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RESEARCH ARTICLE

Characterization of Biofilm Production and Quorum Sensing Phenomenon among Antibiotic Resistant Acinetobacter Baumannii isolated From Wound in Fections in Iraq

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Abstract

Objective(s): Acinetobacter baumannii has emerged as a significant hospital pathogen, quickly becoming resistant to wide range of antimicrobials. It has gained notoriety as a cause of debilitating soft tissue infections in soldiers returning from Iraq and Afghanistan. This study focused on the characterization of biofilm production in Iraqi A. baumannii and its regulation by quorum sensing. Material and Methods: twenty four isolates were collected from Baghdad and Al-Najaf hospitals and re-diagnosed by compact Vitek 2 and genetically using housekeeping gene (*rec A*). Phenotypic detection of biofilm formation was screened by microtiter dish assay, twitching motility assay, and SEM. Quorum sensing phenomenon (QS) was detected using conventional PCR.Results:100% of isolates formed either strong or weak biofilm by microtiter dish assay. Twitching motility test revealed that 79.2% of isolates were motile. SEM analysis showed 79.2%ofisolatesmade different stages of biofilm started with adhesion step and ending with a mushroom like architecture as highly magnification images showed on glass cover slips. PCR analysis of QS showed that91.7% of *A. baumannii* harbored *AHL* gene encoding N-acyl homoserine lactone hydrolase at amplified size 498bp, while 83.3% of *A.* baumanni*i* isolates could harbor aba I gene encoding N-acyl homoserine lactone synthase at amplified size382bp. Conclusion: it could be concluded that *A.* baumannii isolates were capable to produce biofilm and control it using quorum sensing genes expression. This would raise the concern of dissemination of chronic infections among hospitalized patients in two cities: Baghdad and Al-Najaf.

Keywords: *Biofilm, Twitching Motiltiy, Scanning electron microscopy, Quorum sensing.*

Introduction

Biofilm is a microbially derived sessile community which is characterized by cells that are irreversibly attached to a substratum and embedded in a matrix of self-produced extracellular polymeric substances (EPS) (1).It can be divided into distinct stages, from the initial attachment of bacteria to the surface until the formation of mature biofilm with a characteristic of three-dimensional architecture (2).

Adaptation to surface attached growth within a biofilm is accompanied by significant changes in gene and protein expression, as well as metabolic activity which confers resistance to antimicrobial therapy and host mechanisms of clearance **(**1). Bacterial functions such as motility, adhesion, transport, stress response, activation of metabolic pathways and EPS synthesis are required at each stage (2) and usually regulated by quorum sensing. Through this phenomenon, bacteria communicate with each other using chemo tactic particles or pheromones (3), allowing the bacteria to switch between plank tonic phenotype to high cell density biofilm phenotype (4). Recent studies have begun to integrate QS into global regulatory networks and establish its role in

developing and maintaining the structure of bacterial communities (4). Pathogenic bacteria typically use QS in the regulation of genes encoding extracellular virulence factors (5). It has revealed that biofilm producer Acinetobacterbaumannii, a gram-negative cocobacilli with a GC content of 38-47% (6), were more resistant to aminoglycosides, carbepenems, tetracyclines, and sulfonamides compared to non-biofilm producers. This would link to its survival in the hospital environment and to infections associated with medical devices (7), resulting in an important public health problem (8).

In our study, we focus on the characterization of biofilm production among local Iraqi A. baumanniithat were tested for their antibiotic resistant pattern in our previous work (9) using different phenotypic biofilm assays including scanning electron microscopy (SEM), and also screen about quorum sensing genes among mentioned isolates by Conventional PCR.

Material and Methods

Collection and Diagnosis of Bacterial Isolates

Twenty four isolates of *A.* baumannii were obtained from patients having wound infections and admitted to several teaching hospitals in Baghdad and AL- Najaf during a period between March and September 2015. Bacterial diagnosis was performed depending on rapid morphological tests on CHROM agar Orientation followed by identification at the species level using complementary Vitek2 compact automated system (Biomeriux, USA).Isolates were also identified by PCR using housekeeping gene (*rec A*) at the amplified size of 425bp from the origin gene sequence F-CCTGAATCTTCYGGTAAAAC; R-GTTTCTGGGCTGCCAAACATTAC. The primer was designed using Geneious Software/ primer 3.

Phenotypic Detection of Biofilm Production

Microtiter Dish assay Biofilm formation in 96-well microtiter plates was assayed and quantified as previously described by O'Toole (10). All biofilm assays were performed using M63 minimal medium supplemented with glucose, $MgSO_4$, and CAA. Isolates were grown overnight in LB broth at 37°C, the 96-well plate(s) were prepared for the assay. Each isolate suspension was diluted (1:50) into an aliquot of the Biofilm media (M63) and mixed well by swirling and pipetting up and down. Standard strains Pseudomonas PA14 and Pseudomonas ΔPel A mutant were also undergone this step and used as positive and negative control, respectively.

The wells were inoculated (at least 4 wells per each isolate) of the 96-well plate (100µl/well) from the isolate mixture using a multi-channel pipette. The 96-well plate was covered with a lid and incubated at (37°C) for up to 24 hours. After the incubation period, the wells were rinsed twice with distilled water to remove the unattached bacteria. Subsequently, 125 µl of Crystal violate stain (at 0.1% concentration) was added to each well and the control well. The plate was let sit to 10-15 min. The plates were then rinsed twice with distilled water and dried on paper towels. Biofilm formed for each isolate was quantified by measuring their absorbance at 550nm using ELISA system.

Quantify absorbance at 550 nm was read for the triplicate wells using 30% acetic acid in water as the blank. Since there is no universally recognized reference value used for evaluating biofilm formation capacity, in current study, isolates with OD_{550} values greater than that of the negative control were considered positive for biofilm formation. Specifically, those with OD_{550} values greater than that of the negative control, but less than that of the positive control were characterized as weak biofilm formers, while those with OD_{550} values greater than that of positive control were considered strong biofilm formers.

Twitching Motility Assay

Each isolate was tested for its twitching motility character according to (11). Twitch motility plates consisted of M63 medium supplemented with $MgSO_4$, glucose (20%), CAA (20%), and solidified with 1.5% agar-agar. Isolates were stab inoculated with a toothpick through a thin (approximately 3 mm) LB agar layer supplemented with M63

to the bottom of the Petri dish and incubated at 37ºC for 24 hours. After incubation, the

zone of twitching motility between the agar and Petri dish interface was visualized by staining with crystal violet.

Results interpretation was dependent on categorizing these isolates into three groups according to positive (Pseudomonas PA14) and negative (Pseudomonas Δ Pel A mutant) control. If the twitching zone diameter was <5 mm, the isolate is considered as twitching negative. A twitching zone diameter between 5 and 20 mm is considered as an intermediate while >20 mm of twitching zone was considered as a highly motile isolate.

Scanning Electron Microscopy SEM

A sterile cover slip was placed inside LB broth tubes cultured with overnight bacteria. The tubes were incubated horizontally at 37˚C/24 hrs, and then isolates were left for more than one month at room temperature in order to let a pellet of colonies to be formed. Isolates samples were prepared for testing by SEM according to the following steps provided by (12). Each cover slip having biofilm formation appearance was removed from LB broth tube very carefully using sterilized forceps.

Fixation step was done for the cover slip using a chemical fixative like glutaraldehyde (2.5%). The cover slips were incubated at room temperature for approximately 2 hours. The fixative agent was then removed and replaced immediately with distilled water to prevent sample dehydration. Cover slips were then coated with gold/ palladium (Au/Pd) Ratio (80:20) (57mm diameter x 0.1mm thick) using a sputter coater, plasma chambers with low discharge. Clear view of biofilm stages were conducted using a conventional scanning electron microscope (SEM) at a magnification from 5µm to 2mm.

Results

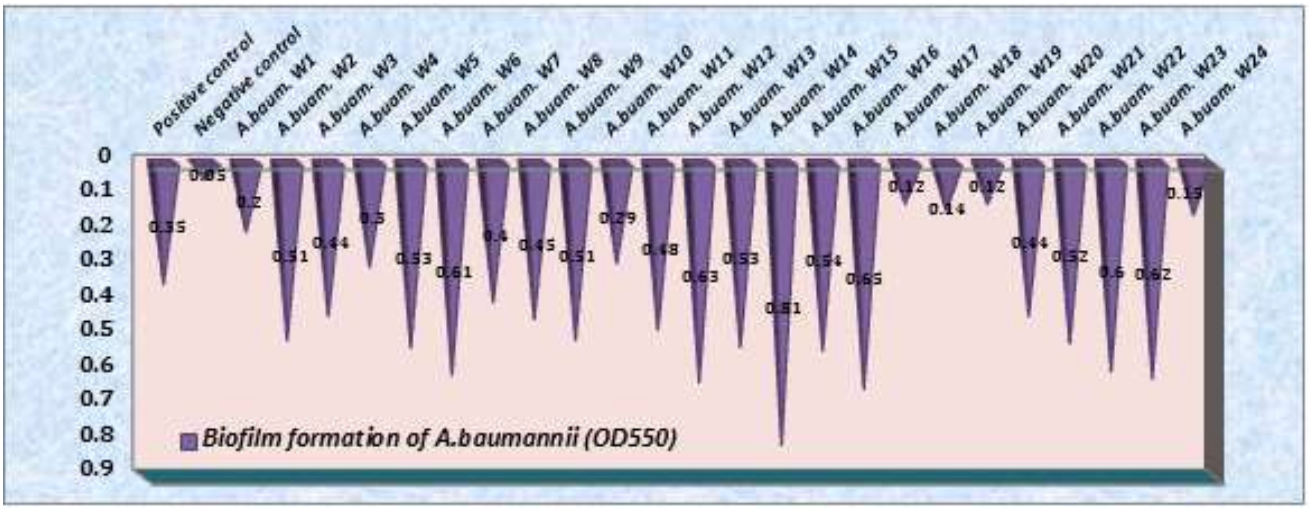
Identification of local isolates

A. baumannii isolates exhibited on CHROM agar Orientation white nontransparent colonies with rod shaped within 24 hr, and they were *A. baumannii*at 99% level by Vitek 2 compact automated system. Results of genotypic detection of housekeeping generic *A*demonstrated that 100% of isolates had*rec A* gene with amplified size of 425bp.

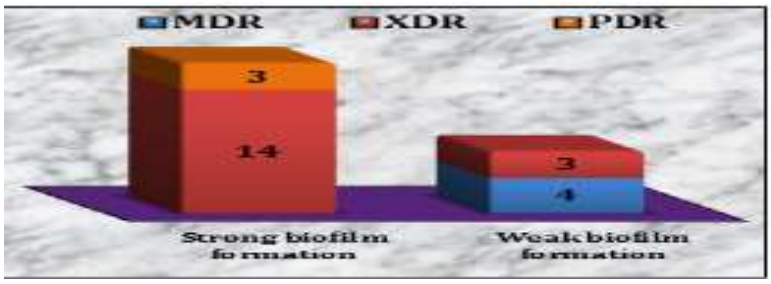
Microtiter Dish assay

Results of current study were compared with *Pseudomonas* P14 (positive control; OD 0.35) and *Pseudomonas* ΔPel A mutant (negative control; OD 0.051) to be classified into either strong or weak biofilm producers using ELISA system at OD 550nm. Reading data revealed that all *A. baumannii* isolates (100%) were biofilm producers. Interestingly, the three PDR *A. baumannii* isolates gave highest OD measure, indicating strong biofilm formation ability.

It could be seen in figure (1-1A) that PDR *A. baumannii* W14 had (OD≥0.81) compared with other isolates. Most extremely drug resistant *A. baumannii* isolates were strongly biofilm producers except for XDR *A. baumannii* W1, W4, and W19 which produce weak biofilm. In contrast to all multidrug resistant *A. baumannii*that produced biofilm weakly (figure 1-1B).



(A)



(B)

Figure (1-1): a) Average of three measures at OD550 conducted by ELISA system for 24 *A. baumannii* isolates compared with positive (*Pseudomonas* P14) and negative control (*Pseudomonas* ΔPel A mutant). b) Classifying the MDR, XDR, and PDA *A. baumannii* isolates into weak or strong biofilm producers.

Twitching Motility pattern

Results showed that 19/24 (79.2%) of *A. baumannii* isolates gave positive results from which 54% of isolates were highly motile isolates that exhibited wide zone of migration more than 20mm after incubation period; while 25% of isolates showed intermediate migration zone of 5-20mm in diameter (figure 1-2).

It was found that PDR *A. baumannii* W14 displayed 30 mm migration zone compared with other PDR isolates (W6 and W23) that exhibited 25 and 29mm, respectively. Also, five *A. baumannii* isolates including (MDR W24 and XDR W1, W4, W19, and W20) were considered as weak biofilm producer in previous test, and they exhibited no twitching motility pattern on M63 medium plate.

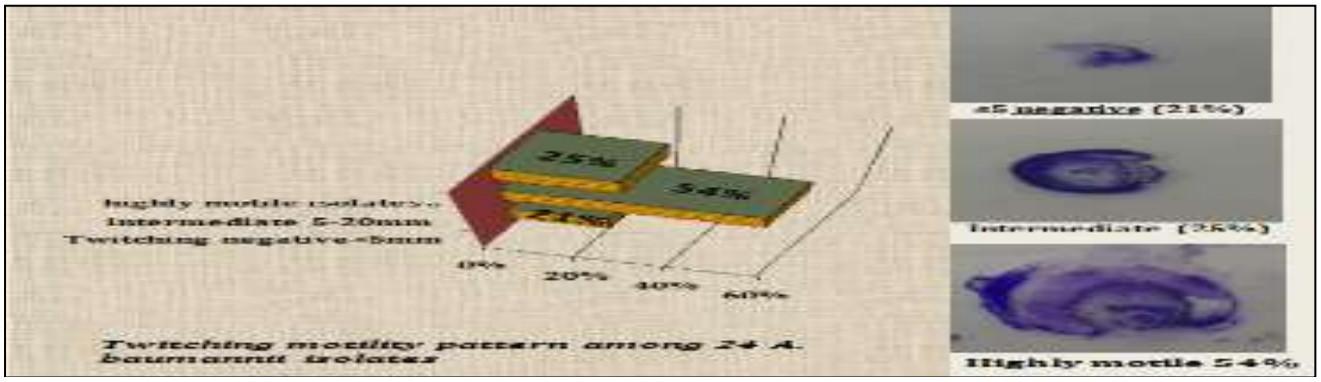


Figure (1-2): Twitching motility profile presented as percentages (left) and migration zone on M63 agar plates (right) for MDR *A. baumannii* W18 (3mm/negative), XDR W17 (10mm/intermediate), and PDR W14 (30mm/high motility).

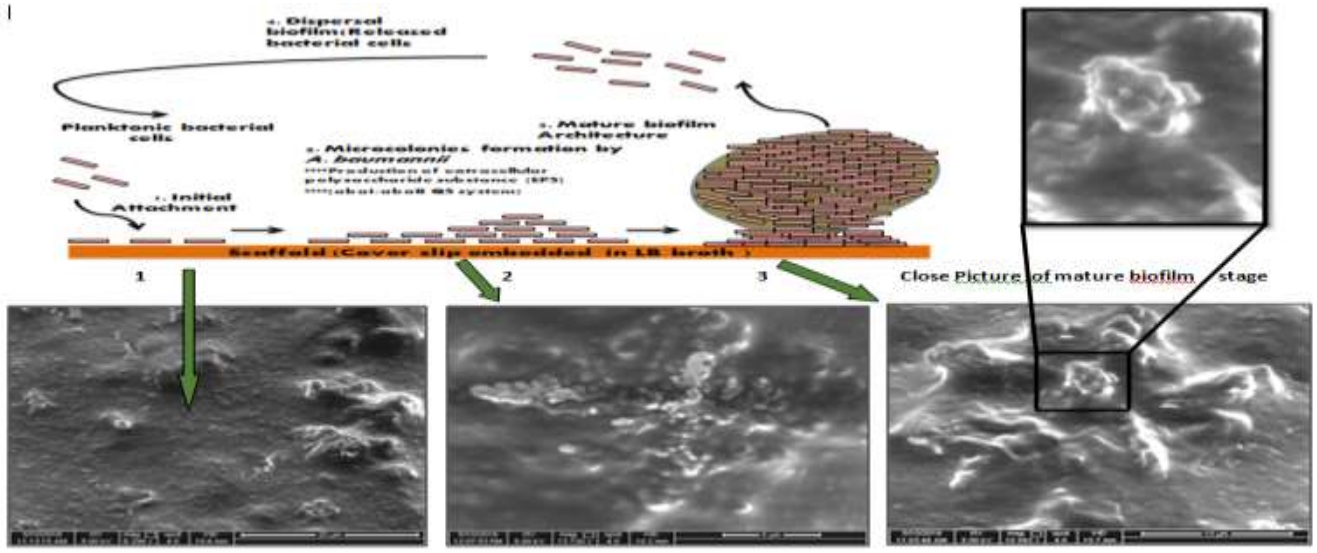
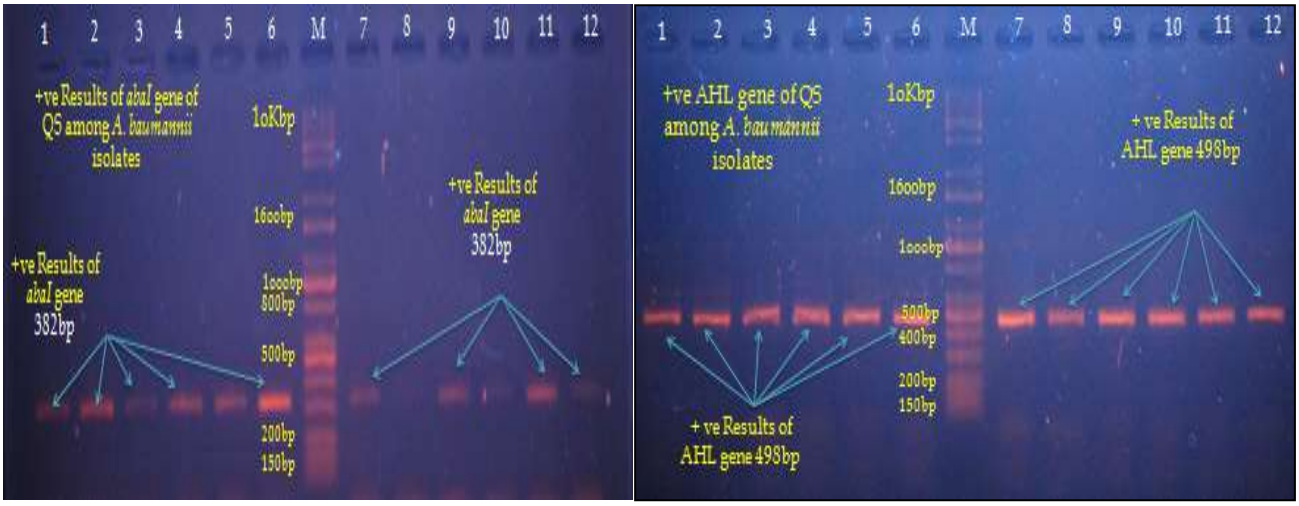


Figure (1-3): SEM images of different biofilm formation stages of PDR *A. baumannii*W14on glass cover slip; 1) Monolayer represents the exopolysaccharides (x20µm); 2) Irreversible stage presented in microcolonies at (x5µm); 3) Clear view of mature stage of biofilm at (x10µm) magnification where cells aggregated in mushroom like shape that had channels for water and nutrition. Images were compared to a diagram designed in the study.

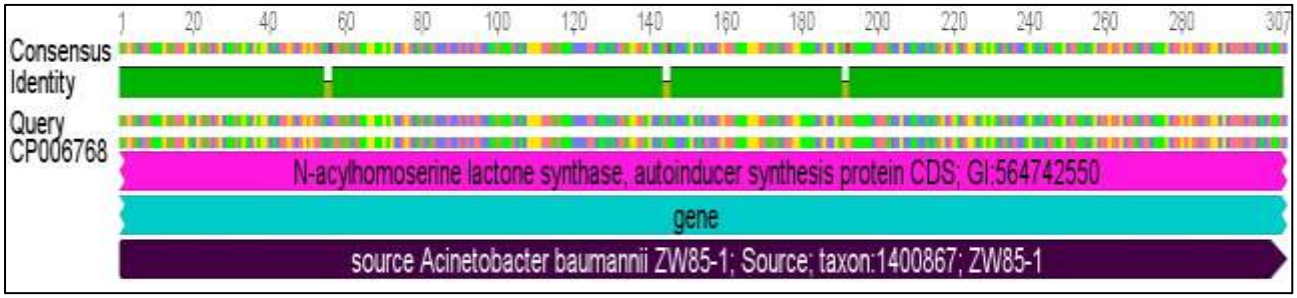
Scanning Electron Microscopy (SEM)

Analysis showed 19/24 of *A. baumannii* isolates made biofilm, suggesting highly significant correlation between biofilm-forming capacity and cell adhesiveness. Clearly, *A. baumannii* isolate that gave positive results in previous two biofilm phenotypic detection methods exhibited high rate biofilm formation on glass cover slip.

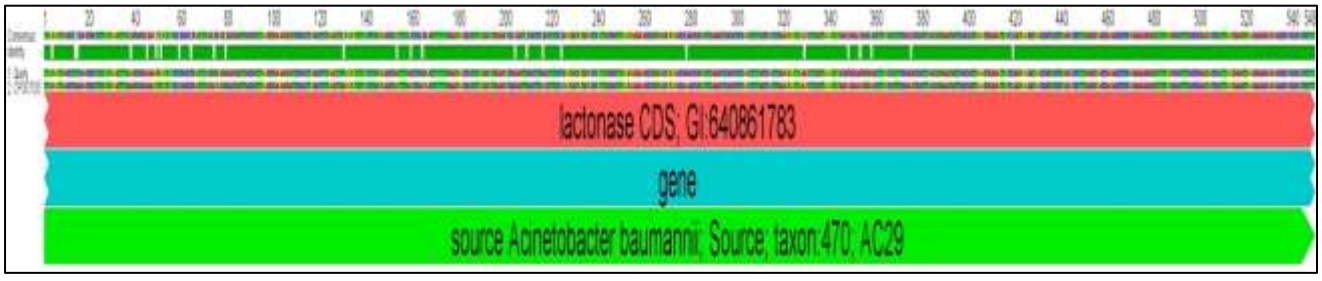
Higher magnification images (figure 1-3 No.1) illustrated monolayer of PDR *A. baumannii*W14 individual cells on cover slips covered with a film that most likely represents the exopolysaccharides which in general consist of carbohydrates, nucleic acids, proteins, and other macromolecules. In No. 2 in the same figure, microcolonies were formed and very close to be differentiated into structured and thick mature stage or so called mushroom like shape as presented in No. 3 (figure 1-3).



(A) **(B)**



(C)



(D)

Figure (1-4): Agarose gel electrophoresis (1% agarose, 5 V/cm for 120min) for QS genes: *AHL* (a) and *abaI* (b) for 24 *A. baumannii* isolates obtained from wound infections. Clear shiny bands represented positive results as compared with 100bp DNA ladder; lane M. c) The amplified *AHL* gene related to PDR *A. baumannii* W14 compared with NCBI standered strainAC29:CP007536. d) The amplified *abaI* gene related to PDR *A. baumannii* W14 compared with NCBI standered strainZW85:CP006768.

PCR Detection of Quorum Sensing Pheromones

Results demonstrated that 22/24 (91.7%) of *A. baumannii* harbored *AHL* gene encoding N-acyl homoserine lactone hydrolase or so called lactonase at amplified size 498 bp as

presented in clear bands compared to 100bp DNA ladder (figure 1-4A). The only two XDR *A. baumannii* W19 and MDR W24 were negatives. Also, results showed that 20/24 (83.3%) of isolates harbored*abaI* gene encoding N-acyl homoserine lactone synthase at amplified size 382bp (figure 1-4B). In current study, data were obtained from sequence of *abaI* and *AHL* genes were submitted to gene Bank of National center of Biotechnology information (NCBI), The European Nucleotide Archive (ENA), and DNA data bank in Japan (DDBJ) under the accession number of (KX266872) for *abaI* gene and accession number (KX349742) for *AHL* gene.

These genes sequences were analyzed and compared with NCBI standard strains. As clear in figure (1-4 C) that *AHL* gene encoding lactonase sequence related to PDR *A. baumannii* W14 was compared with standered strain AC29:CP007535: protien ID (GI:640861783) isolated from Austuralia. The Pariwise identity was (98.4%) with some differences recognized between the local isolate and the recorded NCBI strain as clear with gaps in the upper green identity line. Figure (1-4D) on ther hand represented *abaI* gene sequence related to *A. baumannii* compared with standard strain ZW85:CP006768: protien ID: 564742550 isolated from (China). The Pariwise identity was (94.5%) with some differences apperad in the upper green identity linebetween the local isolate and the recorded NCBI strain.

Discussion

All *A. baumannii* gave the white non transparentcolonies with rod shapedon CHROM agar Orintation as Ajao *et al.*(13) and Manickam*et al.*(14)observed in their study. They further revealed that degraded chromogens would allow easy identification of mixed growth and provide higher detection rates.

Also, Vitek2 Compact automated system was used as the importance of accurate identification of specific species becomes paramount in most modern microbiology laboratories and relies heavily on automated systems (15). Genotypic detection showed100% positivity, supporting the data of the previous diagnostic methods. It was reported that PCR based molecular methods were developed as an alternative ways for accurate identification and classification of bacterial species; especially when amplifying bacterial housekeeping genes (16). As a matter of fact, *rec A* gene encoding an enzyme involved in homologous recombination (17) could be widely applied in bacterial systematics and has proven to be very useful for the identification of bacterial isolates (18).

Results of microtiter dish assay indicated that the development of biofilms by *A. baumannii* would help them in evasion of host defense mechanisms hence become difficult to be eradicated; especially they caused wound infection. Results supported what had been revealed by Rodr´guez-Ban˜o*et al.* (19) and Anbazhagan *et al.* (20).

Gentile *et al.* (21) demonstrated that there was high rate of biofilm production among 60% of highly resistant *A. baumannii* isolates. Abdi-Ali *et al.*(22) revealed that microtiter plate assay exhibited 22%, 18%, 42%, and 23% of *A. baumannii* isolates were classified into strong, moderate, weak, and none biofilm producers, respectively using ELISA at 550nm. Qi *et al.* (23) revealed that highly biofilm producers were multidrug resistant pathogens. Babapour*et al.* (24) reported that 94.3% of *A. baumannii* could produce biofilm using modified microtiter plate assay. They revealed that after reading the OD of the standard microtiter plates by ELISA reader, they added 160 mL of glacial acetic (33% v/v) to each well and again read the absorption, converting the qualitative study to a quantitative one.

Twitching motility test analysis, on other hand, demonstrated that pan drug resistant *A. baumannii* were highly motile isolates compared to multi- or extremely drug resistant isolates; especially PDR W14 isolate. Results agreed with Mussi*et al.* (25) and Eijke lkamp*et al.*(26).

It was reported that twitching motility, a special kind of surface translocation, was considered good sign in biofilm and pellicle formation in *A. baumannii* (26). It has also been reported that different types of motility could be found in *Acinetobacter spp*. isolated from wound infections from hospitalized

soldiers in USA, France, and Iraq (7). Another study reported that 13/48 *A. baumannii* clinical isolated from Walter Reed Army Medical in USA had evidence of surface motility (27). It was believed that *A. baumannii* strains display a motility phenotype depending on autoinducer molecules related to quorum sensing phenomenon (28).

It was further revealed that the production of lipopolysaccharide LPS contributes to the motility phenotype directly, indicating biofilm formation (29). It was also showed that *A. baumannii* clinical isolates had surface motility phenotype on different type of media such as LB and MH (30). It is found that 60 MDR *A. baumannii* isolates showed migration zone of >40mm diameter zone on plates after incubation period of 48h, indicating motility pattern (31).

Scanning Electorn microscopy analysis was very useful in visualizing Biofilm production by *A. Baumannii* isolates. Interestingly, each of the isolates showed high rate of biofilm formation with different stages on cover slips embedded in LB medium and kept statically for long period incubation (30 days). This incubation condition could contribute to high biofilm formation since the solid-liquid interface between a cover slip and an aqueous medium (LB broth) provided an ideal environment for the attachment and growth of microorganisms.

It could be suggested that cover slip would act as a substratum that acquired a conditioning film or coating comprised of primarily proteinaceous material that present in LB broth with respect to the characteristics of the microbial cells that may impact on the rate of microbial attachment. Also, the adhesion stage was considered as an extremely complicatedprocess that could be affected by many factorsincluding change in pH, temperature, nutritional cues, chemical biocides in the medium, and also communication via quorum sensing among isolates. This property of attachment and biofilm formation on glass by *A. baumannii* clinical isolates might be associated with their outstanding antibiotic resistance profile, leading to high capacity to survive in hospital environments and subsequently causes chronic wound infections in hospitalized patients in two cities; Baghdad and Al-Najaf. Results obtained in current study supported what had been found by Brossard and Campagnari (32), Parshanth*et al.* (4); Sahu*et al.* (33).

By using SEM that clinical *A. baumannii* isolates could adhere to the urinary catheter surface cultured in LB at 30°C under shaking conditions for 72 h (34). Also, Bandeira*et al.*(35) showed using SEM and TEM that *A. baumannii* clinical isolates attached to glass cover slips forming biofilm; however under dry conditions. It was further exhibited that global rise of biofilm forming *A. baumannii* poses a major challenge to current treatment options, leading to treatment failure and were difficult to eradicate (36); therefore, there was necessity to study quorum sensing phenomenon among *A. baumannii* as a regulator system (38).

PCR study of Quorum sensing would indicate that biofilm producing *A. baumannii* isolates displayed quorum sensing signals through *luxI/luxR* type that would help in biofilm production. Further, it was clear that two XDR *A. baumannii* W1 and W19 along with MDR *A. baumannii* W18 and W24 showed no band of *abaI* gene.

This would support what had been obtained from biofilm detection methods, where they showed weakly biofilm production and did not exhibit any twitching motility on M63 medium after incubation period. Similar work was done by Niu*et al.*(38) who illustrated the prevalence of *AHL* gene among *A. baumannii* isolates obtained from US military soldiers in Iraq and USA hospitals.

Gonzalez*et al.*(39) also reported that 74% of *A. baumannii* strains produced AI-1 quorum sensing signals when forming biofilm. It was further postulated that *AHL* could enhance biofilm formation in weakly adherent clinical *A. baumannii* isolates when provided exogenously in experiment (4). He *et al.* (40) also reported that*abaI*autoinducer synthase, highly similar to members of the *LuxI* family was required for normal biofilm development.

Conclusion

Phenotypic detection of biofilm formation and its regulation through quorum sensing system among Iraqi *A. baumannii* isolates that could resist wide range of antibiotic classes indicated the disseminating chronic infections among hospitalized patients having wound infections and a big concern of choosing therapeutic regimes to eradicate such pathogen.

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Abbreviation

MDR: Multidrug resistant
XDR: Extremely drug resistant
PDR: Pan drug resistant
SEM: Scanning electron microscopy
QS: Quorum sensing

References

1 Lee HW, Koh Y M, Kim J, Lee JC, Lee YC, Seol SY(2008)et al. Capacity of multidrug-resistant clinical isolates of Acinetobacter baumannii to form biofilm and adhere to epithelial cell surfaces. Clinical Microbiology and Infection; 14 (1): 49-54.

2 Araujo C D, Balestrino D, Roth L, Charbonnel N, Forestier C (2010) Quorum sensing affects biofilm formation through lipopolysaccharide synthesis in Klebsiellapneumonia. Research in Microbiology; 1(10):1-10.

3 Hassan A,Usman J, Kaleem F, Omair M, Khalid A, Iqbal M (2011) Evaluation of different detection methodsof biofilm formation in the clinical isolates. Braz J Infect Dis; 15(4): 305-311.

4 Prashanth K, Vasanth T, Saranathan R, Makki AR, Pagal S (2012) Antibiotic Resistance, Biofilms and Quorum Sensing in Acinetobacter Species, Antibiotic Resistant Bacteria - A Continuous Challenge in the New Millennium, Dr. Marina Pana (Ed.), ISBN: 978-953-51 0472-8.

5 Chan K, Cheng HJ, Chen JW, Yin WF, Ngeow YF (2010) Tandem Mass Spectrometry Detection of Quorum Sensing Activity in Multidrug Resistant Clinical Isolate Acinetobacter baumannii. Scientific World Journal:1-6

6 Howard A, O'Donoghue M, Sleator RD (2012) Acinetobacter baumannii. Virulence; 3(3):243-250.

7 ClemmerKM, Bonomo RA, Rather PN (2011) Genetic analysis of surface motility in Acinetobacter Baumannii. Microbiology; 157:2534–2544.

8 M'hamedi I, Hassaine H, Bellifa S, Lachachi M, Terki IK, Djeribi R (2014) Biofilm formation by Acinetobacter baumanniiisolated from medical devices at the intensive care unit of the University Hospital of Tlemcen (Algeria).African Journal of Microbiology Research; 8 (3): 1-14.

9 Aziz RAR, Al-Jubori SS (2017) Molecular Analysis of Genetic Elements Responsible for XDR in Highly Successful Pathogen Acinetobacter Baumannii Isolated from Clinical Samples of Iraqi Patients. Journal of Global Pharma Technology; 04(9):26-39.

10 O'Toole G A (2011) Microtiter dish biofilm formation assay. JoVE; 47:1-2.

11 O'Toole, GA, Kolter, R (1998) Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. Mol. Microbiol; 30:295–304.

12 Fischer ER, Hansen BT, Nair V, Hoyt F H, Dorward DW (2012) Scanning Electron Microscopy. CurrProtocMicrobiol:1-76.

13 Ajao AO, Robinson G, Lee MS, Ranke TD (2011) Comparison of culture media for detection of Acinetobacter baumanniiin surveillance cultures of critically-ill patients. Eur J Clin Microbiol Infect Dis.; 30(11): 1425–1430.

14 Manikam K, Karlowsky J A, Adam H, Pang (2013) CHROMagar Orientation Medium Reduces Urine Culture Workload. Journal of Clinical Microbiology; 51(2):1179–1183.

15 Sanchez CJ, Mende K, Beckius ML, Akers KS, Romano D R, Wenke JC, (2013) et al. Biofilm formation by clinical isolates and the implications in chronic infections. BMC Infectious Diseases; 13 (47): 1-12.

16 FarahaniA, Mohajeri P Abbasi H (2013) Comparison of Different Phenotypic and Genotypic Methods for the Detection of Methicillin-Resistant Staphylococcus Aureus. N Am J Med Sci.; 5(11): 637–640.

17 Ali R, Al-Achkar K Al-Mariri A Role (2010) of Polymerase Chain Reaction (PCR) in the detection of antibiotic-resistant Staphylococcus aureus. The Egyptian Journal of Medical Human Genetics; 15:293–298.

18 Khosravi A, Sadeghi P, (2015) Sheikhi. Molecular Methods for Identification of Acinetobacter Species by Partial Sequencing of the rpoBand 16S rRNA Genes. Journal of Clinical and Diagnostic Research; 9(7): DC09-DC13.

19 Rodr´guez-Ban˜o J, Martı´S, Soto S, Ferna´ndez-Cuenca F, Cisneros M, Pacho´n J(2008) et al. Biofilm formation in Acinetobacter baumannii: associated

features and clinical implications. Clinical Microbiology and Infection, 14 (3):1-13.

20 Anbazhagan D, Mansor M, Yan GOS, Yusof MY, Hassan H (2012) et al. Detection of Quorum Sensing Signal Molecules and Identification of an Autoinducer Synthase Gene among Biofilm Forming Clinical Isolates of Acinetobacter spp.PLoS ONE 7(7):1-12.

21 Gentile V, Frangipani E, Bonchi C, Minandri F, Runci F (2014) et al.Iron and Acinetobacter baumannii Biofilm Formation. Pathogens; 3:704-719.

22 Abdi-Ali A, Hendiani S, Mohammadi P,Gharavi S (2014) Assessment of Biofilm Formation and Resistance to Imipenem and Ciprofloxacin among Clinical Isolates of Acinetobacter baumanniiin Tehran. Jundishapur J Microbiol; 7(1): 1-5.

23 Qi H L, Zhang C, Song H (2016) Relationship between Antibiotic Resistance, Biofilm Formation, and Biofilm-Specific Resistance in Acinetobacter baumannii. Frontiers in Microbiology; 7:1-10.

24 Babapour E, Haddadi A, Mirnejad R (2016) Biofilm formation in clinical isolates of nosocomial Acinetobacter baumannii and its relationship with multidrug resistance. Asian Pac J Trop Biomed; 6(6): 528–533.

25 Mussi M. Gaddy JA, Arivett B, Actis LA (2010) The Opportunistic Human Pathogen Acinetobacter baumanniiSenses and Responds to Light. Journal of Bacteriology; 192(24): 6336–6345.

26 Eijkeikamp B, Stroeher UH, Hassan KA, Paulsen IT (2011) Adherence and motility characteristics of clinical Acinetobacterbaumannii isolates. FEMS Microbiol Lett; 323: 44–51.

27 Stanton AR (2012) Assessment of motility and hemolytic activity in clinical isolates of Acinetobacter baumannii from University of Kentucky hospital, Lexington, KY. Online Theses and Dissertations:1-134.

28 McQueary C M, Kirkup B C, Zurawski D.VExtracellular (2012) stress and lipopolysaccharide modulate Acinetobacter baumannii surface-associated motility. Journal of Microbiology; 50(3): 434–443.

29 Harding C, Tracy E, Carruthers M, Munson R (2013) Acinetobacter baumannii Strain M2 Produces Type IV Pili Which Play a Role in Natural Transformation and Twitching Motility but Not Surface-Associated Motility. mBio; 4(4):1-13.

30 Kostoulias X, Murray G, Peleg A (2016) Impact of a Cross-Kingdom Signaling Molecule of Candida albicanson Acinetobacter baumanniiPhysiology. Antimicrob Agents Chemother; 60:161–167.

31 Vijayakumar S, Rajenderan S, Biswas I Biofilm (2016) Formation and Motility Depend on the Nature of the Acinetobacter baumannii Clinical Isolates. Front. Public Health; 4(105):1-9.

32 Brossard KA, Campagnari A, (2011) A The Acinetobacter baumannii Biofilm Associated Protein Plays a Role in Adherence to Human Epithelial Cells. Infection and Immunity; 80(1): 228–233.

33 Sahu PK, Pavithra S, Amrita M, Chopade (2013) Characterization of eDNA from the Clinical Strain Acinetobacter baumannii AIIMS 7 and Its Role in Biofilm Formation. The Scientific World Journal:1-11.

34 Basak S, Rajurkar M, Mallick SK (2013) Biofilms: A Challenge to Medical Fraternity in Infection Control. Infection Control:57-74.

35 Bandeira M , Carvalho PA, Duarte A, Jordao L (2014) Exploring Dangerous Connections between Klebsiella pneumoniae Biofilms and Healthcare-Associated Infections. Pathogens; 3:720-731.

36 Haley K, Blanz E, and Kirk L, Gaddy J (2014) Bacterial pathogenesis: Using high-resolution microscopy techniques tostudy host-pathogen interactions. Microscopy: advances in scientific research and education (A. Méndez-Vilas, Ed.).

37 Narayanan A, Nair MS, Venkitanarayanan K, Amalaradjou MAR (2016) Inactivation of Acinetobacter baumannii Biofilms on Polystyrene, Stainless Steel, and Urinary Catheters by Octenidine Dihydrochloride. Frontiers in Microbiology;(7):1-9.

38 Niu C, Clemmer KM, Rather P (2008) Isolation and Characterization of an Autoinducer Synthase from Acinetobacter baumannii. Journal of Bacteriology; 190(9):3386–3392.

39 Gonzalez U, Pharm D, SpencerJ (1998) Aminoglycosides: A Practical Review. Am Fam Physician;58(8):1811-1820.

40 He X, Lu F, Yuan F, Jiang D, Zho P (2015) et al. Biofilm Formation Caused by Clinical Acinetobacter baumannii Isolates Is Associated with Overexpression of the Ade FGH Efflux Pump. Antimicrobial Agents and Chemotherapy; 59(8):4817-4825.

