“THINK BEYOND TO CREATE MIRACLES”

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COMPARATIVE ASSESSMENT OF ANTIBIOTIC SENSITIVITY PROFILES OF BIOFILM-PRODUCING BACTERIAL ISOLATES FROM CLINICAL AND WATER SAMPLES IN ABAKALIKI, EBONYI STATE, NIGERIA


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Abstract:

The objective of this study was to compare the antibiotic sensitivity patterns of biofilm-producing Klebsiella pneumoniae isolated from clinical samples (wound and catheter urine) of hospitalized patients with biofilm-producing Pseudomonas aeruginosa and Klebsiella pneumoniae isolated from drinking water sources (tap and borehole). A total of 50 clinical samples (catheter urine = 30; wound = 20) and fifty water samples (taps = 11; boreholes = 39) were collected. The bacterial isolates were characterized and identified using standard microbiology methods. Antibiotic sensitivity test was carried out using Kirby – Bauer disk diffusion technique and the results were interpreted according to the CSLI standards. Screening for biofilm-producing potentials was done by tube method. A total of 44 Pseudomonas aeruginosa and 6 Klebsiella pneumoniae were isolated from water samples while 24 Klebsiella pneumoniae isolates were obtained from clinical samples of hospitalized patients. Results also showed that none of the clinical samples (catheter urine and wound) was positive for P. aeruginosa. Out of the isolates obtained from water samples, 37 Pseudomonas aeruginosa and all the 6 Klebsiella pneumoniae isolates were positive for biofilm production while all the 24 Klebsiella pneumoniae isolates from clinical samples were positive for biofilm production. Results showed that the P. aeruginosa isolates obtained from taps and boreholes were highly resistant to imipenem (100 %), tetracycline (100 %), ceftriaxone (100 %), followed by cefotaxin (97.7 %), amoxicillin-clavulanic acid (95.4 %), meropenem (86 %), ofloxacin (86 %), aztreonam (79.1 %), ampicillin (76.7 %) ciprofloxacin (69.8 %) and chloramphenicol (85.1 %). P. aeruginosa showed low level of susceptibility to aztreonam (20.9 %), ampicillin (23.3 %), ciprofloxacin (30.2 %) and chloramphenicol (34.9 %). This study revealed that all the K. pneumoniae isolates obtained from water samples were completely resistant (100 %) to imipenem, tetracycline, cefotaxime, cefoxitin, amoxicillin-clavulanic acid, meropenem, aztreonam, ampicillin, ciprofloxacin and chloramphenicol except ofloxacin in which 2.3 % susceptibility frequency was observed. This study also showed that Klebsiella pneumoniae isolates from catheter urine and wound were susceptible to imipenem (100 %), gentamycin (50 %) but resistant to ceftriaxone (91.7 %), cefotaxime (100 %), sulfamethoxazole (100 %), tazobactam (100 %), tobramycin (100 %) and ceftazidime (100 %). This study shows that chloramphenicol and ciprofloxacin are still effective against biofilm-producing P. aeruginosa while ofloxacin had little activity against K. pneumoniae isolated from water samples. All the K. pneumoniae isolates obtained from water samples were completely resistant to imipenem but surprisingly, all the K. pneumoniae isolates obtained from clinical samples were susceptible to imipenem. K. pneumoniae isolates from clinical samples also recorded some level of susceptibility to gentamycin. This study, therefore, shows that antibiotic resistance is more prevalent in biofilm-producing bacterial isolates obtained from water samples (taps and boreholes) than those from clinical samples (catheter urine and wound).

Introduction:

Biofilm was first reported about five decades ago; Sihorkar and Vyas (2001) described Biofilms as matrices of microorganisms that originated extracellular polymeric substances (EPSs) which attach to a solid surface or substratum. Biofilms are currently defined as structured bacterial communities enclosed in a self-produced exopolysaccharide matrix and adherent to abiotic or biological surfaces (Costerton et al., 1995). The attachment of these bacteria to a surface results in an increase to antimicrobial resistance thereby causing treatment failure in hospital and clinics; increasing great hygienic and financial concerns in the environmental and biomedical fields (Sihorkar and Vyas 2001, Bechzer, 2001). Pathogenic bacteria are also able to form biofilms representing potential health risks (Arnon et al., 1997). Biofilm formation can be divided into distinct stages; from the initial attachment of bacteria to the surface; to the formation of mature biofilm with a characteristic three-dimensional architecture. Many bacterial functions are required at each step; such as motility, adhesion, transport, stress response, activation of metabolic pathways and extracellular matrix synthesis (Beloin et al., 2004; Domka et al., 2007). The biofilm producing organisms have an inherent resistance to antibiotics and may be very damaging because of the development of immune complex diseases in the long run (Donlan et al., 2002; Souli et al., 1998). Biofilms are also often the site for quorum sensing; influencing the availability of key nutrients for biofilm formation, chemotaxis towards surface, motility of bacteria, surface adhesion and presence of surfactants are certain factors which influence biofilm formation (Carol and Marcelo, 2005; Thomas and Day, 2007). Bacteria associated with biofilms are much more difficult to eradicate and remove from surfaces than planktons (Simoes et al., 2006). Water is an essential requirement for the survival of living organisms especially human. It is also one of the most important elements for all forms of life. Water is indispensable in the maintenance of life on Earth and essential for the composition and renewal of cells in our body; participates in the composition of our tissues, and transports the most diverse substances throughout the alimentary canal (system) of an organism. As water is indispensable in the circulatory system of an organism, it is also important in the transmission chain of many human diseases since certain microbes which are capable of causing life-threatening disease survive in water (Saka et al., 2011). Pseudomonas aeruginosa and Klebsiella spp are human pathogens that are able to form biofilm on different biotic and abiotic surfaces e.g. water system. They have emerged as a primary source of nosocomial infections including infections of artificial implants, contact lenses and urinary catheters(Chau et al., 2007). Klebsiella pneumoniae especially resistant to many recalcitrant infections and are notoriously difficult to eradicate.
They exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic into biofilms, decreased growth rate and expression of resistance genes (Kim, 2001). According to a recent public announcement from National Institute of Health (NIH), more than 60% of all infections are caused by biofilm-producing organisms such as *Pseudomonas aeruginosa* and *Klebsiella* spp (Kim, 2001).

This study, is therefore designed to compare the antibiotic sensitivity patterns of biofilm-producing *Klebsiella pneumoniae* isolated from wound and catheter urine of hospitalized patients to biofilm producing *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolated from tap and borehole water.

**Materials and Methods**

**Sample Collection**

A total of 50 clinical samples were collected (wound = 20; catheter urine = 30) from patients in Calabar General Hospital, Nigeria. The wound surfaces were swabbed with sterile swab stick while the urine samples were collected with sterile bottles. Fifty (50) water samples were also collected from tap (11) and boreholes (39) from different locations in Abakaliki town, Ebonyi State, Nigeria. The nozzles of both the taps and boreholes were cleaned with cotton wool soaked in 75% ethanol. Every sampling tap/borehole (point) was opened and water was allowed to flow for five minutes (5 minutes) before the water samples were collected with sterile sample bottles. All the samples were transported in an ice pack to the Microbiology laboratory unit of Ebonyi State University, Abakaliki for bacteriological analysis.

**Sample Analysis**

Each wound swabbed sample was inoculated into nutrient broth and incubated at 37 °C for 18-24 hrs. The bottle with growth was streaked on agar plate and incubated at 37 °C for 18-24 hrs, while the urine sample was inoculated on MacConkey agar and Eosin methylene blue (EmB) agar. Growth on MacConkey agar and EmB agar were sub-cultured on Nutrient agar plates and incubated at 37 °C for 18-24 hrs. Pour plate method was used to analyze the water samples. Ten (10) fold serial dilutions were made and dilutions were plated out on plate count agar according to Cheesbrough (2006). Bacteria were further sub-cultured through successive streaking to obtain pure cultures.

**Identification and Characterization of Bacterial isolates**

Identification and characterization of bacterial isolates was carried out using standard microbiological techniques such as Gram staining, motility test, coagulase test, catalase test, indole test, Vogues-Proskauer test, citrate production test, methyl red test and sugar fermentation test (Cheesbrough, 2006).

**Antibiotic Susceptibility test**

Antibiotic sensitivity test on the isolated bacteria (*P. aeruginosa* and *Klebsiella pneumoniae*) was done using Kirby – Bauer disk diffusion method and the results were interpreted according to the guidelines of CLSI standards (2007). The following standard antibiotics: ampicillin, cotrimoxazole, ciprofloxacin, aztreonam, meropenem, tetracycline, chloramphencol, imipenem, cefotaxin, amoxicillin-clavulanic acid and ofloxacin were used on the bacterial isolates obtained from the two water sources (taps and boreholes) while imipenem, gentamycin, cefotaxime, ceftriaxone, sulamethoxazole/threomprim, tazobactam, tobramycin and ceftazime were used on the bacterial isolates obtained from the clinical samples (catheter urine and wound) of hospitalized patients. All the antibiotic discs were obtained from Oxoid, UK.

**Biofilm Formation Assay**

Qualitative method for biofilm formation was conducted using tube method as described by Christensen et al., 2011. A loopful of the test organism was inoculated in 10 ml of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 37 °C for 48 hrs. After incubation, tubes were decanted, washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stains were washed with deionized water and tubes were dried in inverted position. The scoring for the tube method was done in comparison with the results of the control strains. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube (Christensen et al., 2011).

**Results**

**Table 1: Distribution of Bacteria isolated from Water and Clinical Samples**

<table>
<thead>
<tr>
<th>Samples</th>
<th>No of samples tested</th>
<th><em>P. aeruginosa</em></th>
<th><em>K. pneumoniae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Borehole water</td>
<td>39</td>
<td>35 (79.5%)</td>
<td>4 (66.7%)</td>
</tr>
<tr>
<td>Tap water</td>
<td>11</td>
<td>9 (20.5%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Catheter urine</td>
<td>30</td>
<td>nil</td>
<td>21 (87.5%)</td>
</tr>
</tbody>
</table>
Table 2: Antibiotic sensitivity profile of *Klebsiella pneumoniae* isolated from clinical samples (wound and catheter urine)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance</th>
<th>Susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>91.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Tazobactam</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Ceftazime</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3: Antibiotic sensitivity profile of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolated from water samples (borehole and tap)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>Pseudomonas aeruginosa</em></th>
<th><em>Klebsiella pneumonia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible (%)</td>
<td>Resistance (%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>23.3</td>
<td>76.7</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30.2</td>
<td>69.8</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>20.9</td>
<td>79.1</td>
</tr>
<tr>
<td>Meropenem</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>34.9</td>
<td>65.1</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic</td>
<td>4.6</td>
<td>95.4</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>2.3</td>
<td>97.7</td>
</tr>
</tbody>
</table>

Table 4: Total rates of formation of biofilm by *Klebsiella pneumoniae* isolated from both wound and catheter urine

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Mild (weak)</th>
<th>Moderate</th>
<th>Strongly</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>11(55%)</td>
<td>4(20%)</td>
<td>5(25%)</td>
</tr>
</tbody>
</table>

Table 5: Detection of Biofilm-producing Potential of *P. aeruginosa* and *Klebsiella pneumoniae* Isolated from Water Sources

<table>
<thead>
<tr>
<th>ISOLATES</th>
<th>BIOFILM PRODUCERS</th>
<th>NON-BIOFILM PRODUCERS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>37 (84.1 %)</td>
<td>7 (15.9 %)</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>6 (100 %)</td>
<td>0 (0 %)</td>
</tr>
</tbody>
</table>

Discussion

Gram-negative opportunistic pathogens, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, are responsible for causing a spectrum of community-acquired and nosocomial infections; and typically infect patients with indwelling medical devices, especially urinary catheters, on which these microorganism is able to grow as a biofilm. Over the years, studies by various researchers have shown that *Pseudomonas aeruginosa* and *Klebsiella* spp also have the ability to form biofilm on different biotic and abiotic surfaces e.g. water system. The increasingly frequent acquisition of antibiotic resistance by *K. pneumoniae* and *Pseudomonas aeruginosa* has given rise to a global spread of these multidrug-resistant pathogens, mostly at the hospital level. This scenario is exacerbated when it is noted that intrinsic resistance to antimicrobial agents dramatically increases when *K. pneumoniae* and *Pseudomonas aeruginosa* grow as a biofilm.
In this study, forty four (44) *Pseudomonas aeruginosa* and six (6) *Klebsiella pneumoniae* isolates were obtained from 50 water sources (tap and borehole water) while 24 *Klebsiella pneumoniae* isolates were obtained from 50 clinical samples (wound and catheter urine) (Table 1). These isolates were phenotypically characterized using standard Microbiological methods.

*P. aeruginosa* was more prevalent in borehole water (79.5 %) than in tap water (20.5 %) (Table 1). *K. pneumoniae* isolates were also more predominant in borehole water (66.7 %) than in tap water (33.3 %). This shows that borehole water is a larger reservoir for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* than tap water. Results also show that 24 *K. pneumoniae* isolates were obtained from the clinical samples (wound and catheter urine) while surprisingly, no *P. aeruginosa* was isolated in all the 50 clinical samples analyzed (Table 1). This study is in agreement with the work of some researchers. For example, *Klebsiella* is second only to *Escherichia coli* in nosocomial Gram-negative bacteremia (Yinon et al., 1996), as well as in urinary tract infections (UTIs), affecting catheterized patients (16 % and 70 %, respectively) (Niveditha et al., 2012). In fact, *K. pneumoniae* has been reported as a prominent cause of infections in individuals with indwelling urinary catheters (Ronald, 2002; Frank et al., 2009).

As concerns the bacteremia associated with intravascular catheters, an epidemiological study on bloodstream infections carried out in Israel revealed that *K. pneumoniae* was the second most common species (10 %), trailing *S. aureus* (30 %) (Siegmman-Ingra et al., 2000).

The *K. pneumoniae* isolates obtained from clinical samples (wound and catheter urine) were resistant to ceftotaxime (100 %), sulfamethoxazole (100 %), ceftazidime (100 %), followed by ceftriaxone (91.7 %) and gentamycin (50 %) being the least (Table 2). All the *K. pneumoniae* isolates were completely susceptible to imipenem (100 %) and this shows that imipenem is still a relevant antibiotic in the treatment of infections caused by the opportunistic pathogen, *Klebsiella pneumoniae* (Table 2).

In this study, the *Klebsiella pneumoniae* isolates obtained from water samples were found to be completely resistant (100 %) to all the antibiotics used except ofloxacin in which a measure of 2.3 % susceptibility was observed (Table 3). The *Pseudomonas aeruginosa* isolates were resistant to imipenem (100 %), tetracycline (100 %), cotrimoxazole (100 %), followed by cefotaxin (97.7 %), amoxicillin-clavulanic acid (95.4 %), meropenem (86 %), ofloxacin (86 %), aztreonam (79.1 %), ampicillin (76.7 %), ciprofloxacin (69.8 %) and chloramphenicol (65.1 %) being the least. Chloramphenicol and ciprofloxacin were the most effective antibiotics against *P. aeruginosa* with a susceptibility percentage value of 34.9 % and 30.2 % respectively (Table 3).

The biofilm forming potentials of *Klebsiella pneumoniae* isolated from the clinical samples (catheter urine and wound) of hospitalized patients were mild 11(48 %) moderate 4(20 %) and strong 5(25 %) (Table 4). Our study revealed that *Klebsiella pneumoniae* isolated from the clinical samples (catheter urine and wound) collected from Calabar General Hospital produced biofilms. The opportunistic pathogen, *K. pneumoniae*, can give rise to severe diseases, typically nosocomial infections, such as septicemia, pneumonia, UTI and soft tissue infection. *Klebsiella* infections are often considered as a paradigm of hospital-acquired infections. The indiscriminate use of antibiotics has revealed a considerable increase in outbreaks caused by microorganisms resistant to antimicrobial drugs.

Results show that biofilm production was highest in *Klebsiella pneumoniae* (100 %) than in *Pseudomonas aeruginosa* (84.1 %) in the isolates obtained from water samples (Table 5).

In contrast, starting from the early 1970s, *K. pneumoniae* epidemiology and its spectrum of infections significantly changed when this microorganism was established in the hospital environment and became a leading cause of nosocomial infections. In fact, its considerable efficiency of colonization, accompanied by acquired resistance to antibiotics, has enabled *K. pneumoniae* to persist and spread rapidly in healthcare settings. The most common healthcare-associated infections caused by this agent are those involving the urinary tract, wounds, lungs, abdominal cavity, intra-vascular devices, surgical sites, soft tissues and subsequent bacteremia (Gupta, 2002; Ko et al., 2002). In general, a cohort study indicated that the majority of infections associated with different medical devices; including both urinary and intravascular catheters, was caused by *K. pneumoniae*, and a high percentage (about 90 %) of biofilm-producing bacterial isolates causing infection were multidrug resistant (Singhai et al., 2012). Later, in-vitro studies have demonstrated that about 40 % of *K. pneumoniae* isolated not only from urine, but also from sputum, blood and wound swabs, were able to produce biofilm (Yang et al., 2008), as well as that about 63 % of *K. pneumoniae* isolates from urine samples of catheterized patients suffering from UTIs were positive for in-vitro biofilm production (Niveditha et al., 2012).

Alcántar-Curiel and colleagues demonstrated that, among the 69 examined *K. pneumoniae* isolates, 55 were able to produce biofilm. In the same year, Stickler and colleagues, by using a model of a catheterized bladder, firstly investigated the possible role of *K. pneumoniae*, *P. aeruginosa* and other uropathogens in the development of crystalline biofilm on catheter surfaces, demonstrating that these microorganisms are not able to raise the urinary pH and, thus, contribute to the crystalline biofilm formation (Stickler et al., 1998).

*Pseudomonas* and *Klebsiella* species as found in this study and other studies have been implicated in the contamination of water sources. The large number of bacteria isolated in the water samples used in this study contradicts the WHO guidelines for drinking water. Our study revealed that heavy growth of *P. aeruginosa* and *Klebsiella* spp were detected in all the water sources (tap and borehole water) (Table 1). This study is in agreement with the work of some researchers. For example, *Klebsiella* is second only to *Escherichia coli* in nosocomial Gram-negative bacteremia (Yinon et al., 1996), as well as in urinary tract infections (UTIs), affecting catheterized patients (16 % and 70 %, respectively) (Niveditha et al., 2012). In fact, *K. pneumoniae* has been reported as a prominent cause of infections in individuals with indwelling urinary catheters (Ronald, 2002; Frank et al., 2009).
samples. Therefore, by the WHO standard, 90% of all the drinking water sources tested are not really safe for human consumption. Eight K. pneumoniae and 44 P. aeruginosa isolates were obtained in the water samples (borehole and tap) and this is in conformity with the work of Janda and Abbott (2006), who observed the same bacteria in pipeline water samples. The prevalence of drug-resistant microorganisms poses a great challenge to clinicians and is a serious public health problem (Iroha et al., 2010). Antibiotic resistant bacteria are a cause for concern because of possible colonization of the gastrointestinal tract and conjugal transfer of antibiotic resistance to the normal flora leading to more multiple antibiotic resistant organisms (McKenze et al., 1995). The low susceptibility of Pseudomonas aeruginosa to antibiotics has become worrisome in our society today.

The presence of biofilm-producing P. aeruginosa and Klebsiella pneumoniae in the water and the clinical samples used in this present study is worrisome. Out of the 44 P. aeruginosa spp isolated from the water samples, 37 (84.1%) were biofilm producers, 7 (15.9%) were non biofilm producers while all the Klebsiella pneumoniae isolates were surprisingly biofilm producers. This study agrees with the study of Ashoka et al. (2015) who reported high prevalence of biofilm-producing Pseudomonas aeruginosa and Klebsiella pneumoniae from pipeline water. Most bacterial biofilms are responsible for several chronic diseases that are difficult to treat. Biofilm-producing P. aeruginosa and Klebsiella pneumoniae can cause chronic opportunistic infections, which are a serious problem for medical care in industrialized societies, especially for immunocompromised patients and the elderly. Although biofilms have been considered to be highly resistant to most antimicrobial agents and a lot of mechanisms have been proposed to explain the high resistance of biofilms to antimicrobial agents, including restricted penetration of antimicrobial agents into biofilms, slow growth owing to nutrient limitation; expression of genes involved in the general stress response, and emergence of a biofilm-specific phenotype.

Nosocomial Klebsiella and Pseudomonas infections continue to be a heavy burden on the economy and on the life expectancy of patients in developed and developing countries. Thus, further progress in the prevention of hospital-acquired infections will require new approaches to infection control.

Conclusion

Biofilm-producing organisms in drinking water systems and clinical samples can serve as a significant environmental and hospital reservoir for pathogenic microorganisms. The present study revealed very high prevalence of strong antibiotic resistant biofilm-producers (Pseudomonas aeruginosa and Klebsiella pneumoniae); indicating a very serious contamination and poor sanitary standard.

The increasing evidence on the ability of K. pneumoniae and P. aeruginosa to form biofilm, mostly on medical devices and the recent data supporting the correlation of such a behavior with the antibiotic resistance acquisition should alert even more regarding the hazard of these pathogens in hospital settings. The exploration of these virulence factors and the study of new mechanisms to control them could be an important way to counteract K. pneumoniae and P. aeruginosa infections. In particular, the biofilm mode of growth makes bacteria up to 1,000 times more resistant to antibiotic therapy.

However, the presence of P. aeruginosa and K. pneumoniae in both the tap and borehole water indicated that the water source contradicts WHO standard for potable water. Therefore, it is important to establish a comprehensive water safety plan to ensure that water from boreholes and tap is properly treated before use so as to prevent water-borne diseases. Many studies were performed on K. pneumoniae and P. aeruginosa in order to better highlight the mechanisms underlying this resistance; demonstrating that the limitation of the penetration of antibiotic molecules through the biofilm matrix is not the main reason for the increasing resistance, but rather the slow growth rate in the center of biofilm is the major reason (Donlan et al., 2001). In any case, other mechanisms are involved, and further studies are requested as a future challenge to elaborate new concepts in the preventive measures against biofilm-producing Klebsiella pneumoniae and Pseudomonas aeruginosa.

References


