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CORRELATIONBETWEEN ANTIBIOTICS RESISTANCE AND BIOFILM FORMATION BY KLEBSEILLA PNEUMONIAE

Eptissam Younan Pirko*

Assistant Lecture, Diyala University/ College of Medicine Department of microbiology, Diyala Iraq.

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***Correspondence for Author**
Eptissam Younan Pirko
Assistant Lecture, Diyala University/ College of Medicine Department of microbiology, Diyala Iraq.

ABSTERACT

Klebseilla pneumoniae is important gram negative opportunistic pathogenic bacteria associated with nosocomial acquired infections. Aim of the presented study was isolation and identification of Klebseilla pneumoniae and evaluate the prevalence of Klebseilla pneumoniae biofilm formed in microtiter plate by a spectrophometric assay as a risk and virulence factor Also, study the resistance of this bacteria to many antibiotic which used in therapeutic, a fifty samples were collected from different patients from many Hospitals in Baghdad city during the period from February-August 2015. Klebsiella isolates (50 isolates) were as follows: sputum (14), urine (10), burn (9), wound (9) and blood (8). All isolate were identification by culturing on (blood, MacConkey) agar and analysed according to morphological and biochemical tests, API 20 E system as well as vitek 2 system. Biochemical test performed were catalase, Oxidase, IMViC Test (including: indole test, methyl red, vogas prosquar and citrate utilization) as well as urease production, and fermentation of sugars (glucose, sucrose, lactose and manitol, additional to H2S production on Triple sugar iron), so the antimicrobial resistance testing was performed for 12 different antibiotics.Most of isolates were obtained from sputum samples by 28 % (14 isolates) followed urine 20 % (10 isolates) while both of burn and wound have 18 %(9) and finally blood infection 16 % (8 isolates). So (54%) of the isolates were produced strong biofilm, and (22%) were moderate produced biofilm, while (24%) were weak or non- produced biofilm. Bacterial strains were highly resistant to CEF (94.8,90.9)% respectively in K. pneumoniae form and non-form biofilm respectively, so more sensitive (0%)to GEN and IMP in form and non-form biofilm respectively, Also, K. pneumoniae positive biofilm showed (89.7,7.6,89.7,94.8,74.3,46,0,0, 25.6,56.44,

58.94,58.94,35.8,64.1and76.9)% respectively resistance to Ampicillin, Amikacin, Amoxicillin; Ceftriaxone, Cefotaxime; Ciprofloxacin; Gentamicin, Imipenem; nitrofurantoin; pipracillin; tobramycin; tetracycline; Norfloxacin; Nalidixic acid and trimethoprim/sulfamethoxazole respectively, compared to the (81.8,0, 81.8,90.9,63.6,27.2,0,0,27.2,54.5,18.1,36.3,18.1,54.5and27.2) % respectively resistance by non-form biofilm for the respective antibiotics **Conclusion:** Our data indicate all most isolates of Klebseilla pneumoniae form biofilm and these bacteria were more resistance to antibiotics comparative to isolate that non- form biofilm.

KEYWORDS: biofilm; Klebseilla pneumoniae, polystyrene microtiter plates, Antibiotics.

INTRODUCTION

Formation and development of biofilm (microbial city) by bacteria is important stage in the pathogenesis of Klebsiella, and it is relatively common phenomenon among many pathogenic bacteria which is development depending on many biological, chemical and physical factors [Bryers,2008], biofilm composed of polysaccharides, proteins, DNA, surfactants, lipids, glycolipids, membrane vesicles and ions such as Ca^{2+} [Donlan and Costerton 2002], the main molecule that responsible for intercellular adhesion is polysaccharide intercellular adhesion (PIA) which conceder as crucial virulence factors, also poly-N-acetylglucosamine (PNAG) [Mack,1996].

Severity of K. pneumoniae infections causing by virulence factors as fimbriae type (1 and 3), LP, iron-scavening systems, urease and ability to produce many types of adherence factors, capsular polysaccharides, which conceder essential virulence of Klebsiella, these capsular polysaccharides help adhered bacteria to the epithelial cells as well as formed the biofilms on surfaces [Tarkkanen etal,1992], so material of capsular formed fibrillous which covered the surface of bacteria, and it prevents killing bacteria by bactericidal serum factors so, protects it from phagocytosis [Amako, etal, 1988], also trec and suge genes have been demonstrated to affect biofilm formation by modulating CPS production [Wu, etal, 2011].

Biofilms cause chronic infections because they causing increased tolerance to antibiotics and disinfectant chemicals as well as resisting phagocytosis and other components of host immune system (Hoiby, eta,2010), therefore strains which formed biofilm may cause problems in therapy(Wojtyczka,2014), so increased resistant of Klebsiella pneumoniae to antibiotics caused complicated therapy of this infections, so high concentrations of antibiotics

with Prolong treatment are normally needed to removing or reducing the biofilms (Holder, 1993).

K. pneumoniae can carried asymptomatically in throat , nose, skin and in- testinal tract of healthy individuals but can also cause a range of infections in hospitalized patients [Brisse etal,2006].

METHODS

1-Clinical specimens

Two hundred twenty five samples were collected from patient in many Hospitals of Baghdad city during the period from February to August 2015. The Klebsiella isolates (50 isolates) were as follows: sputum (14), urine (10), burn (9), wound (9) and blood (8).

2- Bacterial isolate

All samples collected from specimen (Blood, Burn, Wound, Urine and sputum) were analysed for K.Pneumoniae according to morphological on blood and maCconkey and identification by using api-20 and vitek2-Compact System.

3-Biochemical test

Biochemical test performed were catalase, Oxidase, IMVIC Test (including: indole test, methyl red, vogas prosquar and citrate utilization) as well as urease production, and fermentation of sugars. Sugar fermentation test performed were glucose, sucrose, lactose and manitol, additional to H2S production on Triple sugar iron.

4- Antimicrobial resistance

Antimicrobial resistance testing was performed for 12 different antibiotics by disk diffusion (Kiry bauer method), using the standard protocol for diffusion of antimicrobial agents on Mueller-Hinton agar as described in National Committee for Clinical Laboratory Standards NCCLS guidelines (CLSI, 2012). The antibiotics tested included: Ampicillin, Amikacin, Amoxicillin; Ceftriaxone, Cefotaxime; Ciprofloxacin; Gentamicin, Imipenem; nitrofurantoin; pipracillin; tobramycin; tetracycline; Norfloxacin; Nalidixicacidand Trimethoprim/sulfamethoxazole.

5- Biofilm assay on polystyrene microtiter plates

Ten ml of trypticase soy broth (TSB) with 1% glucose was inoculated with a loopful of test organism from overnight culture on nutrient agar. The TSB broth was incubated at 37oC for

24 hours. culture was diluted 1:100 with fresh medium and filled with 200µl of diluted cultures individually flat bottom tissue culture plates (96 wells), and incubated at 37oC for 24 hours. After incubation, gentle tapping of the plates was done. The wells were washed with 200µl of phosphate buffer saline (pH 7.2) four times to remove free-floating bacteria. Biofilms which remained adherent to the walls fixed with 2% sodium acetate and stained by 0.1% crystal violetm then washed with deionized water to removed Excess stain, Eventually, the absorbance was read at a wavelength of 550 nm using Micro ELISA Reader. Experiments were performed in duplicate and the average of OD values of sterile medium were calculated [Mathur et al, 2006].

Statistical analysis

Analyzed data by using SPSS 17 version.

RESULTS AND DISCUSSION

K. pneumoniae have been known to coexist in wound, burn, cystic fibrosis, urinary tract infections and respiratory tract [Childers et al. 2013].

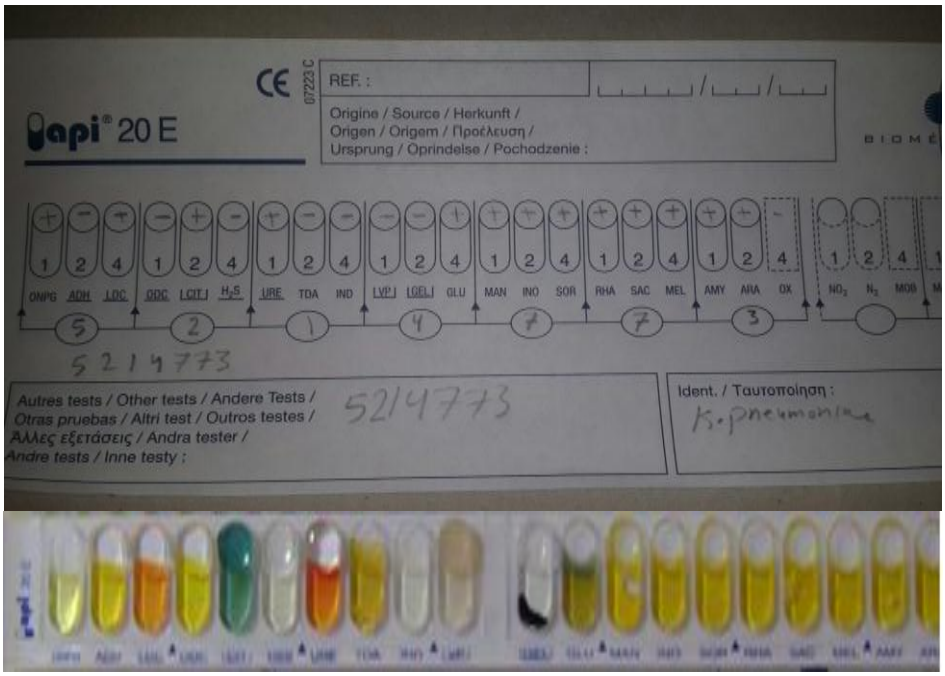

Figure1: API 20 E system of Klebsiella pneumoniae code 5214773.

The result in figure 1 showed API 20 E system useding for identification of Klebsiella pneumoniae which has serial code number as 5214773, these results are quite consistent with the results of Mohammed (2012).

Table1: Number and percentage of Klebsiella pneumoniae isolated from clinical source.

Clinical source	Total sample	Klebseilla pneumonia isolate	
		Number	%
Sputum	50	14	28
Urine	50	10	20
Burn	50	9	18
Wound	50	9	18
Blood	50	8	16
Total	250	50	100

The results in table (1) showed that most of the isolates were obtained from sputum samples by 28% (14 isolates) followed by urine 20% (10 isolates) while both burn and wound have 18% (9) and finally blood infection 16% (8 isolates). This finding was also observed by Ansari et al. (2014) who have ensured that 6 (13.04%) were K. pneumoniae isolated from from different clinical specimens as blood, pus and wounds.

The rate of Klebsiella infection increases urinary tract infection because the capsules of these bacteria are important virulent factors (Roberts, 2000). Similarly Hennequin and Forestier (2007) reported in a previous study the association of biofilm producing bacteria with colonization of urinary catheters [Hennequin and Forestier, 2007].

Table 2: Biochemical test for Klebseilla pneumoniae.

Biochemical test	Results
Catalase	+
Oxidase	-
Indole test	-
Methyl red	-
Vogas prosquar	+
Citrate utilization	+
Urease production	+
Kligler iron agar (KIA)	Acidic/Acidic, with gas,No H2S
Lactose fermentation	+
Motility	–

Table 2 showed all isolate of Klebseilla pneumoniae have positive results for biochemical tests as Catalase, Vogas prosquar, Citrate utilization, Urease production and Lactose fermentation, while negative for Oxidase, Indole test, Methyl red as well as produced Acidic/Acidic, with gas, without H2S nor motility.

Mohammed showed in his study all isolate of Klebseilla pneumoniae have Oxidase negative and catalase positive, H_2S in not produced, reduce nitrates [Mohammed, W.F.2012].

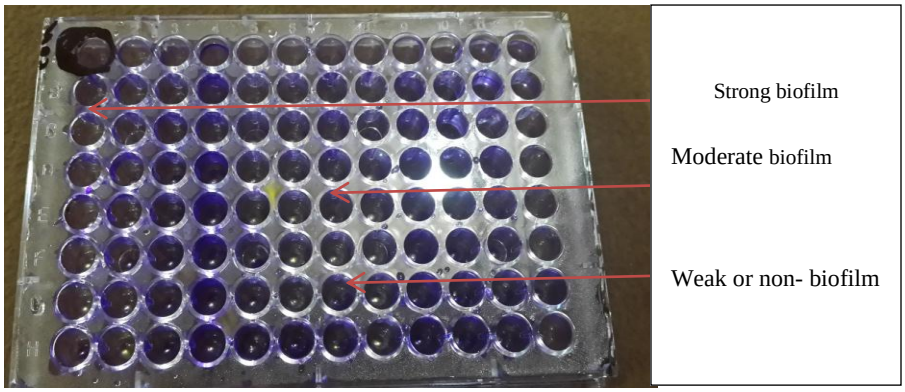

Figure 2: Biofilm of Klebsiella pneumoniae by microtiter plate (Mtp)

In figure (2) appearance the levels of biofilm formed by K. pneumoniae isolates by Microtiter plate assays. Higher levels (strong) of biofilm formation were (No. = 27), moderate were (No. =11) and weak or non- biofilm form (No. =12).

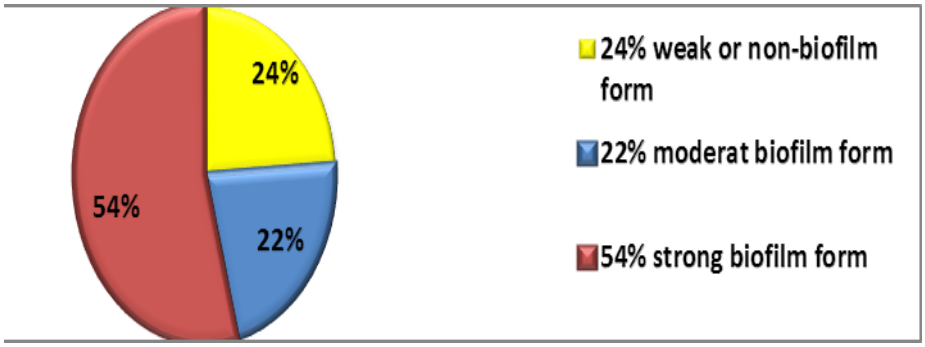

Figure3. Distribution of Klebseilla pneumoniae isolates according to biofilm Formation by microtiter plate (M.T.P).

The results observed in figure (3) that (54%) of the isolates were produced strong biofilm, so (22%) were moderate produced biofilm, while (24%) of the isolates were non or weak produced biofilm ,biofilm form that classification was based according to Mathur et al., 2006. This result was conformed to Ansari et al. (2014) who showed strongly positive of biofilm formed by microtitre assay in 2 (33.33%) Klebsiella spp., while the remaining isolates were either moderate adherent 1 (16.67%) or weak/non-biofilm producers 3 (50%). The importance of trec in gastrointestinal tract colonization suggests that biofilm formation contributes to the establishment and persistence of K. pneumoniae infection. While Yang and Zhang showed in his study 40% of K. pneumoniae isolated from wound swabs, sputum, urine, blood were reported to be able to form an in vitro biofilm [Yang and Zhang, 2008], so about 63% of K. pneumoniae isolates from urine samples form biofilm [Niveditha, etal, 2012].

Table.3 Results of Resistant test of Klebsiella pneumoniae (form and non-form biofilm) isolates against Antibiotics.

Antibiotics	Number of isolates (50)			
	Resistant of Klebsiella pneumoniae (form biofilm) to antibiotics		Resistant of Klebsiella pneumoniae (non-form biofilm) to antibiotics	
	Number (39)	%	Number (11) (12)	%
AMP	35	89.7	9	81.8
AMK	3	7.6	0	0
AMO	35	89.7	9	81.8
CER	37	94.8	10	90.9
CEF	29	74.3	7	63.6
CIP	18	46	3	27.2
GEN	0	0	0	0
IMP	0	0	0	0
NIT	10	25.62	3	27.2
PIP	22	56.44	6	54.5
TOB	23	58.94	2	18.1
TET	23	58.94	4	36.3
Norfloxacin	14	35.8	2	18.1
Nalidixic acid	25	64.1	6	54.5
Trimethoprim/ sulfamethoxazol	30	76.9	3	27.2

AMP=Ampicillin, AMK=Amikacin, AMO=Amoxicillin, CER=Ceftriaxone, CEF=Cefotaxime, CIP=Ciprofloxacin, GEN=Gentamicin, IMP=Imipenem, NIT=nitrofurantoin, PIP=pipracillin, TOB=tobramycin, TET=tetracycline,

The antibiotic resistance pattern of fifty isolates from different clinical source were screened for their resistance to fifteen used antibiotics, table 3 showed the percentages of resistant this bacteria which form biofilm to antibiotics were as follow: 94.8% to CER, 89.7% to both AMP and AMO, So (76.9, 74.3) % respectively to Trimethoprim/Sulfamethoxazole and CEF respectively, while low resistance to AMK (7.6%) while no resistance (0%) compare to that non-form biofilm the percentage of resistance to antibiotics as 90.9% to CER and 81.8% to AMP, AMO but low resistance (18.1%) to TOB and norfloxacin, So 27.2% to CIP, NIT and Trimethoprim/Sulfamethoxazole but no resistance (0%) to AMK, GEN and IMP.

The present results showed klebseilla form biofilm for all antibiotics higher than non-form biofilm, Mohamed, 2012 showed in his study the Klebsiella pneumoniae are resistant to Ampicillin (AMP), Piperacillin/Tazobactam(PIT), Cefazolin(CZ) and Cefotaxime(CTX) while no resistance to Imipenem(IMP) and Amikacin(AK), In Taiwan Lee et al., (2004)

reported that Klebsiella pneumonia (10 isolates) resist to AMP (100%), CZ (50%), CTX (50%), CAZ (40%), GEN (50%), AK (20%) and IMP (0%), so Seyyed et al., (2011) noted that Klebsiella pneumoniae percent resisted to GEN, AMP, CTX, AK, CIP, and IMP respectively as (100, 100, 96 ,70 ,55 10) % respectively.

In present study showed K. pneumoniae producers biofilm showed (89.7,7.6,89.7 ,94.8,74.3,46,0,0,25.6,56.44,58.94,58.94,35.8,64.1and 76.9)% respectively resistance to Ampicillin, Amikacin, Amoxicillin; Ceftriaxone, Cefotaxime; Ciprofloxacin; Gentamicin, Imipenem; nitrofurantoin; pipracillin; tobramycin; tetracycline; Norfloxacin; Nalidixic acid and trimethoprim/sulfamethoxazole respectively, compared to the (81.8,0,81.8,90.9, 63.6,27.2,0,0,27.2,54.5,18.1,36.3,18.1,54.5 and 27.2)% respectively resistance by biofilm non-producers for the respective antibiotics, These results are quite consistent with Subramanian et al, who found in his study K. pneumoniae producers biofilm showed 93.3%, 83.3%, 73.3% and 80% resistance to nalidixic acid, ampicillin, cefotaxime and co-trimoxazole, respectively, compared to the 70%, 60%, 35% and 60% resistance shown by biofilm non-producers for the respective antibiotics [Subramanian, etal., 2012].

While AL-Talib (2006) showed in his study Klebsiella pneumonia resisted as (100%) to both CAZ and IMP while (86%) to GEN and (29%) to CTX. These differences in resistance of some antibiotics may be occurred in some genera or species depending on the drug of choice that has been adopted in that hospitals and the time of using in addition to the nature of the strains. Mechanisms of risistance to antibiotics differ according to the antibiotics themselves (Landgren et al., 2005), as well as isolates which form-biofilm more resistant at least 10 times than their planktonic counterparts (Bellifa, S.et al., 2013).

One of the most commonly studied properties of biofilms is their increased resistance to the effects of antibiotics (Liaqat et al., 2009). Because of cells embedded in biofilms may include limited diffusion of antibiotics into the biofilm or decreased bacterial growth, also some antibiotics can react with matrix of biofilm, as well as, these biofilm functions as a diffusion barrier for some antimicrobial drug, and increasing resistance to antibiotics (Vuotto etal. 2014). On the other hand, cells in biofilms can adapt and form protected phenotypes (Monzon et al., 2001), as well as components of biofilm matrix form a mechanical shield and act to inhibit the effect of antibiotics(Anahit etal.,2015).

Antibiotic resistance is encoded by several genes, many of them can transfer between bacteria

[Jessica, 2015] therefore high resistance to antibiotics in biofilm-associated infections are very difficult to treat (Hall-Stoodley etal., 2004), cause more hazard of this pathogen in hospital settings.

CONCLUSION

Our data indicate the bacteria isolate form biofilm were more reistance to antibiotics from isolate that non- form biofilm.

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REFERENCES

1. AL-Talib H. I. (2006). A bacteriological study in early and late onset neonatal sepsis. college of medicine university of Mosul.
2. Ansari, M. A.; Khan, H.M.; Khan, A.A.; Cameotra, S. S. and Pal,R. Antibiofilm efficacy of silver nanoparticles against biofilm of extended spectrum β-lactamase isolates of Escherichia coli and Klebsiella pneumoniae.Applied Nanoscience, 2014; 4(7): 859-868.
3. Bellifa, S.; Hassaine, H.; Balestrino,D.; Charbonne, N., M'hamedi,I.; Terki,I.K.; Lachachi,M.; Didi,W. and Christiane,F.C. Evaluation of biofilm formation of Klebsiella pneumoniae isolated from medical devices at the University Hospital of Tlemcen, Algeria. African Journal of Microbiology Research, 2013; 7(49): 5558-5564.
4. Brisse, S; Grimont, F. and Grimont,P. (2006). The genus Klebsiella. The Prokaryotes A Handbook on the Biology of Bacteria, 3rd edition, eds Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (Springer, New York), 3rd Ed. Vol 6: Pro- teobacteria: Gamma Subclass.
5. Brooks, G.F.; Carroll, K.C.; Butel, J.S.; and Morse, S.A. Jawetz, Melnick and Adelbergs Medical Microbiology. 24th.ed. the McGraw- Hill Companies, Inc. New York, 2007; P.224-232.
6. Bryers, J.D. Medical biofilms. Biotechnol. Bioeng, 2008; 100: 1–18.
7. Ceri, H.; Olson, M.E.; Stremick, C.; Read, R.R.; Morck, D.; Buret, A. The Calgary biofilm device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J. Clin. Microbiol, 1999; 37: 1771–1776.

8. Childers, B.M., Van Laar, T.A., You, T., Clegg, S., Leung, K.P. MrkD (1P) from Klebsiella pneumoniae IA565 Allows for Co-existence with Pseudomonas aeruginosa and Protection from Protease-mediated Biofilm Detachment. Infect immune, 2013; 81: 4112-20.
9. Donlan, R.M., Costerton, J.W. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev, 2002; 15: 167–193.
10. Gillings, M.R.; Stokes, H.W. Are humans increasing bacterial evolvability? Trends Ecol. Evol, 2012; 27: 346–352.
11. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol, 2004; 2: 95–108.
12. Hoiby, N.; Bjarnsholt,T.; Givskov,M.; Molin,S. and Ciofu,O. Antibiotic resistance of bacterial biofilms. International journal of antimicrobial agents, 2010; 35(4): 322–332.
13. iaqat I, Sumbal F, Sabri AN. Tetracycline and chloramphenicol efficiency against selected biofilm forming bacteria. Curr. Microbiol, 2009; 59(2): 212-220.
14. Robertis, K.; Murray; Peter, A.; Mayes; Dary, K.; Granner and Victor W. Rodwell. Plasma protein, immunoglbulins and clotting factors, Harper's biochemistry text book of biochemistry; 24th edition, losaltos, California, Appelton and Langer, 2000; 707-733.
15. Tarkkanen, A. M., B. L. Allen, P. H. Williams, M. Kauppi, K. Haahtela, A. Siitonen, I. Orskov, F. Orskov, S. Clegg, and T. K. Korhonen. Fimbriation, capsulation, and iron-scavenging systems of Klebsiella strains associated with human urinary tract infection. Infect. Immun, 1992; 60: 1187-1192.
16. Vuotto, C.; Longo, F.; Pia Balice, M.; Donelli, G. and Varaldo, P.E.Antibiotic Resistance Related to Biofilm Formation inKlebsiella pneumoniae. Pathogens, 2014; 3: 743-758.
17. Amako, K.; Meno, Y.; Takade, A. Fine structures of the capsules of Klebsiella pneumoniae and Escherichia coli K1. J. Bacteriol, 1988; 170: 4960–4962.
18. Anahit, P.; Michael, G. and Ian, T. P. Antibiotic Discovery: Combatting Bacterial Resistance in Cells and in Biofilm Communities, Molecules, 2015; 20: 5286-5298.
19. -Clinical and Laboratory Standards Institute. (2012). Performance Standards for Antimicrobial Susceptibility Testing, 22nd Informational Supplement, M-100-S22.Vol.32, No.3, Wayne, Pa, USA.
20. -Holder IA. P. Aeruginosa burn infections: pathogenesis and treatment. Pseudomonas aeruginosa as an opportunistic pathogen. Springer 1993.
21. Hennequin C, Forestier C. Influence of capsule and extended- spectrum beta-lactamases encoding plasmids upon Klebsiella pneumoniae adhesion. Res. Microbiol, 2007; 158:

339-347.

22. Jessica, M.A.; Blair,M. A.; Webber, A.J.; Baylay, D.O. Ogbolu and Laura J.V. Piddock. Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology, 2015; 13: 42–51.
23. Landgren M.; Oden H.; Kuhn I.; Osterlund A. and Kahlmeter G. Diversity among 2481 Escherichia coli from women with community-acquired lower urinary tract infections in 17 countries. J Antimicrob Chemother, 2005; 55: 928–937.
24. Lee N.; Chen S.; Tang R. and Hwang B. Neonatal bacteremia in a neonatal intensive care unit: analysis of causative organisms and antimicrobial. J. chin. Med. Assoc. 67: 15-20.
25. Mack, D.; Fischer, W.; Krokotsch, A.; Leopold, K.; Hartmann, R.; Egge, H.; Laufs, R. The intercellular adhesion involved in biofilm accumulation Staphylococcus epidermidis linear beta-1, 6-linked glycosaminoglycan: purification and structural analysis. J. Bacteriol, 1996; 178: 175–183.
26. -Mathur,T.; Singhal, S.; Khan, S.; Upadhyay, D.J.; Fatma,T.and Rattan, A. Detection of biofilm formation among the clinical isolates of Staphylococci: An evaluation of three different screening methods. Indian Journal of Medical Microbiology, 2006; 24(1): 25-29.
27. Monzon,M.; Oteiza, C.; Leiva, J. and Amorena, B. Synergy of different antibiotic combinations in biofilms of Staphylococcus epidermidis. J. Antimicrob. Chemoter. 2001; 48: 793-801.
28. -Mohammed, W.F. Study on some Bacteriological and Immunological aspects of Septicemia in Neonate in Baghdad.College of Science. University of Al-Mustansiriya, master of Science in Biology/Microbiology.
29. Niveditha, S.; Pramodhini, S.; Umadevi, S.; Kumar, S.; Stephen, S. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). J. Clin. Diagn. Res, 2012; 6: 1478–1482.
30. Podschun, R.; Ullmann, U. Klebsiella spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin. Microbiol. Rev, 1998; 11: 589–603.
31. R. Antibiofilm efficacy of silver nanoparticles against biofilm of extended spectrum b-lactamase isolates of Escherichia coli and Klebsiella pneumoniae. Appl Nanosci, 2014; 4: 859–868.
32. Seyyed Mohammad H. A.; Azar D. K.; Masood D.; Farshid K.; Seyyed M.M. and Mohammad R. A. Identification of bacterial agents and antimicrobial susceptibility of

neonatal sepsis: A 54-month study in a tertiary hospital. African Journal of Microbiology Research, 2011; 5(5): 528-531.

33. Subramanian, P.; Shanmugam, N.; Sivaraman, U.; Kumar, S.; Selvaraj, S. Antiobiotic resistance pattern of biofilm-forming uropathogens isolated from catheterized patients in Pondicherry, India. Australas. Med. J, 2012; 5: 344–348.
34. Wojtyczka, R.D; Kamila, O.; Małgorzata, K.; Danuta, I.; Arkadiusz, D.; Tomasz, M.; Michał, K.;Maria, M. and Tomasz J. Wąsik. Biofilm Formation and Antimicrobial Susceptibility of Staphylococcus epidermidis Strains from a Hospital Environment. Int. J. Environ. Res. Public Health, 2014; 11: 4619-4633.
35. Wu, M.C.; Lin, T.L.; Hsieh, P.F.; Yang, H.C.; Wang, J.T. Isolation of genes involved in biofilm formation of a Klebsiella pneumoniae strain causing pyogenic liver abscess. PLoS One, 2011.
36. Yang, D.; Zhang, Z. Biofilm-forming Klebsiella pneumoniae strains have greater likelihood of producing extended-spectrum beta-lactamases. J. Hosp. Infect, 2008; 68: 369–371.
37. Zgair AK, ChhibberS. Adhering ability of Stenotrophomonas maltophilia is Dependent on Growth Conditions. Microbiol, 2011; 80: 466-471.