CLINICAL SCIENCE

DETECTION OF BIOFILM PRODUCTION AND ANTIMICROBIAL SUSCEPTIBILITY IN CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII* AND *PSEUDOMONAS AERUGINOSA*

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Abstract

Acinetobacter baumannii and *Pseudomonas aeruginosa* are commensal which commonly colonize humans. As a result of their ubiquitous nature, reservoirs in hospital environment and resistance to many antimicrobial agents they are responsible for hospital – acquired infections. Additionally treatment of these infections is difficult because of the ability for biofilm formation. Aim of the paper was to determine the association between biofilm formation on medical devices and antibiotic resistance profile, compared to respiratory samples in clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Material and methods: The study comprised 50 clinical samples (36 from medical devices and 14 as a control group from respiratory secretions). *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were identified by routine microbiological methods. Modification of the microtiter plate assay described by Stepanovic et al. was used to investigate the formation of biofilm. The antimicrobial susceptibility testing was performed according to EUCAST guidelines. Results: Of the 50 analyzed strains, 16 (32%) were non-biofilm producers, and 34 (68%) were producing biofilms. Out of these, 29 (58%) were from medical devices, and 5 (10%) from the control group. Acinetobacter baumannii showed biofilm formation in 19 (67.9%), of which 17 (60.7%) from medical devices, and 2 (7.1%) from control group. *Pseudomonas aeruginosa* produced biofilm in 15 (68.1%), of which 12 (54.5%) from medical devices, and 3 (13.6%) from the control group. Multidrug resistance was detected in 40 (80%). All strains of *Acinetobacter baumannii* were multidrug resistant (MDR). For *Pseudomonas aeruginosa*, 11 (73.3%) biofilm forming isolates were MDR, and 1 (14.2%) non-biofilm forming isolate was MDR. Conclusion: Biofilm production was higher in strains from medical devices. Eighty percent of isolates were MDR. This is a serious challenge for treatment of these hospital-acquired infections.

КЛИНИЧКИ ИСТРАЖУВАЊА

ДЕТЕКЦИЈА НА СПОСОБНОСТА ЗА ФОРМИРАЊЕ БИОФИЛМ И АНТИМИКРОБНАТА ОСЕТЛИВОСТ НА КЛИНИЧКИТЕ ИЗОЛАТИ НА *ACINETOBACTER BAUMANNII* И *PSEUDOMONAS AERUGINOSA*

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Извадок

Acinetobacter baumannii и *Pseudomonas aeruginosa* се комензали кои нормално ги колонизираат луѓето. Нивната убиквитарна природа, присуството во хоспиталната средина како и резистенцијата кон голем број антимикробни средства ги прави одговорни за инфекциите кои се стекнуваат во болничка средина. Дополнително, третманот на овие инфекции е отежнат поради способноста за продукција на биофилм. Целта на оваа студија беше да се одреди способноста на *Acinetobacter baumannii* и *Pseudomonas aeruginosa* да продуцираат биофилм на медицински помагала и нивната резистенција кон антимикробни средства споредена со истите изолати изолирани од примероци од респираторен тракт. Материјал и методи: Примероци од 50 пациенти (36 од медицински помагала, 14 како контролна група од респираторни секрети). *Acinetobacter baumannii* и *Pseudomonas aeruginosa* беа идентификувани со рутински микробиолошки методи. Модификација на микротитарскиот метод опишан од Stepanovic et al. бешеупотребен за детекција на продукција на биофилм. Тестирањето за антимикробната осетливост беше препроведено според препораките на EUCAST (Европски комитет за тестирање на антимикробна осетливост). Резултати: Од 50 анализирани соеви, 16 (32%), не создадоа биофилм, додека, пак, 34 (68%) продуцираа биофилм. Од овие, 29 (58 %) беа од медицински помагала, а 5 (10%) од контролната група. Деветнаесет соеви на *Acinetobacter baumannii* (69.7%) имаа способност за продукција на биофилм. Од нив, 17 (60.7%) соеви потекнуваа од медицински помагала, а 2 (7.1%) од контролната група. Петнаесет соеви на *Pseudomonasaeruginosa* (68.1%) имаа способност за продукција на биофилм. Од нив, 12 (54.5%) соеви потекнуваа од медицински помагала, а 3 (13.6%) од контролната група. Мултирезистентност беше детектирана во вкупно 40 (80%) соеви. Сите соеви на *Acinetobacter baumannii*беа мултирезистентни. Кај *Pseudomonas aeruginosa*,11 (73.3%) соеви што продуцираа биофилм беа мултирезистентни, додека, пак, 1 (14.2%) сој без способност за продукција на биофилм, беше мултирезистентен. Заклучок: Создавањето на биофилм е повисоко кај соевите од медицинските помагала. Осумдесет и еден процент од изолатите беа мултирезистентни. Тоа е сериозен предизвик за третман на овие болнички стекнати инфекции.

Introduction

Acinetobacter and *Pseudomonas* are biochemically inert, opportunistic bacteria that are commensals which commonly colonize humans and animals. These Gram-negative bacteria have the ability to persist and multiply on abiotic surfaces.[1] Although they are ubiquitous in nature, *Acinetobacter* and *Pseudomonas* have a key role and are responsible for hospital-acquired infections, particularly in patients with compromised immune system.[2,3] The highest frequency of infections is found in the intensive care units (ICUs), in which they are most common causative agents of ventilator-associated pneumonia, catheter-associated-bacteremia, urinary tract infections or surgical site infections and are associated with high morbidity and mortality. The increased use of indwelling medical devices, such as central venous catheterization, mechanical ventilation and antimicrobial therapy are considered as crucial factors for upraise of these infections.[4,5]

Both bacteria are intrinsically resistant to many antibacterial agents, but also can acquire resistance to almost any antibiotic. Resistance mechanisms include production of beta lactamase, efflux pumps, or modification of specific target sites. Multidrug resistance (MDR) can occur as a result of single mechanism, or action of different mechanisms in a single isolate.[6] Extensively drug resistant (XDR) and pandrug resistant (PDR) strains are cumulating in various countries.[7] Additionally, treatment of these infections is difficult, because *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

have ability to form biofilms.[8]

Biofilms are defined as structured aggregate of bacterial cells, surrounded by extracellular polymeric substance (EPS), which they self - produce and are embedded in, attached to a biotic or abiotic surface. Bacteria in these biofilms are more protected from host immune response, antibiotics and adverse environmental conditions than the free-living planktonic cells. In this communities, bacteria are producing chemical signaling QR (quorum sensing) molecules for cell-to-cell communication during changes in the environment such as temperature, oxygen level, pH etc. Moreover, antibacterial agents when administrated below the minimum inhibitory concentration (MIC) act like stressors and induce biofilm formation.[8, 9]

Extracellular polymeric substance (EPS) is crucial element which provides attachment to surface, adhesion of cells and aggregation. It functions as a three-dimensional structure that enables unity, stability and protection from antimicrobial agents and immune system of the host.[10]

Bacteria in biofilms have a higher resistance against antibiotics than their planktonic cells. This is thought to be caused by different factors such as: the extracellular matrix which acts like a barrier and prevents penetration of the drug, different compounds in the matrix inactivating the drug, metabolic alterations in bacteria within the biofilm, a large number of bacteria compared to available antibiotic and close proximity of bacteria enables exchange of genetic material.[11]

Foreign body implants in vivo are coated with blood components such as collagen, fibrin, fibronectin, etc. which represents a higher risk factor for biofilm formation. Consequently, this results in severe hospital acquired-infections. These biofilms on medical devices with high resistance to antibacterial drugs and constant reinfections pose a huge danger for chronic infections, tissue damage and therapeutic failure.[11, 12]

According to the published data *Acinetobacter* and *Pseudomonas* are the most common nosocomial Gram-negative pathogens, which is contributed to their high resistance to the known antibiotics and persistence in hospital environment. This notorious persistence is attributed to formation of biofilm as one of the virulence factors of these bacteria and consequently causing device-related infections.[8, 13] In this study, we had aim to determine the rates of biofilm production and antibiotic resistance profile among the clinical strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in association with the origin of the sample.

Materials and methods

Our study comprised a total of 50 samples taken from hospitalized patients. Thirty-six of them were from medical devices: tip from central venous catheter, swabs from endotracheal and tracheostomy tubes, swabs from abdominal drain tubes, cerebrospinal liquor from ventricular shunt and hemoculture from catheter. Fourteen samples as a control group were from respiratory secretions (sputum, tracheal aspirate and bronchoalveolar lavage). All of the samples were derived from the University Clinical Center in Skopje and were submitted for routine laboratory testing at the Institute of Microbiology and parasitology, Faculty of Medicine.

Acinetobacter baumannii and *Pseudomonas aeruginosa* were initially identified using standard laboratory methods including growth on blood agar at 37°C, Gram stain and biochemical tests. Final identification was done with Vitek 2 system (Biomerieux, France).

After the identification, few colonies with identical morphology were stored in trypticase soy broth supplemented with 20 % glycerol at -80°C until further analysis.

The formation of biofilm was assessed with the method previously described by Stepanovic *et al*. with certain modifications.[14]

Bacterial cultures were refreshed from trypticase soy broth (after defreezing, one whole loop was inoculated on blood agar and incubated aerobically for 18 – 24 hours at 37°C). After verifying purity of the strain, few colonies were suspended in saline to acquire McFarland 0.5 suspension containing (~10 8 CFU/ml). This was performed using a photometric device. This blend was homogenized by vortexing for 1 minute.

Tissue culture plates (Laboglob, Germany) with 96 polystyrene, flat bottomed wells were filled with 180 μl trypticase soya broth (TSB) supplemented with 1% dextrose (medium for biofilm cultivation). From the previously prepared bacterial suspension 20 μl were added to each

well. Three wells were used for negative control and contained 200 µl TSB with 1% dextrose per well. Because phenotypic expression of biofilm formation is highly susceptible to different in vitro conditions, to minimize errors, each strain was tested in three wells (triplicate) and each test was carried out two times. The inoculated plates were covered with a lid and incubated aerobically 24 hours at 35 – 37°C under static conditions.

After these 24 hours, the supernatant containing the unattached bacterial cells was gently removed with a pipette and discarded and the plates were washed three times with 300 µl PBS (pH = 7.2) and then drained in an inverted position. Long fixation was done for 20 minutes with 150 µl methanol, thereafter the plates were emptied and left to air dry at room temperature.

To visualize the biofilm formed on each well, at the bottom and at the walls 150 µl crystal violet was used for 15 minutes. This is a cationic dye that stains negatively charged biofilm constituents based on ionic interactions. The stain was removed gently with micropipette and discarded, excess stain was removed with running tap water and the plates were air dried at room temperature. 150 µl 95% ethanol was added for 30 minutes at room temperature to resolubilize the dye from attached cells. Addition of alcohol enables indirect measurement of bacteria attached to the wells.

The absorbance of each well with ELISA microplate reader at 570 nm was measured. Average (mean) OD values were calculated for all tested strains and negative controls, because all tests were performed two times and in triplicate. The cut–off value (ODc) is defined as three standard deviations (SD) above the average OD of the negative controls. For each microtiter plate the cut–off value was determined.

The strains were divided in four categories for easier interpretation of the results, based upon previously calculated mean OD values: OD ≤ ODc = no biofilm formation; ODc < OD ≤ 2*ODc = weak biofilm formation; 2*ODc < OD ≤ 4* ODc = moderate biofilm formation; 4*ODC < OD = strong biofilm formation.

Standard disc diffusion method guidelines[15] by EUCAST (The European Committee on Antimicrobial Susceptibility Testing) were used to test susceptibility to frequently prescribed antibiotics which are active against these bacteria. Antibacterial categories included in the test were: beta–lactams, aminoglycosides, quinolones, sulfonamides and polymyxins. Commercial antibiotic discs (Oxoid, England) that were used for both bacteria were: piperacillin – tazobactam (PTZ, 36 µg), imipenem (IMI, 10 µl), meropenem (MER, 10 µg), ceftriaxone (CAZ, 10 µg), cefepime (FEP, 30 µg), amikacin (AM, 30 µg), ciprofloxacin (CIP, 5 µg). For *Pseudomonas aeruginosa* additionally were used: tobramycin (TB, 10 µg) and levofloxacin (LEV, 5 µg) and for *Acinetobacter baumannii*: ampicillin (AMP, 10 µg), amoxicillin – clavulanate (AMC, 30 µg), ertapenem (ETP, 10µg), cefuroxime (CXM, 30µg), ceftriaxone (CRO, 30µg), gentamicin (GM, 10 µg) and co-trimoxazole (SXT, 25 µg).

Muller Hinton agar (Oxoid) with antibiotic discs was incubated for 24

hours at 37°C and examined for inhibition zones.

Susceptibility for colistin was done with broth microdilution test (Merlin) to determine the MIC according to EUCAST.[16]

Statistical analysis

Biofilm detection by tissue culture plate method was graded as weak/none, moderate and strong. High and moderate production was considered positive and weak/none biofilm production was considered negative. Association of two or more set of variables was analyzed using the Chi – square test. A p value < 0.05 was considered as statistically significant. IBM SPSS version 28 was used for data analysis.

Results

A total number of 50 isolates were obtained. Bacteriological profile included *Acinetobacter baumannii* (n=28) and *Pseudomonas aeruginosa* (n=22). Thirty six were isolated from indwelling medical devices, *Acinetobacter baumannii* (n=21), *Pseudomonas aeruginosa* (n=15) and fourteen respiratory samples served as a control group, *Acinetobacter baumannii* (n=7) and *Pseudomonas aeruginosa* (n=7). In this study, strains were evaluated for their ability to pro-
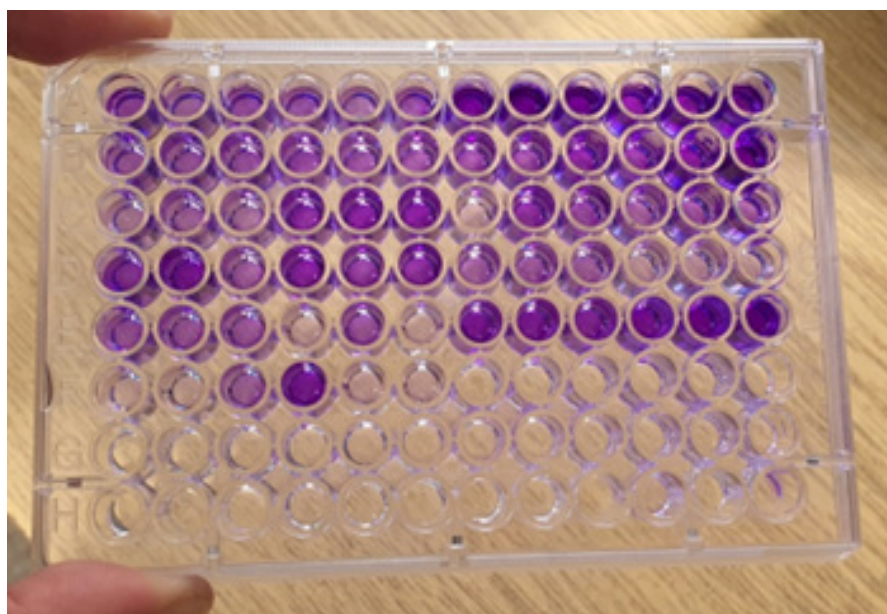
Fig 1. Biofilm formation by the TCP method

duce biofilms by the tissue culture plate method (TCP) Figure 1.

Of the 50 bacterial strains tested, 16 (32%) were non-biofilm producers while 34 (68%) produced biofilms. From those which were biofilm producers, 29 (58%) were from medical devices and 5 (10 %) from the control group.

From 28 isolates of *Acinetobacter baumannii*, biofilm production was detected in 19 (67.9%). In 15 (68.1%) isolates of *Pseudomonas aeruginosa* formation of biofilm was detected.

Out of these, 14 (28%) isolates showed a strong biofilm production, 20 (40%) moderate and 16 (32%) showed weak/ none production.

Acinetobacter baumannii isolated from medical devices showed biofilm formation in 17 (60.7%) strains and only 2 (7.1%) strains were producing biofilm from the control group.

Pseudomonas aeruginosa isolated from medical devices showed biofilm formation in 12 (54.5%) strains and in 3 (13.6%) strains from the control group. The rate of biofilm production between strains from medical devices and control group was found to be statistically significant. The Pearson's chi – square showed a strong statistical significance ($\chi2$ = 9.314; df = 1, p = 0.002), with p < 0.05 (Table 1).

Table 1. Detection of biofilm formation by Tissue culture plate method

Organism	Total number of isolates (N)	Number of BF* isolates by TCP† from medical devices N (%)	Number of BF* isolates by TCP† from control group N (%)
Acinetobacter baummanii	28	17 (60.7%)	2 (7.2%)
Pseudomonas aeruginosa	22	12 (54.5%)	3 (13.7%)
Total number of isolates	50		

*biofilm forming; †tissue culture plate method

Quantification of biofilm formation is shown in Table 2. Seven (25%) strains of Acinetobacter baumannii from indwelling medical devices were strong biofilm producers, 10 (35.7%) were moderate producers and 4 (14.2%) were weak/none biofilm producers. From the control group, there were no strong biofilm producers, 2 (7.1%) were moderate producers and 5 (17.8%) were weak/none biofilm producers.

Five (22.7%) strains of Pseudomonas aeruginosa isolated from medical devices were strong biofilm producers, 7 (31.8%) were moderate and 3 (13.6%) strains were weak/none biofilm producers. From the control group, 2 (9.09%) strains were strong biofilm producers, 1 (4.5%) was moderate and 4 (18.1%) strains were weak/none biofilm producers.

Table 2. Quantification of biofilm formation

| Biofilm formation | Isolates from medical devices | | Control group | |
	Acinetobacter baumannii N (%)	Pseudomonas aeruginosa N (%)	Acinetobacter baumannii N (%)	Pseudomonas aeruginosa N (%)
Strong	7 (33.3%)	5 (33.3%)	0	2 (28.5%)
Moderate	10 (47.6%)	7 (46.6%)	2 (28.5%)	1 (14.2%)
Weak/None	4 (19.04%)	3 (20%)	5 (71.4%)	4 (57.1%)
Total	21 (100%)	15 (100%)	7 (100%)	7 (100%)

All isolates of *Acinetobacter baumannii* (n=28,100%) were resistant to ampicillin, amoxicillin – clavulanate, piperacillin – tazobactam, ertapenem, imipenem, meropenem, cefuroxime, ceftazidime, ceftriaxone, cefepime. Seventeen (89.4%) strains isolated from medical devices showed resistance to gentamicin, 15 (78.9%) isolates showed resistance to trimethoprim – sulfamethoxazole and 1 (5.2%) isolate was resistant to colistin. Out of non–biofilm forming isolates, 8 (88.8%) were resistant to gentamicin and trimethoprim– sulfamethoxazole (Table 3).

Table 3. Antibiotic resistance pattern of biofilm forming and non–biofilm forming Acinetobacter baumannii isolates

Antibiotics	Resistance in BF* isolates Total N=19 (100%)	Resistance in NBF† isolates Total N=9 (100%)
Ampicillin	19 (100%)	9 (100%)
Amoxicillin– clavulanate	19 (100%)	9 (100%)
Piperacillin– tazobactam	19 (100%)	9 (100%)
Ertapenem	19 (100%)	9 (100%)
Imipenem	19 (100%)	9 (100%)
Meropenem	19 (100%)	9 (100%)
Cefuroxime	19 (100%)	9 (100%)
Ceftazidime	19 (100%)	9 (100%)
Ceftriaxone	19 (100%)	9 (100%)
Cefepime	19 (100%)	9 (100%)
Gentamicin	17 (89.4%)	8 (88.8%)
Amikacin	19 (100%)	9 (100%)
Ciprofloxacin	19 (100%)	9 (100%)
Trimethoprim– sulfamethoxazole	15 (78.9%)	8 (88.8%)
Colistin	1 (5.2%)	0

*biofilm forming; †non-biofilm forming

The antimicrobial resistance pattern of *Pseudomonas aeruginosa* isolates which were biofilm producing and non–biofilm producing, is shown in Table 4. A high resistance in biofilm forming isolates was detected against piperacillin–tazobactam 14 (93.3%), followed by cefepime 12 (80%), ceftazidime 11 (73.3%), meropenem, tobramycin, ciprofloxacin, levofloxacin 10 (66.6 %), amikacin 9 (60%), imipenem 6 (40%) and colistin 1 (6.6%). Non-biofilm producing isolates showed resistance to ciprofloxacin, levofloxacin, cefepime 4 (57.1%), piperacillin – tazobactam, imipenem, meropenem, cefepime 3 (42.8%), tobramycin 1 (14.2%).

Table 4. Antibiotic resistance pattern of biofilm forming and non–biofilm forming Pseudomonas aeruginosa isolates

Antibiotics	Resistance in BF* isolates Total N=15 (100%)	Resistance in NBF† isolates Total N=7 (100%)
Piperacillin tazobactam	14(93.3%)	3(42.8%)
Imipenem	6(40%)	3(42.8%)
Meropenem	10(66.6%)	3(42.8%)
Cefepime	12(80%)	3(42.8%)
Ceftazidime	11(73.3%)	4(57.1%)
Tobramycin	10(66.6%)	1(14.2%)
Amikacin	9(60%)	0
Ciprofloxacin	10(66.6%)	4(57.1%)
Levofloxacin	10(66.6%)	4(57.1%)
Colistin	1(6.6%)	0

*biofilm forming; †non-biofilm forming

Multidrug resistance (MDR) was seen in 40 (80%) isolates, of which 30 (88.2%) biofilm forming isolates and 10 (62.5%) non–biofilm producing isolates were MDR. All isolates of *Acinetobacter baumannii*, 19 (100%) of biofilm forming and 9 (100%) of non–biofilm forming, were MDR. Eleven (73.3%) of biofilm forming Pseudomonas aeruginosa isolates were MDR and 1 (14.2%) non–biofilm forming isolate was MDR. In P*seudomonas aeruginosa* isolates, there was a statistically significant difference in MDR in relation to biofilm production ($\chi2$= 6.712; df = 1; p = 0.001), with p< 0.05 (Table 5).

Table 5. Comparison of multidrug resistance among biofilm forming and non–biofilm forming isolates

Organism	Number of BF* isolates	BF* MDR†	Number of NBF‡ isolates	NBF‡ MDR† N (%)	p value
Acinetobacter baumannii	19	19 (100%)	9	9 (100%)	/
Pseudomonas aeruginosa	15	11 (73.3%)	7	1 (14.2%)	0.001
Total	34	30 (88.2%)	16	10 (62.5%)	

*biofilm forming; †multu–drug resistant; ‡non-biofilm forming

Discussion

In our study out of the 50 isolates, 29 (58%) from medical devices and 5 (10%) from the control group, had the ability to form biofilm. Our results showed that there was a statistically significant difference in biofilm formation in association with origin of the sample (p = 0.002). Analysis of the antibiotic susceptibility of all isolated strains allowed classifying the bacteria in MDR and non–MDR strains. Our data detected 80% and 20% of MDR and non–MDR strains, respectively. Thirty MDR strains (88.2%) were biofilm forming and ten MDR strains (62.5%) were non–biofilm forming. All isolates (n=28) of *Acinetobacter baummannii* were MDR with no difference between biofilm forming and non – biofilm forming, but there was a statistically significant difference in *Pseudomonas aeruginosa* strains which were MDR in association with biofilm formation (p = 0.001).

In the present study, biofilm production in isolates of *Acinetobacter baumannii* from medical devices was observed in 17/21 (60.7%), out of which 25% were strong and 35.7% were moderate producers. In 2/7 (28.5%) isolates from the control group moderate biofilm formation was detected. This result shows that strains from other microbiological samples have significantly lower ability for biofilm production compared to strains from medical devices. This was supported by the results obtained by Revdiwala *et al.*[17] who noted 17/23 (73.9%) biofilm forming isolates from inserted devices, 69.5% were moderate and 4.3% strong producers, while only in 1 isolate from non-ventilator associated respiratory tract infection biofilm formation was detected by Rodriguez et *al.*[18] On the other hand, Lee HW et *al.*[19] in their study demonstrated that all 23 clinical isolates of *Acinetobacter baumanni* had the ability to form biofilm.

In our study, *Pseudomonas aeruginosa* isolates from medical devices had the ability for biofilm formation in 12/15 (54.5%), 5 (33.3%) strong and 7 (46.6%) moderate producers. In 3/7 (13.7%) isolates from the control group, 2 (28.5%) were strong producers from patients with cystic fibrosis and 1 (14.2%) isolate was a moderate producer. These results were in agreement of those presented by Danin PE et *al.*[20] who demonstrated

that from 22 biofilm forming isolates, the most frequent organism found in endotracheal tubes was Pseudomonas aeruginosa (58.3%). Diez–Aguilar M et al.[21] in their study demonstrated that from 53 respiratory samples from patients with cystic fibrosis, 32 (60.4%) were biofilm producers.

All *Acinetobacter baumannii* strains in our study were resistant to commonly prescribed antibiotics (100%), except in biofilm forming isolates where two were sensitive to gentamicin and four to trimethoprim sulfamethoxazole. One (5.2%) isolate was resistant to all antibacterial categories including colistin. In contrast to this, the study by Eze EC et *al.*[22] noted resistance of 17% to all antibiotics in all categories. In non–biofilm forming isolates, one isolate was sensitive to gentamicin and one to trimethoprim sulfamethoxazole. This susceptibility pattern was supported by Konca K et al.[23] who analyzed the antimicrobial susceptibility in MDR *Acinetobacter baumannii* strains. Resistance to colistin was 2.2% and to trimethoprim – sulfamethoxazole 73.9%, so these antibiotics are therapy choice.

For biofilm forming isolates of *Pseudomonas aeruginosa*, high resistance was detected for beta-lactams, 93.3%, 80% and 73.3% for piperacillin–tazobactam, cefepime and ceftazidime respectively, followed by fluoroquinolones 66.6%. This is in contrast with the results obtained by Folliero V et al.[24] who noted, based on the sensitivity level of biofilm forming isolates, that fluoroquinolones were a potential treatment for these infections. Low resistance was noted for amikacin (60 %). In our study, one strain (6.6%) was resistant to colistin. Non–biofilm forming isolates showed resistance to fluoroquinolones with 57.1%, followed by beta-lactams with 42.8%. Low resistance of 14.2% for tobramycin and all strains sensitive to amikacin shows that these antibiotics are therapy choice in patients without cystic fibrosis which has been supported by Tanriverdi E et al.[25] They found that treatment with inhalation of tobramycin decreased hospitalization rates and improved the symptoms.

In the current study, all isolates (n=28) of *Acinetobacter baummannii* were MDR with no difference between biofilm forming and non–biofilm forming. As for Pseudomonas aeruginosa, 11/15 (73.3%) biofilm forming isolates were MDR and only 1/7 (14.2%) non–biofilm forming strain was MDR. This coincided with the results of Abidi SH et al.[26] who showed that production of biofilm was higher among MDR *Pseudomonas aeruginosa* strains than in non-MDR strains.

We documented a high rate of biofilm production in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolated from medical devices. These bacteria as hospital pathogens are responsible for chronic and multidrug resistant infections. This represents a serious challenge to clinicians in the treatment and care of these patients. Antibiotics belonging to the class of polymyxin E (colistin) and in small percentages aminoglycosides and trimethoprim – sulfamethoxazole, are effective for biofilm producing strains.

Conclusion

This study was based only on phenotypic method for biofilm detection. This is a simple, reliable, accurate

method and can be utilized for biofilm screening. Future studies with molecular methods should identify genes responsible for biofilm production and resistance to antimicrobial agents.

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