbiological evidence implicating the catheter as a source of the bloodstream is determined. Some diagnostic methods require catheter removal, like the semi quantitative roll-plate catheter culture, which is one of the most frequently studied diagnostic techniques (Amin Nirma et al., 2013). The limitation of this method is that it only detects colonization of the external surface of the catheter rather than intraluminal colonization. Another method is Quantitative catheter segment cultures; the advantage of sonication or vortexing is that these methods help release organisms from both the external and internal surfaces of the CVC, but the disadvantage is that it might not be clinically relevant, whereas relevant planktonic organisms might be killed (Mette Møller et al., 2013).

The factors triggering biofilm development may vary from organism to organism. However, it is clear that these factors have a profound impact on the transition of planktonic to biofilm form attributing to catheter colonization, further ending up in persistent and resistant blood stream infections (Alhede et al., 2011). This study aimed to evidence the relationship of the failure of the basic rules for handling the catheter and the biofilm formation, we used a highly sensitive methodology, SEM, and other tools like data collection by observing the handling and care of CVC, microbiological isolation methods and their correlation with the clinical criteria in patients.

2. Material and methods

2.1. Patient population

A prospective and transversal study was conducted at the Department of Neonatology, Female Surgery and Male Surgery of a Hospital at San Luis Potosi, Mexico, which is a tertiary care hospital, in the period from April until September 2013.

This study was submitted and approved by the Ethics Committee of the Hospital with the number 39–12 to carry out in only three areas of the hospital, which have high incidence of central catheters-day.

2.2. Inclusion criteria

The inclusion criteria for the observation of risk factors for catheter care, management and for the microbiological procedures for samples were adult and neonate patients having at least 48 h of hospitalization requiring a CVC. Patients were excluded if they were transferred to another department.

2.3. Observation of risk factors related to CRI

A total of 126 different patients with CVC were included on the observational data of risk factors related to CRI during the installation and maintenance of the catheter. The observation of risk factors included the conditions of handling maintenance and care of the catheter like the type of dressing, sterile gloves, face mask, sterile gown, sterile fields and environmental conditions. The observational data related to the development of CRI was carried out following the guidelines for the prevention of intravascular catheter- related infections of the CDC for care and management of CVC (Macias et al., 2010). A detailed clinical history and examination was done for each subject in order to know characteristics such as age, sex, duration of use of CVC, site of CVC, swelling/purulence around the CVC, possible signs of infection, date and reason of removal of the catheter and microorganisms isolated from CVC tips when the roll plate and sonication methods were performed.

2.4. Sample collection

The study was carried out on 126 central venous catheter tips; all consecutive catheters were derived from adult and neonate patients with different diseases, samples were properly collected, shipped and processed. When catheters were removed, the distal part (the tip) of the catheter was aseptically dissected in three segments, each with a size of two centimeters long. Two segments were subsequently cultured by two comparative culture methods, roll plate method and sonication, and the other segment was examined by scanning electron microscopy (SEM).

2.5. Microbiological procedures

Catheter tips were processed by using both semi quantitative roll plate method (Maki method) and sonication. The reference standard of tip culture is a semi quantitative method described by Maki et al., with a cut off of 15CFU to distinguish microbial contamination of catheters from significant colonization (Mette Møller et al., 2013). This semi quantitative roll plate method was performed by rolling the external surface of a catheter tip back and forth on the surface of a blood agar plate supplemented with 10% sheep blood (Becton Dickinson Mexico, Diagnostics system BDDS) at least three times and then incubating the plate for 24 h at 5% CO_2 and 37 °C, with this condition we could isolated the aerobic and microaerophilic microorganism, after which the number of CFU were quantitated as described in detail elsewhere. Sonication method was performed by placing the catheter in 5 mL of 0.9% NaCl, after vortex by 30 s, it was sonicated for 1 min (Ultrasonic Cleaner, model SB- 3200DT (N), Freq. 40 KHz, Zhejiang, China (Mainland)) and then vortex again for 30 s. One hundred microliters of the sonication fluid was cultured on Triptic Soy agar (BD Bioxon, Mexico), allowing a detection limit of $\geq$100CFU/catheter tip (Alhede et al., 2011). Microorganisms recovered from the plates were identified and counted by standard microbiological methods.