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Biofilm Formation by Bacteria Isolated from Intravenous Catheters

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ABSTRACT

Background: Reports on the association of nosocomial bacterial infections with indwelling medical devices such as intravenous catheters (IVC) has increased in recent years. The potential to form biofilm on these devices seems to be the main reason for establishment of such infections. The aim of this study was to measure the potential of biofilm formation by bacterial isolates from IVCs.

***Methods*:** Seventy-one IVCs were collected from hospitalized patients in ICU, NICU, hematology and oncology wards at Taleghani Hospital from Jan 2010 to Jan 2011. The bacterial isolates were identified using the standard biochemical tests and the potential to form biofilms was determined by the microtiter plate assay method (MTP) and colony morphology using Congo red agar plates (CRA).

Results: Overall, 54 (71%) IVCs were colonized and 76 bacteria were isolated among which, 64 (84.2%) were coagulase negative staphylococci (CoNS), 3 (3.9%) *S. aureus*, 3 (3.9%) *Enterococcus spp.*, 2 (2.6%) *E. coli* and 4 (5.3%) were miscellaneous isolates not further identified. Among the CoNS, biofilm formation was observed in 68.7% and 82.8% of bacteria using MTP and CRA methods, respectively. *S. aureus* and *E. coli* isolates also were biofilm producers but *Enterococcus* and other unknown isolates were biofilm negative.

Conclusions: Our results confirm that the prevalent biofilm forming bacteria on IVCs were CoNS and that was the reason for high rates of nosocomial infections.

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Introduction

Microbial biofilm formation is identified as the cause of a variety of infections including dental plaques, upper respiratory tract infections, peritonitis, urogenital tract infections and also the diseases related to medical devices (1, 2). Meanwhile, microbial biofilm formation on medical devices such as catheters is among the most important causes of infection in humans (2-4). The bacterial population of biofilms may be formed by the same or multiple species depending on the type of the device and duration of its use in patients. The bacteria in biofilms are located in an extracellular matrix, mainly composed of polysaccharides which can act as a filter to entrap minerals or host serum components. Moreover, bacterial species in biofilms show resistance to antibiotics and the host immune system. The chronic bacterial infections associated with biofilms cannot be eradicated by conventional antibiotic therapies (5-7). There are several reasons for antibiotic resistance of microorganisms present in biofilms. Apart from the mechanisms of intrinsic resistance of microorganisms to antibiotics, resistance of bacteria in biofilms may be acquired due to transmission of genetic elements to the susceptible organisms in biofilms. Furthermore, the extracellular matrix provides a barrier for penetration of some antibiotics (7). A wide range of organisms including pathogenic bacteria and fungi causes microbial infections associated with biofilms. These microorganisms can form single or multiple species biofilms. The biofilm-forming bacteria in indwelling medical devices (IMD) include the Gram-positive bacteria such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, the viridans streptococci and the Gram-negative types such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (5-8). These organisms may be acquired from human carriers (skin of the patients or hospital staff) or environment (tap water or other environmental sources). In recent years, coagulase negative staphylococci (CoNS), especially *Staphylococcus epidermidis*, have been identified as the main cause of IMD-related infections and nosocomial bacteremia mostly due to their potential to form biofilm as the main virulence factor (5-7). In this regard, we aimed to identify the common bacterial strains contaminating catheters and to determine their ability to from biofilms in vitro.

Material and method

In this study, 71 intravenous catheters were collected from patients hospitalized in ICU (n=17), NICU (n=22), hematology (n=18) and oncology (n=14) wards of the Educational-Medical Center of Taleghani Hospital between January 2010 to January 2011. Immediately after collection, the catheter tips were cut using sterile scissors and placed in capped tubes containing 5 ml nutrient broth. The samples were transferred to the laboratory in less than 2 hours. In addition, 4 sterile intravenous catheters (IVC) were treated the same way and used as controls. The bacteria contaminating catheters were isolated using Maki's technique; the catheter tips were rolled back and forth on blood agar plates containing 5% sheep blood and were incubated at 37 °C for 48 hours (9). The obtained colonies were transferred to nutrient agar plates and incubated to obtain pure cultures. The purified colonies were Gram stained and further identified using standard microbiology techniques. The following tests were performed to identify Gram-positive cocci: catalase production, bacitracin resistance, coagulase production, mannitol fermentation and DNase production. The tests used for identification of Gram-negative bacilli were oxidase, lactose fermentation, MRVP, Simmons' citrate, urease, indole production, hydrogen sulfide production and TSI. Biofilm forming ability was tested using two different techniques, microtiter plate (MTP) and Congo red agar (CRA) (8, 10). In MTP method, the overnight bacterial culture (24 hrs) was diluted

1:100 in TSB (trypticase soy broth) and then 200 µl of each bacterium was added into 4 wells of a sterile flat bottom 96-well microtiter plate. The plates were incubated at 37 °C for 22-24 hours. After removing the supernatant fluid, each well was washed three times using 200 µl of phosphate buffered saline (PBS; pH = 7-7.2). The plates were dried at room temperature for 1 hour and the resulting biofilms were stained using a solution of 0.1% safranin in water for 1 minute. The plates were then washed three times with tap water and dried at room temperature. Eventually, the absorbance was read at a wavelength of 492 nm using Micro ELISA Reader (Stat Fax 2100, Awareness Technology). The experiment was performed on four different days for each bacterium.

Table 1. Distribution of bacterial isolates among different wards.
* Described in the results section. Hem, hematology; Onc, oncology.

Organism	ICU	NICU	Hem	Onc
CoNS	16	18	20	10
S. aureus	3	-	-	-
E. coli	-	-	1	1
Enterococcus spp.	-	-	-	3
other bacteria*	-	-	2	2

If the mean absorbance was less than or equal to 0.12, it was reported as biofilm-negative, greater than 0.24 considered as strong biofilm producer and between 0.12 and 0.24 were weak biofilm producers (8). In all biofilm formation experiments, RP62A (biofilm-positive) and RP62NA (biofilm-negative) strains of *S. epidermidis* were used as controls (8).

In the CRA method, the potential to form biofilm was checked by observing the morphology of bacterial colonies on Congo red agar plates (21 g Mueller-Hinton broth, 36 g sucrose, 0.8 g Congo red and 15 g agar) using streak plate method and incubation at 37 °C for 24 to 48 hours. Dry black colonies were considered biofilm-positive and pink colonies as biofilm-negative (10, 11).

Result

Of the 71 examined intravenous catheters, 54 (71%) were contaminated with bacteria including 11 catheters from ICU, 15 from NICU, 16 from hematology and 12 from oncology wards. A total of 76 bacteria were isolated where at least 1 and at most 3 bacteria were isolated from each infected catheter. The isolates included 64 (84.2%) CoNS, 3 (3.9%) *S. aureus*, 3 (3.9%) enterococci, 2 (2.6%) *Escherichia coli* and 4 (5.3%) other bacteria including 1 large oxidase-negative Gram-negative coccus, 1 small non-spore forming Gram-positive Bacillus and 2 large non-spore forming Gram-positive bacilli which were not further identified. The distribution of isolates among the catheters obtained from different wards is presented in Table 1. The results of biofilm formation using the MTP method are shown in Fig. 1. In total, of the 76 isolated bacteria, 49 (64.5%) were biofilm-producers, of which, 24 (49%) produced strong biofilms and 25 (51%) were weak biofilm-producers. The potential to form biofilm was similar among the isolates obtained from different wards. Among the coagulase negative staphylococci, 44 isolates (68.7%) produced biofilm. Moreover, all *S. aureus* and *E. coli* isolates were biofilm-positive, enterococci and the unidentified bacteria were biofilm-negative (Figure 1).

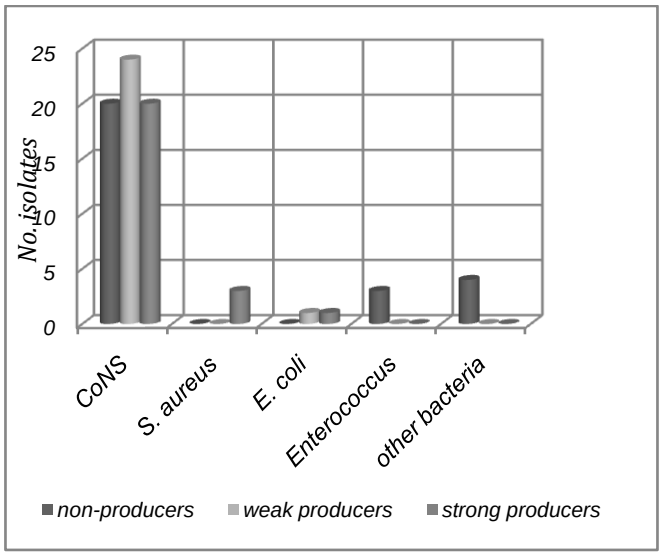

Figure 1. Biofilm formation among the bacteria isolated from IVCs based on the MTP method. Other bacteria are the unidentified organisms mentioned in the results.

The results obtained from the CRA method (Figure 2), showed biofilm-forming ability in 58 (76.3%) bacterial isolates where 53 (91.4%) were CoNS. Similar to the MTP method, all isolates of *S. aureus* and *E. coli* produced biofilms.

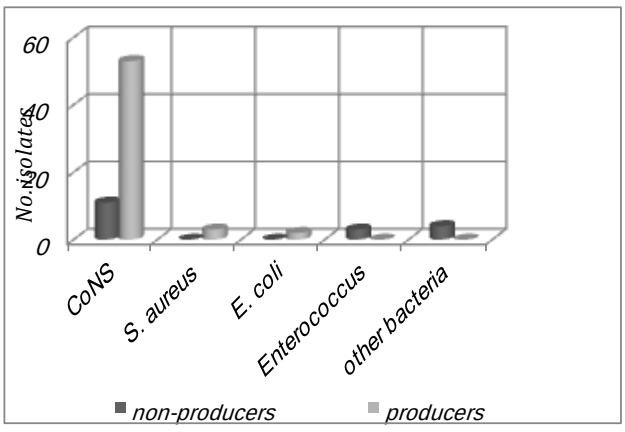

Figure 2. Biofilm formation among the bacteria isolated from IVCs based on the CRA method. Other bacteria are the unidentified organisms mentioned in the results.

Discussion

Contamination of IMD with bacteria is a common and well-known occurrence. It has been shown that non-pathogenic bacteria such as CoNS are capable of causing infection if they can produce biofilm on these devices and the corresponding chronic infections cannot be easily treated (5, 7, 12). Among various types of IMD, urinary catheters, IVCs and central venous catheters (CVC) are frequently used in modern medicine. Most cases of bacteremia associated with *S. epidermidis* are due to the ability of the microorganism to produce biofilm on catheter surfaces. Successively, the bacteria can enter the blood stream and eventually reach host tissues (13-15). The incidence of bacteremia in immune-compromised patients can lead to patients' death (13, 15). Furthermore, most studies have shown that CoNS are the predominant organisms contaminating IVCs (6, 14, 14, 16). In the present study, of the 71 examined IVCs, 54 (71%) were contaminated with bacteria and CoNS were the predominant microorganisms isolated. Catheter infection rates have been different in various reports. A variety of reasons including good hygiene practice at medical centers, observing sterile conditions at the time of catheterization, duration of catheterization, etc. are involved. Considering the presence of staphylococci as normal skin flora, the microorganisms can reach catheters through skin abrasions. The catheter provides a favorable environment for the formation of biofilm and establishment of infection (17). In this study, a total of 76 bacterial strains were isolated from IVCs consisting of 64 CoNS, 3 *S. aureus*, 3 enterococci, 2 *E. coli*, and 4 unidentified bacteria. Biofilm formation by the CoNS was 68.7% using MTP and 82.8% in CRA method. The other isolates except enterococci and unidentified bacteria were also able to produce biofilms. In a study in 1997, Ziebuhr *et al* showed that 87% of the *S. epidermidis* strains isolated from catheters produced biofilm (18).

In 2001, Arciola *et al* showed that 48.5% of *S. epidermidis* and 60.8% of *S. aureus* strains isolated from IVCs produced biofilm (19). In 1990, Kotilainen *et al* reported biofilm formation in 53% of the CoNS isolates from IVCs where 97% of the strains were *S. epidermidis* and 3% were *S. hominis* (20). In 2009, Eftekhar *et al* showed that more than 50% of *S. epidermidis* strains isolated from skin and nosocomial infections were able to produce biofilm. Furthermore, there was no significant difference between the two groups of isolates in terms of biofilm formation using MTP (52% and 56%, respectively) and CRA methods (64% and 68%, respectively) (10). In addition, it has been shown that the relationship between the presence of *ica* genes (responsible for biofilm formation) and biofilm formation by *S. epidermidis* strains was more consistent with CRA compared to MTP method (10,11,21). In the present study, the percentage of biofilm-producing bacteria was approximately 14% higher using the CRA method. With respect to the fact that CRA is a qualitative method, much easier and less expensive than MTP, its use for detection of biofilm production and thus potential microbial pathogenicity can be of importance. There are several factors involved in the pathogenesis of pathogenic and opportunistic bacteria. Among those factors, the ability to produce biofilm is of great importance. The results of this research and many other studies conducted around the world show that skin inhabiting bacteria such as CoNS which are usually non-pathogenic, can cause infection after reaching catheters and producing biofilms. In case of resistance of bacteria to common antibiotics, these infections can even endanger the life of the host.

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Conflict of interest

None declared conflicts of interest.

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