Biofilm formation by *Acinetobacter baumannii* isolated from medical devices at the intensive care unit of the University Hospital of Tlemcen (Algeria)

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*Acinetobacter baumannii* is an opportunistic pathogen responsible for nosocomial infections due to biofilm formation on the surface of implantable medical devices. Thirty (30) strains of *A. baumannii* were isolated from medical devices and tested for their ability to form a biofilm. The factors that may influence this process, such as the hydrophobicity of the bacterial wall, temperature, duration of implantation and the nature of the medical device, were also investigated. Strains were able to form a biofilm; however this process was more substantial at 30°C than at 37°C and was maximal after 96 h of incubation. Strains seem to adhere better to silicone and latex than to polyvinylchloride (PVC) and no apparent relationship was found between hydrophobicity and biofilm formation.

Key words: *Acinetobacter baumannii*, biofilm, medical devices.

INTRODUCTION

Biofilm is a microbially derived sessile community which is characterized by cells that are irreversibly attached to a substratum or interface with each other, and embedded in a matrix of self-produced extracellular polymeric substances (Lee et al., 2008). Biofilm formation has been linked to the survival of pathogenic bacteria in the hospital environment and has been connected to infections associated with indwelling medical devices (Martí et al., 2011). The widespread use of medical devices has caused a great advance in the management of many diseases. Indwelling medical devices are being increasingly used for the treatment of functional deficits in numerous medical fields (Abd El-Baky, 2012). Despite the considerable success achieved with new material devices, these abiotic surfaces are susceptible to bacterial colonization which creates an important public health problem (Treter and Macedo, 2011). The temporary implantation of a vascular catheter, a urinary catheter or an endotracheal tube can become a site for bacterial adhesion and infection (Espinasse et al., 2010). More than 60% of hospital-acquired infections worldwide are due to bacteria forming biofilms on medical devices (Lichter et al., 2009; Treter and Macedo, 2011). The medical consequence of these devices-related infections can be life threatening and may lead to device removal. In such a situation, the management of these devices can be a difficult and costly affair (Singh et al., 2011).

Although medical devices may differ widely in design...
and use of characteristics, specific factors determine susceptibility of a device to microbial contamination and biofilm formation. For example, duration of use, flow rate and composition of the medium in or on the device, conditioning films on the device, and device material construction all may influence biofilm formation (Donlan, 2001). In fact, in vitro studies have shown that the most significant factors influencing biofilm formation on the surface of a synthetic implant are: its structure and hydrophobicity and the species of bacteria involved (von Eiff et al., 2005). Biofilms on indwelling medical devices may be composed of gram positive or gram negative microorganisms (Linski et al., 2009). These microorganisms may originate from the patient’s skin or mucous membranes during implantation. Sometimes, the pathogens may also be acquired from the hands of the surgical or clinical staff (Kokare et al., 2009). Acinetobacter baumannii is one of the common bacteria associated with biofilms on indwelling medical devices causing bacteremia, urinary tract infections, secondary meningitis, and pneumonia (King et al., 2009; McQueary and Actis, 2011). Some of the challenges in the prevention and treatment of infections caused by this opportunistic pathogen are its remarkable widespread resistance to different antibiotics and its ability to persist in nosocomial environments (Tomaras et al., 2003). In fact Acinetobacter baumannii can survive on fingertips and inanimate objects such as glass, plastic and other environmental surfaces, even after exposure to dry conditions (desiccation, nutrient starvation and antimicrobial treatments), during extended periods of time, and the environment can be a transmission route in some outbreaks (Tomaras et al., 2003; Gaddy and Actis, 2009; Espinal et al., 2012). The ability of Acinetobacter strains to adhere to surfaces is an important mechanism in the pathogenicity of these bacteria. Although the adhesion ability is determined by specific factors, such as adhesins, and non-specific factors, such as hydrophobicity and cellular surface electrical discharge, it varies among strains (Costa et al., 2006).

With regard to the role of Acinetobacter baumannii in medical device-related infections, the purpose of this paper is to study for the first time in Algeria, the ability of clinical Acinetobacter baumannii isolated from medical devices to form biofilm. The factors influencing this process such as cell surface hydrophobicity (CSH), temperature and time of implantation of medical devices were investigated. The nature of the medical device surface was also studied by comparing the adhesion ability of strains on three different biomaterials (silicone, latex and polyvinylchloride) used in medical device manufacturing.

**MATERIALS AND METHODS**

**Bacterial strains**

A total of 30 strains of Acinetobacter baumannii were studied. Isolates were collected from urinary catheters, endotracheal tubes and central venous catheters in the intensive care unit of the University Hospital of Tlemcen (Algeria). The strains were identified by macroscopic, microscopic, and biochemical tests, including an oxidase test and using the API 20 NE system (bioMerieux SA, Lyon, France). The capacity of strains to grow at 41 and 44°C was also used for identification of Acinetobacter baumannii.

**Antibiotic resistance**

Antimicrobial susceptibility testing was performed for 10 different therapeutically relevant antibiotics using the standard protocol for diffusion of antimicrobial agents on Mueller-Hinton agar as described in National Committee for Clinical Laboratory Standards NCCLS guidelines (CLSI, 2003). The antibiotics tested included: ticarcillin, ticarcillin/clavulanic acid, cefazidim, imipenem, gentamycin, amikacin, tobramycin, ciprofloxacin, trimethoprim/sulfamethoxazole and colistin.

**Quantification and kinetics of biofilm formation to polystyrene**

The biofilm formation was performed in 96-well plates according to the procedure of O'Toole and Kolter (1998). Its growth was determined in Luria Bertoni broth using an initial OD600 of 0.01 and incubated at 37 and 30°C for 24 h without shaking. Two wells were left uninoculated and for use as negative controls. The biofilm was stained with 0.5% crystal violet (w/v) for 20 min at room temperature and the wells were washed to remove the unbound crystal violet. Biofilm formation was finally quantified at 570 nm after solubilisation in 95% ethanol. The bacterial isolates were considered to be positive for biofilm formation when the readings obtained were at least twice greater than the negative control. The strain Acinetobacter baumannii (ATCC 19606) was used as a positive control.

The kinetics of Biofilm formation was performed by extending the incubation time to 6 days.

**Hydrophobicity assays**

The hydrophobicity of the bacterial wall was evaluated with the MATH protocol (Rosenberg, 1984) using hexadecane as a solvent. A. baumannii strains were grown in 50 ml of Luria Bertani (LB) and incubated for 18 h at 37°C. The cells were recovered by centrifugation (5000 rpm for 15 min). The pellet obtained was then washed after two successive centrifugations with PBS (Phosphate Buffered Saline pH 7.1) and suspended in the same buffer at an initial optical density (OD) between 0.8 and 1 at 600 nm. A volume of 0.3 ml of each solvent was added to 1.8 ml of bacterial suspension and the whole is vortexed for 2 min. After 20 min settling, the optical density (OD) of the aqueous phase was measured at 600 nm and the percentage of adhesion to solvent was then calculated using the following equation:

\[
\text{CSH}\% = \frac{\text{OD}_{\text{solvent}} - \text{OD}_{\text{aqueous phase}}}{\text{OD}_{\text{solvent}}} \times 100
\]

**Adhesion to silicone, latex and polyvinylchloride**

Samples of sections 1 cm² from each support were introduced into tubes containing 5 ml of an A. baumannii bacterial suspension adjusted to an OD600 of 0.1 and incubated at 37°C for 24 h. The supports were recuperated, thoroughly washed with sterile distilled water and placed in 5 ml of PBS (pH 7.1). Sonication was performed three times using the WiseClean WUC-D06H ultrasound for 5 min, and interrupted at regular intervals by vortexes of 20 s. A series of dilutions 1/10 to 1/100000 was performed for each sample, and plated on nutrient agar. After 24 h of incubation, the
Table 1. Distribution of strains according to the medical device, implants duration and antibiotic resistance pattern.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Medical devices</th>
<th>Duration of medical devices implantation (days)</th>
<th>Antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1</td>
<td>Central venous catheter</td>
<td>10</td>
<td>TIC TCC CAZ AK TM SXT CIP</td>
</tr>
<tr>
<td>AB2</td>
<td>Central venous catheter</td>
<td>8</td>
<td>TIC TCC CAZ AK TM SXT CIP</td>
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<tr>
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<td>Urinary catheter</td>
<td>6</td>
<td>TIC TCC CAZ GN AK TM SXT CIP</td>
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<tr>
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<td>8</td>
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</tr>
<tr>
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<td>10</td>
<td>TIC TCC CAZ GN AK SXT CIP</td>
</tr>
<tr>
<td>AB6</td>
<td>Endotracheal tube</td>
<td>17</td>
<td>TIC TCC CAZ IMP GN AK SXT CIP</td>
</tr>
<tr>
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<td>15</td>
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<td>Urinary catheter</td>
<td>4</td>
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<tr>
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<td>Urinary catheter</td>
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</tr>
<tr>
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</tr>
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<td>AB30</td>
<td>Endotracheal tube</td>
<td>19</td>
<td>TIC TCC CAZ GN AK SXT CIP TM</td>
</tr>
</tbody>
</table>

AB, A. baumannii; TIC, ticarcillin; TCC, ticarcillin/clavulanic acid; CAZ, ceftazidim; IMP, imipenem, TM, tobramycin; GN, gentamycin; AK, amikacin; Cip, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole.

Colonies were counted at 37°C. The experiments were carried out in triplicate.

RESULTS

Characterization of the strains

An endotracheal tube, urinary catheters and central venous catheters used in patients catheterized between 4 and 21 days were collected. Eleven (11) strains were isolated from the endotracheal tube, 10 from urinary catheters and nine from central venous catheters (Table 1). The antimicrobial profiles showed that all isolates were resistant to ticarcillin, ticarcillin/clavulanic acid, Ceftazidim and trimethoprim/sulfamethoxazole. Twenty-seven (27) isolates were resistant to ciprofloxacin, 27 to amikacin, 20 to Gentamycin, 15 to imipenem, 10 to tobramycin and finally all isolates were susceptible to colistin.

Biofilm formation

A set of 30 A. baumannii strains were tested for their ability to form a biofilm by crystal violet staining. All strains had ability to form biofilm, however this process was more significant at 30°C than at 37°C, with values of OD570 (mean ± SD) varying between 0.63 ± 0.07 and 1.36 ± 0.1 and between 0.18 ± 0.06 and 1.23 ± 0.09, respectively (Figure 1).

The results of the kinetics of biofilm formation showed that a maximum OD value was reached after 72 h of incubation. However, a decrease in the density of the biofilm was noted after 96 h of incubation (Figure 2).
Figure 1. Quantification of biofilm formed by Strains of A. baumannii in Microplate at 30 and 37°C. Values are means ± SD of three independent experiments.

Figure 2. Kinetics of biofilm formation of A. baumannii.

Cell surface hydrophobicity

The study results of the hydrophobicity of A. baumannii strains indicated that out of the 30 strains tested, more than half, that is 18 (60%) of them, were hydrophilic with a percentage that varies between 1.25 and 15%. Moderately hydrophobic strains amounted to 7 (23%) with a percentage between 21.4 and 33.76%, and finally only 5 (17%) strains were hydrophobic with a percentage ranging from 42.5 to 90, 13% (Figure 3).

Adhesion to biomaterial

The strains AB25, AB29 and AB3 were selected for use in this study. They were chosen because of their high capacity to form a biofilm and their different hydrophobicity characters. The results of the adhesion of strains to the three different surfaces indicated that all strains had the same behavior, exhibiting a greater adhesion to silicone which was closely followed by latex. Finally, polyvinylchloride (PVC) comes last with a much lower adhesion compared to the two other surfaces (Figure 4).

DISCUSSION

During the last decades, A. baumannii has emerged globally as an important nosocomial pathogen that gives rise to outbreaks of colonization and infection of critically ill, hospitalized patients (de Breij et al., 2009). One reason for the emergence of this pathogen may be its persistence in hospital wards, in particular in the intensive
Figure 3. Cell surface hydrophobicity of *A. baumannii* strains. CSH was determined based on the difference of the OD of bacterial before and after adsorption to hexadecane, weak (0 - 20%), moderate (21 - 50%) and strong CSH > 40%.

Figure 4. *A. baumannii* biofilm formation (CFU/ml) on Latex, Silicone and PVC for 24h of incubation.

Infections of hospitalized patients with Acinetobacter spp., often preceded by colonization, are frequently associated with invasive procedures and implantable medical devices. This process may be facilitated by the ability of a strain to form a biofilm (Wroblewska et al., 2008). It has become apparent that biofilm formation is a common trait of *A. baumannii* clinical isolates (McQueary and Actis, 2011). Several studies have demonstrated a high propensity among *A. baumannii* clinical isolates to form biofilms and a significant association of biofilms with multiple drug resistance and device-associated infections (Sechi et al., 2004; Rao et al., 2008; Rodríguez-Baño et al., 2008). In this study all *A. baumannii* strains were isolated from the endotracheal tube, urinary and central venous catheters at the intensive care unit of the University Hospital of Tlemcen formed biofilms, however this process is found to be more significant at 30 than at 37°C. This fact could explain the observed persistence of the members of the *A. baumannii* group in the inanimate hospital environment (Marti et al., 2011). Espinal et al. (2012) showed a relationship between the biofilm formation and the survival of *A. baumannii* clinical isolates, confirming the fact that isolates which produce biofilms survive longer than their non-biofilm forming counterparts on dry surfaces. The ability of *A. baumannii* to persist in nosocomial environments was also attributed to its widespread resistance to different antibiotics. In fact, the resistance profile revealed a remarkable resistance to most of the antibiotic agents tested, along with a significant susceptibility to colistin.

The duration of the device implantation significantly influences the biofilm formation (Domka et al., 2007). The kinetics of biofilm formation established in our study...
showed that for up to 96 h of incubation, strains of \textit{A. baumannii} adhere strongly and continuously. This first period corresponds to significant biofilm formation and the second to biofilm dispersion, with a release of bacterial cells into the culture medium (Djeribi et al., 2012). These observations suggest that the duration of implantation of the medical device must be reduced while it can go up to 21 days in our Intensive Care Unit, as shown in Table 1. Indeed, the risk of catheter-related infection is mainly connected with the time during which the catheter remains inserted. It is estimated that the risk increases by 5% each day (Mączyńska et al., 2010).

Cell surface hydrophobicity (CSH) of \textit{A. baumannii} strains is also known to be associated with pathogenicity, bacterial adhesion and biofilm formation (Costa et al., 2006; Pour et al., 2011). Accordingly, the hydrophobicity of the isolates was evaluated by determining the affinity of cells to hexadecane (Rosenberg, 1984). The results obtained from the MATH method revealed that the majority of \textit{A. baumannii} strains isolated from a hydrophobic medical device surface showed a hydrophilic character which contradicts several studies which admitted that the hydrophobic cells tend to adhere to a hydrophobic substrate, while the hydrophilic cells tend to adhere to a hydrophilic substrate (Costa et al., 2006; Djeribi et al., 2013). It is suggested that there is a positive correlation between the degree of bacterial hydrophobicity and the adhesion to abiotic surfaces (Costa et al., 2006; Pour et al., 2011). However, in this study, no apparent relationship was detected between hydrophobicity and biofilm formation, as the most hydrophilic strain AB9 formed a similar biofilm to the most hydrophobic one AB18. According to McQueary and Actis (2011), cell hydrophobicity is not a good predictor of the properties of \textit{A. baumannii} biofilms; in contrast to other bacterial pathogens such as \textit{Neisseria meningitides} and \textit{Stenotrophomonas maltophilia}, which both display a direct correlation between surface hydrophobicity and biofilm formation on glass and plastic, respectively (Yi et al., 2004; Di Bonaventura et al., 2008; McQueary and Actis, 2011).

Another important factor influencing biofilm formation is the type of catheter and the chemical composition of the material it is made of (Mączyńska et al., 2010; Espinal et al., 2012). In this study, tests for adhesion to latex, silicone and PVC surfaces were carried out as these materials are used in the fabrication of implantable medical devices. The strains showed less adhesion to PVC as compared to silicone and latex. These results are in accordance with other studies performed by many laboratories where it has been reported that microbial adherence to biomaterials occurs in the following order: latex > silicone > PVC > Teflon > Polyurethane > stainless steel > titanium (Abd El-Baky, 2012). Dwornicz et al. (2003) found that \textit{Enterococcus faecalis} adheres highly to silicone and siliconized latex than to PVC. However, Maczynska et al. (2010) demonstrated that PVC was the biomaterial that was best colonized by \textit{klebsiella} strains. These contradictions can be explained by the fact that various bacteria species probably prefer a certain chemical composition of the biomaterial to which their adhesion is the strongest (Mączyńska et al., 2010).

Indeed, the material surface characteristics play an important role in bacterial adhesion. These characteristics include the material's surface-charge, the hydrophobicity and the surface roughness or physical configuration (Katsikogianni and Missirlis, 2004).

**Conclusion**

This paper show the great ability of \textit{A. baumannii} strains to form a biofilm as well as the difference in the intensity of biofilm on three different biomaterials used in the manufacture of medical devices demonstrating the influence of chemical composition of the biomaterial on biofilm formation. Factors that may influence this process have also been demonstrated, as this can help in preventing diseases associated with infections caused by implanted medical devices. On the other hand, a better understanding of biofilm formation by \textit{A. baumannii} is needed in order to provide new strategies to minimize the susceptibility of the device surface to colonization by this opportunistic pathogen.

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**REFERENCES**


