International Journal of Sciences & Applied Research

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Biofilm production in multidrug resistant *Acinetobacter baumanii*

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Abstract

Acinetobacter baumanii (A. baumanii) is an important nosocomial pathogen causing colonization and infection of critically ill, hospitalized patients. Persistence of multidrug resistance (MDR) *A. baumannii* in hospital environment together with ability to form biofilm added to the virulence of the organism. This guided us to detect biofilm production and its association with MDR among the different clinical isolates of A. baumannii. During the present study, 68 clinical strains of A. baumannii, isolated from hospitalized patients, were examined for biofilm formation. Most (98.5%) of the clinical isolates were biofilm producers especially from ICU samples and they were multidrug resistant, even polymixin resistant isolates are slowly emerging. Furthermore, no relation was found between the ability of biofilm production and antibiotic resistance pattern.

Keywords: Acinetobacter baumannii - MDR- biofilm

Introduction

Acinetobacter baumanii (A. baumanii) is now among the most important nosocomial pathogens, because of its increasing isolation in clinical settings, causing a wide range of infections (bloodstream and urinary tract infections, ventilator-associated pneumonia, wound, skin and soft-tissue infections) often associated with high morbidity and mortality rates (Karakoc et al., 2013). It ranks second only to *P. aeruginosa* among the nosocomial, aerobic, nonfermentative, gram negative bacilli pathogen (Rao et al., 2008) and third among the causative agents infections in intensive care units (Martí et al., 2011).

Infections of hospitalized patients with *Acinetobacter spp*., are often preceded by colonization and 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. baumanii* clinical isolates (McQueary and Actis, 2011).

The *A. baumanii* multi-drug resistant (MDR) phenotype seems to play an important role in the remarkable capacity to persist and spread in the hospital environment, together with its ability to colonize both biotic and abiotic surfaces (Gurung et al., 2013). Regarding its interaction with host cells, pili-mediated adherence to epithelial cells is considered the initial step for colonization and subsequent host infection (Lee et al., 2006). Its ability to form biofilm on abiotic surfaces plays an important role in causing nosocomial infections, due to the surface

colonization of hospital equipment and indwelling medical devices, such as urinary catheters, central venous catheters , endotracheal tubes, etc. (Djeribi et al., 2012). Because of the presence of dormant cells, it can survive for a long time under desiccated conditions (Gayoso et al., 2014).

Espinal et al. (2012) confirmed the fact that isolates which produce biofilms survive longer than non-biofilm formers on dry surfaces. So, it is becoming evident that biofilm-forming ability can be considered one of the main virulence factors of *A. baumanii* clinical isolates (Rodriguez-Bano et al., 2008; Gurung et al., 2013). Several studies have demonstrated a significant association of biofilms with multiple drug resistance and device-associated infections (Rao et al., 2008; Rodríguez-Baño et al., 2008).

The objective of our study was to detect biofilm production and its association with MDR among the different clinical isolates of *A. baumannii* in our hospital.

Materials and methods

1. Bacterial isolates:

In the current study, isolates of *A. baumannii* were obtained from Al-Noor specialist hospital, Microbiology Laboratory, Makkah, KSA, from various clinical samples between March and July 2014. Clinical data were collected including; sex, age, type of specimen as well as departments involved.

2. Confirming the identity of *A. baumannii* strains:

Verifying the identity was conducted using colony morphology, microscopic examination and oxidase test. Oxidase-negative Gram-negative bacilli were further identified by Microscan (Siemens Microscan WalkAway system, Germany) to separate *A. baumannii*.

3. Antimicrobial susceptibility tests:

Minimal inhibitory concentration (MIC) test: was done using Microscan (Siemens Microscan WalkAway system, Germany).

4. Biofilm formation

Biofilm formation was determined as follows. Overnight cultures were diluted to 0.5 McFerland using Brain Heart Infusion broth as a growth medium (Oxoid, Madrid, Spain), deposited in 96-well plates and incubated at 37^{o}C for 48 h without shaking. Biofilm was washed well twice using saline, stained with 0.4% Crystal Violet (w/v) and quantified at 590 nm after solubilization with 95% ethanol. The experiment was performed in triplicates. OD590 values for each well were subtracted from those of the blank, which only contained Brain Heart Infusion broth without inoculum (Stepanovic et al., 2007; Yanti et al., 2009). All samples were tested in triplicates.

5. Biofilm calculation: The optical density (ODs) of each strain was obtained by the arithmetic mean of the absorbance of three wells and this value was compared with the mean absorbance of negative controls (ODnc). The following classification was used for the determination of biofilm formation: no biofilm production (ODs=ODnc), weak biofilm production (ODnc<ODs≥2.ODnc), moderate biofilm production (2.ODnc<ODs≥4.ODnc) and strong biofilm production (4ODnc<ODs) (Rodrigues et al., 2010).

Results

A total number of 68 *A. baumannii* isolates were included in the study; all isolates were obtained from clinical samples received from intensive care unit (ICU), medical wards and surgical wards. The study included 56 males and 12 females with age ranged from 5 to 105 years old. Thirty-sex isolates (53%) were from ICU while 32 isolates (47%) from medical wards and surgical wards (non ICU) (**Table 1**).

Table 1: Relation between biofilm production of isolates and ward

Ward	No. & % of isolates (68)	Neg.	Weak	Moderate
ICU	36 (53%)	0%	11 (31%)	25 (69%)
Non ICU	32 (47%)	1 (3%)	11 (34%)	20 (63%)

The highest isolation rates of *A. baumannii* was from wound swap (37%) followed by sputum (35%), then blood culture (12%), body fluid (6%), urine (4%), IV tip catheter (4%) and culture of tissue (1%).

Ninety-seven (97%) of *A. baumannii* isolates from different clinical samples were resistant to Imipenem, (96%) resistant to Meropenem, (94%) to Ciprofloxacin, Mezlocillin, (93%) to Cefepime, Cefotaxime, ceftazidime, Levofloxacin, Piperacillin, Tetracycline, (91%) to Amikacin, (88%) to Gentamicin, Tobramicin. While only (59%) of isolates were resistant to Trimethoprim /sulphamethoxazole and (7%) to Colistin. Results are shown in the Figure 1.

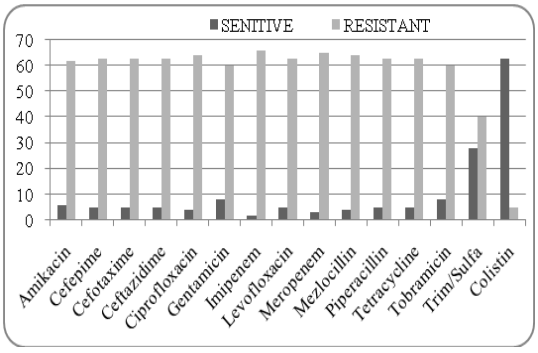

Fig. 1: The antibiotic sensitivity patterns of *A. baumanii* isolates.

Our study showed that only one isolate (1.5%) was negative for biofilm production, while (98.5%) of isolates were biofilm producers as follow: 22 (32.5%) isolates were weak biofilm producers and 45 (66%) were moderate biofilm producers (**Fig. 2**). Higher rate of biofilm production (moderate biofilm producers) was found in patients in ICU (69%) than those in non ICU (63%) (**Table 1**).

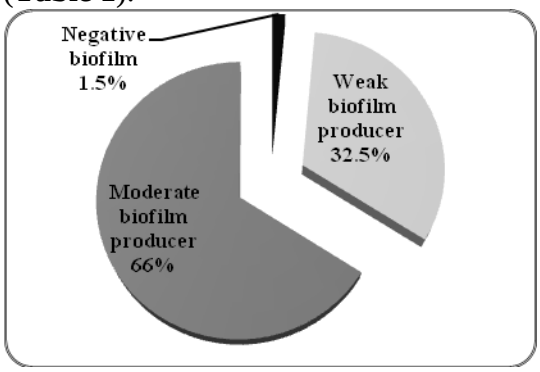

Fig. 2: Distribution of isolates according to biofilm production.

A. baumanii isolates showed higher biofilm production (moderate biofilm producers) in IV tip catheter 100%, tissue 100%, blood 75% and sputum 75%, urine (67%), wound (52%) and body fluid (50%) (**Fig. 3**).

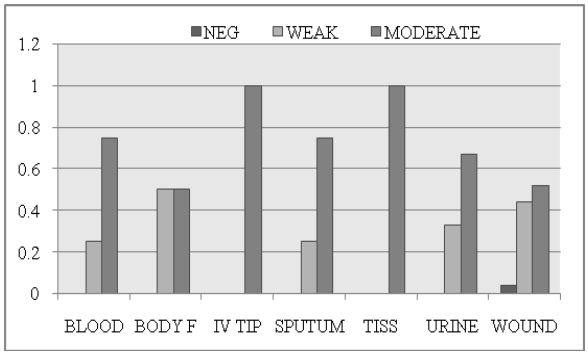

Fig. 3: Relation between biofilm production of isolates and type of clinical sample.

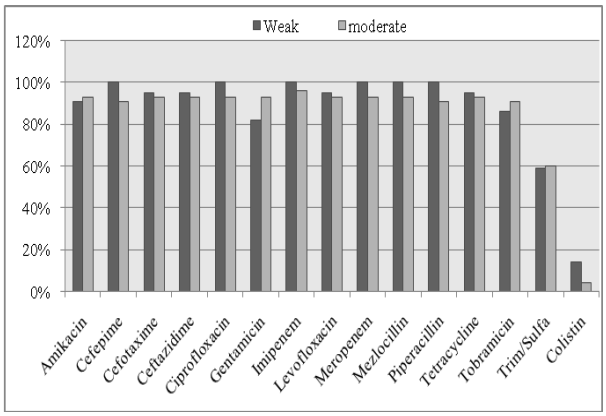

Fig. 4: The antibiotic resistance patterns of biofilm producing *A. baumanii* isolates.

Both weak and moderate biofilm producing *A. baumanii* isolates showed similar antibiotic resistance patterns. Results are shown in the **Figure 4**.

Discussion

Adhesiveness and biofilm-forming ability in *A. baumannii* play a pivotal role in the host-pathogen interactions and in medical device-associated infections (Longo et al., 2014). Mechanisms responsible for antimicrobial resistance in organisms producing biofilms may be delayed penetration of the antimicrobial agents through the biofilm matrix, altered growth rate of biofilm organisms, and other physiological changes due to the biofilm mode of growth (Donlan and Costerton, 2002). Thus, the ability to form biofilm could be an effective strategy to enhance the survival and persistence under stressed conditions like host invasion or following antibiotic treatment (Rao et al., 2008).

This study was done on 68 *A. baumannii* isolates from different clinical samples over the period of 5 months to detect biofilm production and its association with MDR among the different clinical isolates in our hospital. The majority of the study group were males with age ranged from 5 to 105 years old. More than half of isolates (53%) were from ICU while the remaining were from medical wards and surgical wards (non ICU).The highest isolation rates of *A. baumannii* was from wound swap (37%) followed by sputum (35%), then blood culture (12%), body fluid (6%), urine (4%), IV tip catheter (4%) and culture of tissue (1%).

All isolates were MDR with high resistance pattern to all antibiotics except for colistin. The highest resistance rate was for Imipenem and meropenem (97)%, (96%) respectively. This resistance to carbapenems is most often mediated by oxacillinases (OXAs) and less frequently by metallo-b-actamases (MBLs) (Azimi et al., 2013). Resistance pattern for colistin was 7 % which was similar to Nahar et al. (2013). However, in our study this was distributed in ICU and non ICU patients. In their study it was distributed only in ICU isolates, while Non ICU isolates are 100 % sensitive.

Almost all isolates (98.5%) were biofilm producer. Our results was greater than that of Rao et al. (2008) & Rodriguez-Bano et al. (2008) & Martí et al. (2011) as they showed (62-63%) biofilm production and to that of Dheepa et al. (2011) (60%) biofilm production and Gurung et al. (2013) (50%). While, they were similar to that of M'hamedi et al. (2014) where all the isolates were biofilm producers (100%). They used Semi-quantitative evaluation of biofilm formation using Micro-titer plate method as in our study. M'hamedi et al. (2014) added that biofilm formation was 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 (Martí et al., 2011).

This difference can be explained that Rodriguez-Bano et al. (2008) and Martí et al. (2011) considered cut off value for biofilm forming as 3(SD) above the mean ODc of the negative control. Rao et al. (2008) and Dheepa et al. (2011) considered weakly adherent isolates along with non adherent strain as non-biofilm producers and Gurung et al. (2013) used qualitative Test tube method.

Sixty six (66%) of our isolates showed moderate biofilm formation and 32.5% showed weak biofilm formation and no isolates showed strong biofilm formation. This can be explained that the ability to adhere varies among different *A. baumannii* clinical isolates (Lee et al., 2006). They exhibited great variability in biofilm formation, which could be classified into high-, medium- and low-producers. These findings were in agreement with other reports (de Breij et al., 2006).

Moderate biofilm producing A. baumanii was found little more (69%) in patients in ICU than those in non ICU (63%) and the

remaining was weak biofilm former. Higher rate of biofilm production was found in patients on device in ICU according to Nahar, et al. (2013) study who showed that biofilm plays a role in the pathogenesis of some device-associated *Acinetobacter* infections. While this percentage was far from their results as biofilm forming capacity of *Acinetobacter* species was significantly higher in ICU (87.5%) than Non ICU isolates (55.0%). This may be due to difference in cut off value for biofilm formation, as they considered (3SD) above the mean ODc of the negative control.

A. baumanii showed higher biofilm production (moderate biofilm producers) in IV tip catheter (100%), tissue (100%), blood (75%) and sputum (75%), urine (67%), wound (52%) and body fluid (50%). Our results were the same as Cevahir et al. (2008) results regarding blood (75%) and body fluid (50%) and near their result regarding urine (75%), while far from theirs regarding in wound swab (80%) and in sputum (50%). Although it was determined that biofilm-producing isolates were more commonly collected from wound compared to urine and blood (Sanchez et al., 2013), our results regarding wound swab were the same as Nahar et al. (2013) result of non ICU *Acinetobacter* isolates (50%).

This difference in result can be explained by difference in geographical distribution, number of isolates tested in each study, difference in cut off value considered for biofilm production and by Wroblewska et al. (2008) observations who found no correlation between the ability of biofilm formation and site of isolation of A. baumannii.

Both weak and moderate biofilm producing A. baumanii isolates showed similar antibiotic resistance patterns. This was in accordance to Wroblewska et al. (2008) who found no correlation between the ability of biofilm formation and carbapenem resistance. While was in contrast to Dheepa et al. (2011) and Gurung et al. (2013) studies that showed that antibiotic resistance was significantly higher among biofilm producing *A. baumannii* than non-producer. This can be explained that Dheepa et al. (2011) considered weakly adherent isolates along with non adherent strain as non-biofilm producers, and Gurung et al. (2013) used qualitative Test tube method for detection of biofilm, while we used quantitative Microtitre-plate method that has been widely reported in the literature and is considered to be the de facto gold standard (Cremet et al., 2013).

Lee et al. (2008) showed in their study that as a consequence of biofilm production, ability of *Acinetobacter* spp. to transfer genes horizontally might also enhance within these micro-communities and facilitate the spread of antimicrobial resistance. However, following antibiotic policy such as early aggressive antibiotic prophylaxis or therapy and chronic suppressive therapy reduces biofilm production in device related infections. Also, Novel treatment strategies such as phage therapy, quorum-sensing inhibition, and induced biofilm-dispersion have been documented (Hoiby et al., 2010).

Conclusion

In this study we found high percentage of biofilm production along with high antibiotic resistance pattern to penicillin, cephalosporin, aminoglycosides, quinolone and carbapenem group of drugs. Finding (7%) polymixin resistant *A. baumannii* is alarming. Moderate biofilm producer were more than weak biofilm producers and were more in ICU than non ICU isolates. More found in IV tip catheter, tissue, blood, sputum and urine than weak biofilm producers and nearly equal in wound and body fluid. No difference was observed between weak and moderate biofilm producing isolates regarding antibiotic sensitivity. So, no relation was found between biofilm formation and antibiotic resistance pattern. We recommend that the

percentage of biofilm production seen in individual hospital along with their antibiotic susceptibility pattern should be done to formulate hospital antibiotic policy. Also, early aggressive antibiotic therapy and chronic suppressive therapy should be followed to reduce biofilm production

Acknowledgments
Our grateful for Dr. Abdulrahman El-Sawy for his support throughout the research.

Conflict of interest
None

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