ISSN 0975-8542

Journal of Global Pharma Technology

Available Online at www.jgpt.co.in

RESEARCH ARTICLE

Characterization of Biofilm Production in Antibiotic Resistant Klebsiella Pneumonia Isolated From Different Clinical Samples in Iraqi Hospitals

Rawa Abdul Redha Aziz[1]*, Sawsan Sajid Al-Jubori[2]

[1] *Department of Biology, Collage of Science, Kufa University, AL-Najaf, Iraq.*

[2]*Department of Biology, Collage of Science, Al-Mustansiriyah University, Baghdad, Iraq.*

***Corresponding author: Rawa Abdul Redha Aziz**

Abstract

Objective(s) *Klebsiella pneumonia* is a causative agent of chronic infections leading to increase morbidity and mortality; especially when performing biofilm (1). Therefore, biofilm formation has been linked to the survival of pathogenic bacteria in the hospital environment, leading to susceptible bacterial colonization which creates an important public health problem. Therefore, to characterize biofilm production in local Iraqi *K. pneumonia* isolated from different clinical samples in Iraqi hospitals using phenotypic biofilm assays including scanning electron microscopy. Material and Methods local isolates were collected from different Iraqi hospitals and re-diagnosed by compact Vitek 2 and genetically by using housekeeping gene (16s rRNA and 23s rRNA 639 bp). Phenotypic detection of biofilm formation among isolates was screened by using micro titer dish assay, twitching motility assay, and scanning electron microscopy (SEM).Results: It was found that 22/24 (91.67%) of isolates could form biofilm (OD≥0.68). Twitching motility test revealed that 20/24 (80.3%) of isolates could move on the M63 medium. Scanning electron microscopy showed 83.3% of K. pneumonia isolates made biofilm in different stages started with adhesion step and ending with a mushroom like architecture as highly magnification images showed on glass cover slips embedded statically in LB broth. It was interesting that K. pneumonia S2 demonstrated a dense mat of cells aggregates on cover slips generating different biofilm formation step. Conclusion *K.* pneumonia could produce biofilm, describing the ability to resist many kinds of antibiotics and rising a concern of disseminating chronic infection among hospitalized patients in many hospitals in Baghdad.

Keywords: *Biofilm, Twitching motility, ELISA, Scanning electron microscopy.*

Introduction

K. pneumoniae is a gram-negative, nonmetal, ubiquitous, facultative anaerobic and rod-shaped bacterium (1). It is also an opportunistic pathogen that can cause many clinical outcomes including UTIs, bacterimea, meningitis, skin and soft tissue infections, hospital and community-acquired pneumonia, ankylosing spondylitis, cholecystitis, osteomyelitis (2). *K. pneumoniae* has included among the six ESKAPE pathogens (3) responsible for roughly 70% of Gram-negative infections in hospital intensive care units (ICU) (4).It has emerged as a common cause of serious epidemic and nosocomial infections in hospitals, resulting in high morbidity and mortality (5; 6).It is demonstrated that the mortality rate was 46/82 (56%) according to retrospective cohort study from tertiary care of bloodstream infection and 9/9 (100%) according to case-control study of nosocomial *K. pneumoniae* infections in Asia Pacific region(7).Biofilm producer *K. pneumonia* could be resistant to wide range of antibiotics (8; 9). Biofilm is a microbially derived sessile community which is characterized by cells that are irreversibly attached to a substratum or interface with

©2009-2017, JGPT. All Rights Reserved

each other, and embedded in a matrix of self-produced extracellular polymeric substances (10). 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 (10). Many pathogenic and nosocomial bacteria have been observed to predominantly exist as biofilms, in both natural environments and within infected tissues as polymicrobial communities (11).

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 (9). Bacterial functions such as motility, adhesion, transport, stress response, activation of metabolic pathways and extracellular matrix synthesis are required at each step of biofilm production (12).

In our study, we focus on the characterization of biofilm production in local Iraqi *K. pneumonia* isolated from clinical samples in Iraqi hospitals and tested for antibiotic resistant pattern in our previous work (13) using phenotypic biofilm assays including scanning electron microscopy (SEM).

Material and Methods

Collection and Diagnosis of Bacterial Isolates

Twenty four isolates of *K.pneumonia* were obtained from patients admitted to severalteaching hospitals in Baghdad during a period between March 2015 to September 2015 from urinary tract infections (5 isolates), bacteraemia (7 isolates), wound swabs from Burn Unit (4 isolates), 2 isolates were collected from each sputum and ear, and pus, and finally 1 isolate collected fromboth of wounds infections and stool. Isolates were cultured on CHROM agar Orientation plates. Also, they were re-diagnosed by Vitek 2 compact automated system (Biomeriux, USA), and the probability was 99% *Klebsiella pneumonia ssp pneumonia.*

Genotyping Detection for Isolates

Bacteria were diagnosed on the genetic level using amplified housekeeping gene (16s rRNA and 23s rRNA)designed using Geneious Software/ primer 3.The amplified size was 639 bpfrom the origin gene sequence F-3' TGTACACACCGCCCGTC-5'; R-3'GGTACTTAGATGTTTCAGTTC-5'. Total DNA was extracted using Genomic DNA Extraction Kit (Wizards, Promega, USA) following manufacture's protocol for Gram – negative bacteria from overnight bacterial growth.

The extract was used as a DNA template for the PCR process. The mixture of PCRis composed from 12.5 of Go Taq®Green Master Mix (2x), 5 µl template DNA, 1.5 µl primers (for each) final concentration (0.6pmol/µl) and nuclease free water up to 25 µl (4.5 µl). Uniplex PCR products of the segment (16SrRNA and 23s rRNA) were visualized using 1% agarose gel electrophoresis.

Phenotypic Detection of Biofilm Production among Local Isolates

Microtiter Dish Assay

Biofilm formation in 96-well microtiter plates was assayed and quantified as previously described by O'Toole (14). 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 ∆Pel 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.

©2009-2017, JGPT. All Rights Reserved

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.

Then the average and the standard deviation (SD) of absorbance values for each set of triplicate standards and triplicate samples were calculated. 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 (15). 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 37°C 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 ∆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 (16).

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

Results showed that all isolates exhibited on CHROM agar Orientation metallic blue colonies (figure 1-1), and also appeared to be *Klebsiella pneumonia ssp. pneumonia* at 99% level by Vitek 2 compact automated system.Results of genotypic detection ofhousekeeping gene 16SrRNA and 23SrRNA through isolates showed100% positivity with amplified size of 639 bp.

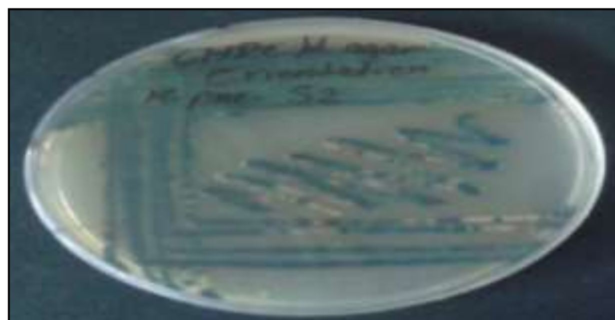

Figure (1-1): K. Pneumonias isolates Showed Metallic Blue Colonies on CHROM Agar Orientation.

Micro titer Dish Assay

Biofilm formed by each isolate was quantified by measuring its absorbance at OD550nm using ELISA system and

©2009-2017, JGPT. All Rights Reserved

compared the results with positive (*Pseudomonas* P14) and negative control (*Pseudomonas*ΔPel A mutant) to be classified into either strong or weak biofilm producers (OD 0.35 and 0.05, respectively). Reading data demonstrated that 22/24 (91.7%) of *K. pneumonaie* isolates gave positive results as presented in table (1-1).

It was clear from the results that MDR *K. pneumoniae* U2 and S1 were not biofilm producers compared to other MDR isolates that were weak biofilm producers; however, XDR *K. pneumoniae* S2 gave (OD≥0.68) when measuring its absorbance by ELISA system followed by XDR b4 and XDR b5 that gave (OD≥0.62 and 0.65, respectively); showing that XDR *K. pneumoniae* isolates gave the highest rate of biofilm productionusing this method (figure 1-2).

Table (1-1): Results of phenotypic detection method of biofilm among 24 *K. pneumoniae* isolates.

No. of isolates	Phenotypic detection of biofilm formation among isolates		
	Microtiter Dish assay readings by ELISA at 550nm average OD± SD	Biofilm formation pattern	Twitching motility assay mm
Positive control (*Pseudonmonas* PA14)	0.4±0.001	Strong	3
Negative control (*Pseudomonas* ΔPel A mutant)	0.05±0.02	None producer	None producer
MDR K. pne.b1	0.33±0.02	weak	6
MDR K. pne.b2	0.31±0.1	weak	9
MDR K. pne.b3	0.45±0.001	strong	6
XDR K. pne.b4	0.62±0.01	strong	25
XDR K. pne.b5	0.65±0.01	strong	30
XDR K. pne.b6	0.53±0.01	strong	22
MDR K. pne.b7	0.32±0.004	weak	3
MDR K. pne.U1	0.14±0.01	weak	0
MDR K. pne.U2	0.01±0.02	none	13
XDR K. pne.U3	0.44±0.02	strong	10
MDR K. pne.U4	0.33±0.01	weak	7
MDR K. pne.U5	0.32±0.01	weak	3
MDR K. pne.S1	0.02±0.01	None	0
XDR K. pne.S2	0.68±0.01	Strong	35
MDR K. pne.W1	0.44±0.02	Strong	23
XDR K. pne.ES1	0.46±0.02	strong	11
XDR K. pne.ES2	0.56±0.01	strong	25
MDR K. pne.Bu1	0.27±0.01	weak	10
XDR K. pne.Bu2	0.56±0.02	strong	20
MDR K. pne.Bu3	0.59±0.01	strong	15
XDR K. pne.Bu4	0.66±0.004	strong	28
XDR K. pne.P1	0.56±0.01	Strong	23
MDR K. pne.P2	0.26±0.01	Weak	7
MDR K. pne. St1	0.48±0.03	Strong	24

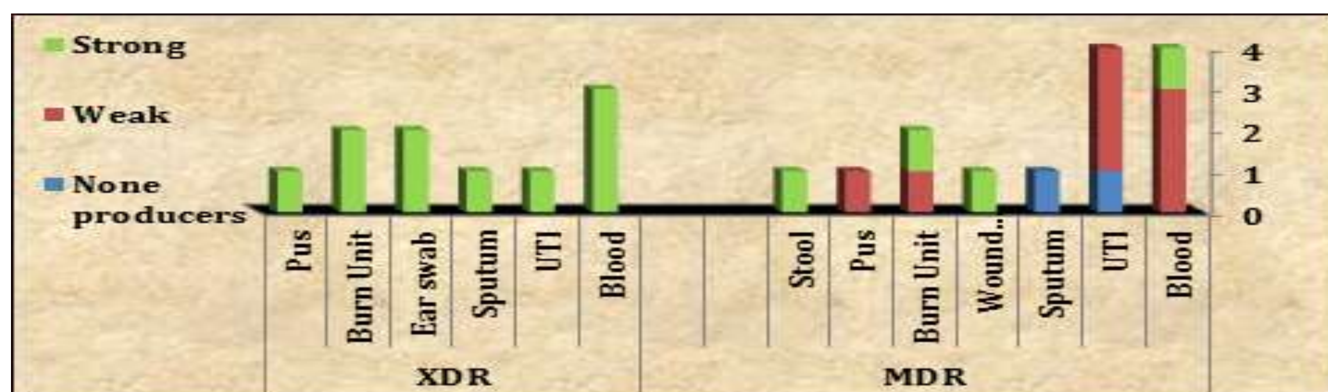

Figure (1-2): Classifying the MDR and XDR *K. pneumoniae* isolates into none- , weak, or strong biofilm producers. Results obtained from microtiter dish assay in current study

©2009-2017, JGPT. All Rights Reserved

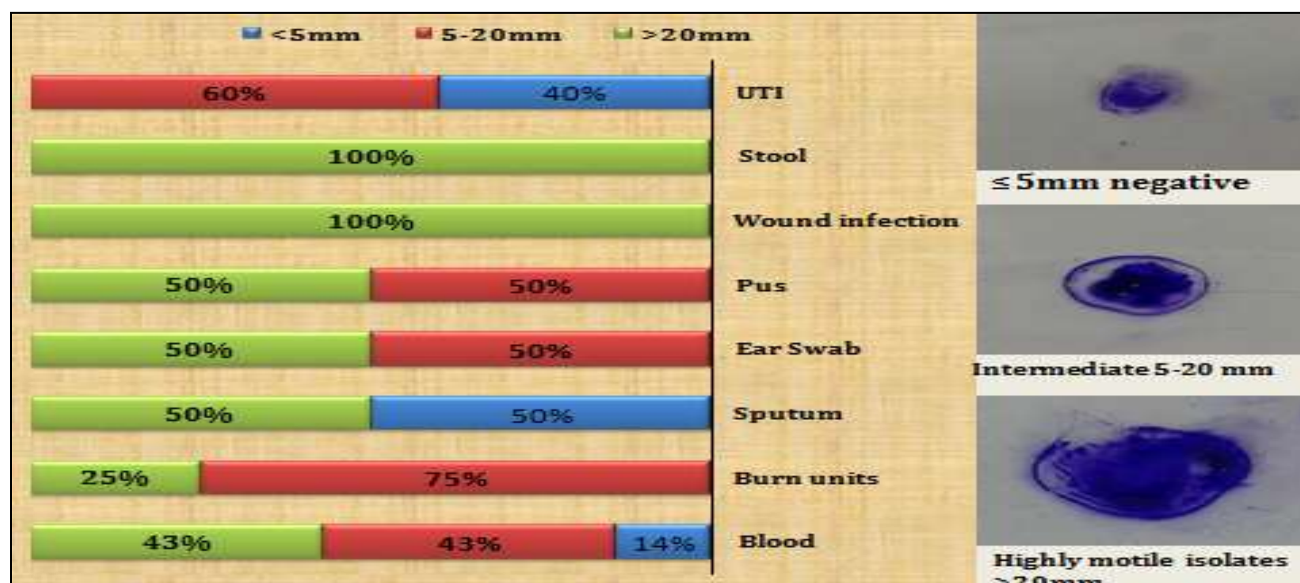

Figure (1-3): Twitching motility profile presented as percentages (left) among 24 *K. pneumoniae* isolates collected from different clinical samples and migration zone on M63 agar plates (right) for *K. pneumoniae* U1 (3mm/negative), ES1 (11mm/intermediate), and S2 (35mm/high motility).

Twitching Motility Pattern

The twenty four *K. pneumoniae* isolates were also tested for their twitching motility pattern on M63 agar plates. Results demonstrated that there was 20/24 (80.3%) of *K. pneumonia* isolates exhibited variable twitching motility patterns after incubation period (Table 1-1). Figure (1-3) represent the variable percentages of twitching motility profile among different clinical sources. XDR *K. pneumoniae* S2 found to be the highly motile isolate that exhibited 35mm in diameter immigration zone on M63 agar plates, followed by XDR Bu4 and XDR b5 which showed 30 and 28mm immigration zones, respectively.

Scanning Electron Microscopy (SEM)

The twenty four antibiotic resistant *K. pneumoniae* isolates were also undergone SEM analysis to have an understanding view of biofilm formation among those isolates related to different clinical samples obtained locally. SEM analysis showed that 20/24 *K. pneumoniae* isolates formed biofilm with different stages on glass liquid interface.

It was interesting that each biofilm producing *K. pneumonaie* isolate demonstrateda dense mat of cells aggregates after the incubation period on cover slips (figure 1-4). This tight cell aggregate could be due to factors involved in aggregative adhesions (type 1 or type 3 fimbriae), luxuriant capsules that all *K. pneumoniae* isolates exhibited earlier, and the lipopolysaccharides (LPS) which allow bacteria to grow as a biofilm. Highly magnification images presented in figure (1-4 No.1) showed condense monolayer formed by XDR*K. pneumoniae* S2 which generated a large amount of extracellular components that interact with organic and inorganic molecules in the LB medium to create the glycocalyx, consequently forming microcolonies as shown in No.2 in the same figure.

Mature biofilm architecture was also identified for this bacterium and found to be heterogeneous, containing microcolonies of bacterial cells embedded in an exopolysaccaharide (EPS) matrix and separated from other microcolonies by water channels that allow easy distribution of nutrients and oxygen (figure 1-4No.3).The property of highly antimicrobial resistant*K. pneumoniae* attachment and high rate of biofilm formation on glass showed by SEM placed them among the infectious bacterial pathogens that could be most challenging to control

©2009-2017, JGPT. All Rights Reserved

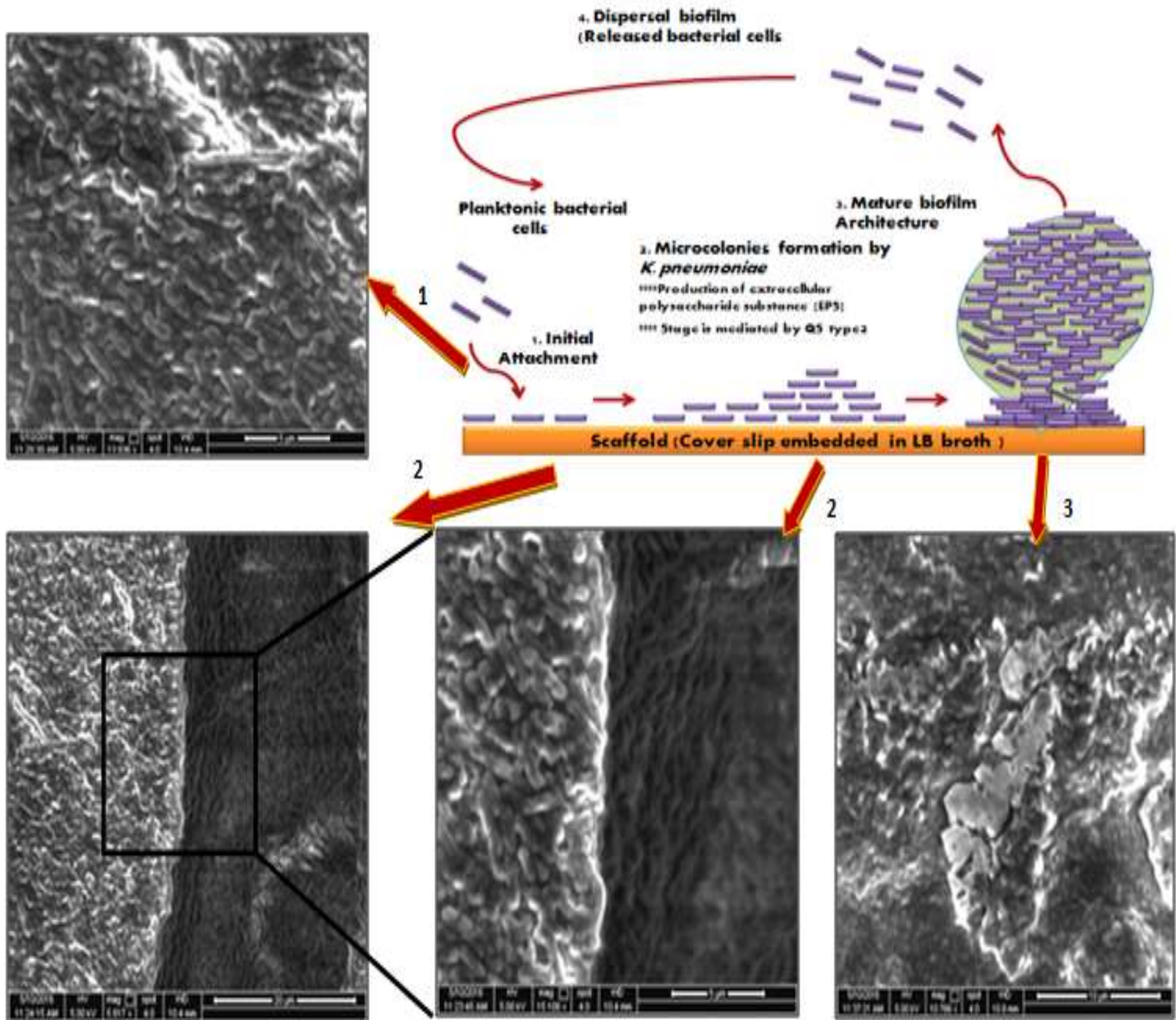

Figure (1-4): SEM images of different biofilm formation stages of *K.pneumoniae* S2on glass cover slip; 1) Adherent stage and attachment of cells tightly (x5µm), 2) (x5µm and x20µm) of irreversible stage presented in microcolonies; 3) Mature stage of biofilm at (x10µm) magnification where cells aggregated in 3D shape that had channels for water and nutrition

Discussions

All *K. pneumoniae* isolated from different clinical samples gave the right color for Enteriobacteriaceae family on CHROM agar Orintation. Same observation was detected by Ajao *et al.*(17) and Manickam *et al.*(18) who both further revealed that degraded chromogens would allow easy identification of mixed growth and provides higher detection rates. Also, Vitek2 Compact automated system was used in current study since the importance of accurate identification of specific species becomes paramount in most modern microbiology laboratories and relies heavily on automated systems (12); especially this automated system which provides rapid and accurate identification to the species level (19).Genotypic detection showed100% positivity among isolates with amplified size of 639 bp, supportting what had been explained for *K. pneumoniae* identification by phenotypic methods above. The identification to the species level was known to be difficult due to similarity of biochemical profiles among species for the same genus, such as *K. planticola* or *K. oxytoca* and *K. pneumoniae* subsp.

pneumoniae that cannot be differentiated by phenotypic tests (20). Consequently, conventional PCR has been used to amplify the highly conserved rRNA genes (16S, 23S, and 5S) which are ideal candidates for

©2009-2017, JGPT. All Rights Reserved

bacterial identification and evolutionary studies (21). As reported from sequence analysis studies, 16S and 23S rRNA could be used to identify *K. pneumoniae* subsp. *pneumoniae* to the species level (22). Besides, Dong *et al.*(23) postulated the importance of 16S-23S internal transcribed spacer to detect *K. pneumoniae* at species level.

Results of microtiter plates showed the capability of different *K. pneumoniae*isolated from clinical source to produce either strong or weak biofilm. Strongest biofilm formed by *K. pneumoniae* isolated from sputum followed by isolate from bacterimeae. Results agreed with Langstraat *et al.*(24) and Jagnow and Clegg (25). Martino *et al.*(26)also demonstrated that only 48% of *K. pneumonia* isolates were moderate biofilm forming strains on polystyrene dishes.

However, Maldonado *et al.* (27) postulated the capability of different *Klebsiella* isolates from clinical source to produce biofilm. They found that *Klebsiella pneumoniae subsp pneumoniae* isolated from UTIs was able to form biofilm (OD> 0.5) at 550nm. Oleiwi and Abid (28) showed that 8 *K. pneumonia* isolated from drinking water in different areas of Baghdad can strongly produce biofilm with mean of (OD>0.7).

Results of twitching motility assay showed that MDR *K. pneumoniae* isolate U2 and S1 could not exhibit any motility pattern along with MDR*K. pneumoniae* b7 and U1 which showed motility for less than 5mm, and this would agree with their results in microtiter dish assay; concluding that they were not biofilm producers.

Few studies in literature have been addressing the twitching motility of *K. pneumoniae*; however, Liaqat*et al.* (29) observed twitching motility in order to determine any effect in the biofilm forming capability of *K. pneumonia*isolates. They further revealed that all the isolates tested were efficient biofilm-formers and had this motility pattern.

Highly magnification images achieved by Scanning electron microscopy showed that local *K. pneumoniae* isolates were capable to produce strong biofilm with different stages; especially the interesting tight mate of extensively *K. pneumoniae* isolated from sputum specimen attached to each other and to glass substratum. Results were supported by Balestrino*et al.* (30) and Pour *et al.* (31).

It was further reported that biofilm characteristic of *K. pneumonia* isolates causing pneumoniae were most studied due to enhance the ability of this bacterium to transfer resistance markers to other clinical strains in mixed infection (31). Abd El-Baky (32) postulated that 22% of *Klebseilla* spp. was the most prevalent microorganism examined for biofilm formation using SEM.It was also reported that there were denser and high cell aggregates on cover slips leading to microcolonies formation that separated by water channels (33).

Both Bellifa*et al.* (34) and Jamal *et al.*(35)demonstrated that most biofilm forming *K. pneumonia* strains were strongly adhere to glass slides and were at least 10 times more antibiotic resistant than their planktonic counterparts. They referred the high adherence with the presence of type 3 fimbriae (36; 37).

Conclusion

Phenotypic detection and scanning electron microscopy (SEM) of local *K. pneumoniae* isolated from different clinical samples among hospitals in Baghdad indicated big concern of disseminating chronic infection among hospitalized patients; especially biofilm producer *K. pneumonia* that could resist wide range of antibiotics by various methods.

Acknowledgment

Special thanks to Prof. Sawsan Sajid Al-Jubori for her guidance, support, and provide lab and equipments that made experiments run easily. Dr. Hadeel Kareem Musafer from College of Science, Al Mustansiriya University, Baghdad/ Iraq is acknowledged for her help in experiments. Dr. Naofal Hussain from College of Science, Kufa University, Al-Najaf Al-Ashraf/ Iraq is acknowledged for running Scanning electron microscopy (SEM) for all isolates.

Abbreviation

MDR: Multidrug resistant
XDR: Extremely drug resistant

©2009-2017, JGPT. All Rights Reserved

PDR: Pan drug resistant

SEM: Scanning electron microscopy

QS: Quorum sensing

References

1 Ramos PP, Picão R C, Almeida LBG, Nicolás MF (2014) Comparative analysis of the complete genome of KPC-2-producing Klebsiella pneumoniae Kp13 reveals remarkable genome plasticity and a wide repertoire of virulence and resistance mechanisms. BMC Genomics; 15(54):1-16.

2 Jagessar RC, Alleyne R Antimicrobial (2001) potency of the aqueous extract of leaves of Terminalia catappa. Academic Research International; 1(3):362-371.

3 Rice LB (2008) Federal Funding for the Study of Antimicrobial Resistancein Nosocomial Pathogens: No ESKAPE. JID; 197:1079-1081.

4 Bogner A, Jouneau PH, Thollet G, BassetD, GauthierCA (2007) history of scanning electron microscopy developments: Towards“wet-STEM” imaging. Micron; 38: 390–401.

5 Holt K E, Wertheim H, Zadokse NR, Bakerg S, Whitehouse CA Danced D, Jenney A, et al.Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. PNAS2015:E3574–E3581.

6 AkbarAB, (2014) Isolation and Identification of Multi-Drug Resistant Strains of Non- Lactose fermenting bacteria from Clinical Isolates. Open Journal of Microbiology; 4:115-123.

7 Giske C G, Monnet D L, Cars O, CarmeliY (2008) Clinical and Economic Impact of Common Multidrug-ResistantGram-Negative Bacilli. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY; 52 (3): 813–821.

8 Boddicker J D, Anderson R A, Jagnow J, Clegg S (2006) Signature-Tagged Mutagenesis of Klebsiella pneumoniae To Identify Genes That Influence Biofilm Formation on Extracellular Matrix Material. INFECTION AND IMMUNITY;74 (8):4590–4597

9 Araujo C D, Balestrino D, Roth L, Charbonnel N, Forestier C (2010) Quorum sensing affects biofilm formation through lipopolysaccharide synthesis in Klebsiella pneumonia. Research in Microbiology.

10 Lee H W, 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.

11 Clemmer K M, Bonomo R A, Rather PN (2011)Genetic analysis of surface motility in Acinetobacter baumannii. Microbiology; 157:2534–2544.

12 Akers SK, Mende K, Yu X, Beckius M L, Aggarwal D, Li P,et (2014) al. Biofilms and persistent wound infections in United States military trauma patients: a case–control analysis. BMC Infectious Diseases; 14 (190):1-11.

13 Aziz RA R Al-Jubori S S (2016) Genetic Elements Responsible for extreme drug resistance (XDR) in Klebsiella pnumoniae var pnumoniae isolated from clinical samples of Iraqi patients. WJPR; 5 (5):1-23.

14 O’Toole, GA (2011) Microtiter dish biofilm formation assay. JoVE.; 47:1-2.

15 O’Toole, G A, Kolter, R Flagellar (1998) twitching motility are necessary for Pseudomonas aeruginosa biofilm development. Mol. Microbiol; 30:295–304.

16 Fischer ER, Hansen B T, Nair V, Hoyt F H, Dorward DW (2012) Scanning Electron Microscopy. Curr Protoc Microbiol: 1-76.

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

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

19 Manchanda V, Sinha Sanchaita S, Singh NP (2010) Multidrug resistant Acinetobacter. J Global Infect Dis; 2:291-304.

20 Wang B, China S, Knopf D, Gilles M, Laskin A (2016) Probing Individual Ice Nucleation Events with Environmental Scanning Electron Microscopy. Geophysical Research Abstracts; 18:1-1.

21 Elgaml A, Hassan R, Barwa R, El-Naggar W (2013) Analysis of 16S ribosomal RNA gene segments for the diagnosis of Gram negative pathogenic bacteria isolated from urinary tract infections. African Journal of Microbiology Research; 7(23):2862-2869.

22 Srinivasan R, Karaoz U, Volegova M, MacKichan J (2015) Use of 16S rRNA Gene for Identification of a Broad Range of Clinically Relevant Bacterial Pathogens. PLoS ONE; 10(2): e0117617.

©2009-2017, JGPT. All Rights Reserved

23 Dong D, Cober E, Reichter S S Impact (2015) of therapy and strain type on outcomes in urinary tract infections caused by carbapenem-resistant Klebsiella pneumoniae. J Antimicrob Chemother 2015; 70: 1203–1211.

24 Langstraat J, Bohes M, Clegg S Type (2001) 3 Fimbrial Shaft (MrkA) of Klebsiella pneumoniae, but Not the Fimbrial Adhesin (MrkD), Facilitates Biofilm Formation. Infection and Immunity. INFECTION AND IMMUNITY; 69(9):5805–5812.

25 JagnowJ, Clegg S (2003) Klebsiella pneumoniae MrkD-mediated biofilm formation on extracellular matrix- and collagencoated surfaces. Microbiology2003;149, 2397–2405.

26 Martino P D, Cafferini N Joly B (2003) Klebsiella pneumoniae type 3 pili facilitate adherence and biofilm formation on abiotic surfaces, 161: 595–603.

27 Maldonado N, Silva C, Cecilia M, Macias M A (2007) simple technique to detect Klebsiella biofilm-forming-strains. Inhibitory potential of Lactobacillus fermentum CRL 1058 whole cells and products; 69(9):5- 12.

28 Oleiwi H Abid S (2014) Role of Extracted Genomic DNA on Biofilm Formationby Pseudomonas aeruginosa and Klebsiella pneumoniae in vitro. Ibn Al-HaithamJour. for Pure & Appl. Sci.; 27 (3):1-11.

29 Liaqat I, Sumbal F, Sabri AN (2009).Tetracycline and Chloramphenicol Efficiency Against Selected Biofilm Forming Bacteria. Current Microbiology; 59 (2): 212–220.

30 Balestrino D., Haagensen J. A. J., Rich C., and Forestier C.Characterization of Type 2 Quorum Sensing in Klebsiella pneumoniaand Relationship with Biofilm Formation. JOURNAL OF BACTERIOLOGY 2005; 187 98). pp. 2870–2880.

31 Pour N K, Dusan . H, Rokhbakhsh F. Bioflim (2011) formation by Acinetobacter baumannii strains isolated from urinary tract infection and urinary catheters. FEMS Immunol Med Microbiol; 62: 328–338.

32 Abd El-Baky RM (2012) Application of Scanning Electron Microscopy for the Morphological Study of Biofilm in Medical Devices, Scanning Electron Microscopy, Dr. Viacheslav Kazmiruk (Ed.). ISBN:; 51:978-953.

33 Myszka K Czaczyk K (2012) Mechanisms Determining Bacterial Biofilm Resistance to Antimicrobial Factors. Antimicrobial Agents: 213-238.

34 Bellifa S, Hassaine H, Balestrino D, Charbonnel N, Lachachi M, Didi W (2013) et al. Evaluation of biofilm formation of Klebsiella pneumoniae isolated from medical devices at the University Hospital of Tlemcen, Algeria. African Journal of Microbiology Research; 7(49):5558-5564.

35 Jamal M, Hussain T, Andleeb S (2015) Inhibition of clinical multi-drug resistant Klebsiella pneumoniae biofilm by Siphoviridae bacteriophage Z. Sci Lett; 3(3):122-126.

36 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.

37 Bala M, Gupta S, Aggarwal P, Manhas A (2017) Biofilm producing multidrug resistant Acinetobacter species from a tertiary care hospital: a therapeutic challenge. IJRMS; 4(7):1-14.

©2009-2017, JGPT. All Rights Reserved