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Evaluation of biofilm formation of *Klebsiella pneumoniae* isolated from medical devices at the University Hospital of Tlemcen, Algeria

Samia Bellifa[1]*, Hafida Hassaine[1], Damien Balestrino[2], Nicolas Charbonnel[2], Imane M'hamedi[1], Ibtissem Kara Terki[1], Merieme Lachachi[1], Wafae Didi[1] and Christiane Forestier[2]

[1]Laboratoire de microbiologie appliquée à l'agroalimentaire au biomédical et a l'environnement (LAMAABE), Université de Tlemcen, Algérie.
[2]Clermont Université, UMR CNRS 6023 Laboratoire Microorganismes : Génome Environnement (LMGE) Université d'Auvergne, Clermont-Ferrand, France.

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Isolates of *Klebsiella pneumoniae* are responsible for opportunistic infections in humans, particularly of the urinary respiratory tracts. These bacteria express type 3 fimbriae that have been implicated in binding to eukaryotic cells and matrix proteins. Twenty four (24) *K. pneumoniae* strains isolated from medical devices were studied. Their capacity to form biofilm was assessed using two types of materials, polyvinylchloride (PVC) and glass of microfermenter in static or kinetic conditions. Strains with adherence to PVC also cling strongly to glass slides. We determined the *in vitro* effects of three antimicrobial agents against planktonic and biofilm forms of *K. pneumoniae* and we demonstrated that isolates of the biofilm form were at least 10-25 times more resistant than the planktonic form. Most strains of *K. pneumoniae* harbored the *mrkD* gene and exhibited a strong ability to adhere to inert surfaces.

Key words: Biofilm, Fimbriae, *Klebsiella pneumoniae*, polyvinylchloride (PVC), microfermenter.

INTRODUCTION

Klebsiella pneumoniae is an opportunistic pathogen that infects immunocompromised patients who are hospitalized or suffering from severe underlying diseases, such as chronic pulmonary obstruction or diabetes mellitus (Allen et al., 1991). These bacterial infections can lead to complications, including urinary tract infections, septicemia and pneumonia in the elderly or in patients with predisposing factors (Williams and Tomas, 1990). Infections due to *K. pneumoniae* are particularly devastating with a mortality rate between 25 and 60% (Ellis, 1998).

K. pneumoniae is naturally present at low concentrations in the environment, but also in the gastrointestinal tract and natural cavities of humans, and constitute aggregates called biofilms. Furthermore, formation of biofilms by *K. pneumoniae* on urinary catheters and intravenous and prosthetic heart valves has been documentted (Farber and Wolff, 1993; Galdiero et al., 1987; Liu, 1993). This property is considered as an important virulence factor for *K. pneumoniae* (Jagnow and Clegg, 2003). Biofilms are currently defined as structured bacterial communities en-closed in a self-produced exopolysaccharide *matrix* and adherent to abiotic or biological surfaces (Costerton et al., 1995). Biofilm formation can be divided into distinct stages, from the initial attachment

*Corresponding author. E-mail : samia.bellifa@yahoo.fr.

of bacteria to the surface to the formation of mature biofilm with a characteristic three-dimensional architecture. Many bacterial functions are required at each step, such as motility, adhesion, transport, stress response, activation of metabolic path-ways and extracellular matrix synthesis (Beloin et al., 2004; Domka et al., 2007). Biofilm producing bacteria are responsible for many recalcitrant infections and are notoriously difficult to eradicate. They exhibit resistance to antibiotics by various methods like restricted pene-tration of antibiotic into biofilms, decreased growth rate and expression of resistance genes (Kim, 2001). Most *K. pneumoniae* isolates express two types of fimbrial adhesins; type 1 and type 3 fimbriae (Schroll et al., 2010).

The expression of type 3 fimbriae has been shown to promote biofilm formation on biotic as well as abiotic surfaces (Di Martino et al., 2003; Jagnow and Clegg, 2003; Langstraat et al., 2001; Struve et al., 2009). These fimbriae belong to the chaperone-usher class of fimbriae and are encoded by five genes (*mrk ABCDF*) arranged in the same transcriptional orientation (Donlan, 2001; Duguid, 1959). This operon called *mrk* comprises the structural genes and those encoding the polypeptides required for assembly of the structure to the surface of the bacterium (Allen et al., 1991). The MrkD adhesin has been shown to mediate adhesion to collagen structures (Tarkkanen, 1990). In this study, biofilm formation by *K. pneumoniae* isolated from medical devices in Algeria was studied for the first time. The ability of these isolates to adhere on abiotic surfaces was investigated in a static (microplates) or kinetic (microfermenter) biofilm formation model. Polyvinylchloride (PVC) or glass slides of microfermenter were used as substrate. The aim was to assess the adherence capacities of the isolates to PVC and glass substrates. In addition, we looked for the presence of type 3 pili-encoding *mrkD* gene.

MATERIALS AND METHODS

K. pneumoniae strains

A total of 24 *K. pneumoniae* isolates were analysed in this study. Isolates were collected from urinary catheter and endotracheal tubes during various services at the University Hospital of Tlemcen (Intensive Care, Urology, and Neurology). All patients had serious disease and were undergoing longterm catheterization. Isolates were identified by conventional biochemical methods and completed with the API 20E (bioMerieux SA, Lyon, France) test kit.

Antibiotic resistance

The antibiotic resistance of isolates was tested against selected antibiotics using the standard protocol for dissemination of antimicrobial agents on Mueller- Hinton agar as described by the Clinical and Laboratory Standard Institute (CLSI, 2006). The antibiotic discs were obtained from Oxoid, England.

This test was used to determine the resistance patterns of the isolated strains. The Extended Spectrum Beta-Lactamase phenotype (ESBL) production was detected by the double disk synergy test (Jarlier et al., 1988).

Formation of biofilm in 96-well microplate

The ability of isolates to form biofilms on PVC plates was determined according to the procedure of O'Toole and Kolter (1998). Ten microlitres of a 1/10 dilution of an 18 h culture in M63B1 medium supplemented with 0.4 % glucose (0.1 optical density), was inoculated into the wells of a 96- multiwell plate (BD Falcon) . Each well contained 150 µL of the same medium. The microplates were incubated for 8 h at 37°C. After incubation, 150 µl of 0.5% (w/v) crystal violet solution was added to each well and the plate incubated for 15 min at room temperature. The plate was then washed, the dye was solubilized in 0.95% ethanol, and absorbance at 570 nm was determined by an ELISA plate reader (Expert, Plus, Asys). Isolates were considered as efficient in biofilm-forming when absorbance at 595 nm after crystal violet staining was equal to or more than 0.15. The data presented were derived from a single experiment which was performed in triplicate.

Formation of biofilm in microfermenters

Biofilm assays were performed in a dynamic microfermenter model (Developed by JM Ghigo) with M63B1+0.4% Glu medium for 24 h. Biofilms were grown either on the glass spatula of the microfermentor slides attached to the removable spatula of the microfermenters. Microfermentors were inoculated by placing the removable spatula for 1 min in 15 ml of a 0.1 optical density (OD) unit suspension from an overnight culture grown in minimal media. The spatula was then rinsed in 15 ml of sterile physiological water. After 24 h, the biofilm biomass was quantified by plating dilutions onto Lysogeny Broth (LB) agar plates to determine the number of colony forming units (CFUs) and by measuring OD (De Araujo et al., 2010).

Determination of MICs and biofilm susceptibility assay

Minimal inhibitory concentrations (MICs)

Minimal inhibitory concentrations (MICs) defined as the lowest concentration of antibiotic which inhibits the growth of a planktonic bacterial population were determined by the microtiter method as described in National Committee for Clinical Laboratory Standards NCCLS guidelines (CLSI, 2006). MICs were performed in 96-well microplates and results were recorded after incubation at 37°C for 18 h. We determined the MICs for three antimicrobial agents: gentamycin, cefotaxime and ciprofloxacin.

Biofilm susceptibility assay

Biofilms were allowed to form as described above. After incubation, the 96-well microplates were rinsed and placed in contact with various concentrations of antibiotics. The sealed plates were incubated at 37°C for 24 h. After incubation, the biofilm was washed three times with sterile PBS (pH 7.2), and the treated biofilm was scraped and transferred to another microplate. After 24 h of incubation at 37°C, the microplates were then observed for any visible growth of bacteria detached from the biofilm and by comparing the initial OD to that obtained after incubation with various concentrations of antibiotic and OD of the control without antibiotic.

Detection of the type3 pili-encoding gene

Bacteria were grown overnight with shaking at 37°C in 10 ml of LB. Briefly, 1 ml of the bacterial suspension was centrifuged (13,000 × *g*, 5 min), the pellet was recovered and resuspended in 300 µL of sterile water and heated to 90°C for 10 min. After centrifugation (13,000 × *g,* 5 min), the supernatant was directly used as template

Table 1. Distribution of strains according to the type of units, the medical device, implants duration and antibiotic resistance pattern.

Strain	Unit	Medical device	The duration of the device implantation	Antibiotic resistance pattern
Kp1	Intensive Care	Endotracheal tube	7 days	Amp, CAZ, CTX,OFX,CF
Kp4	Intensive Care	Endotracheal tube	72 h	Amp, CAZ, CTX, CF
Kp10	Intensive Care	Endotracheal tube	5 days	Amp, CAZ,CTX,CF
Kp5	Intensive Care	Endotracheal tube	7 days	Amp, CAZ, CTX, OFX, CF, Gn, TB, Cip
Kp15	Intensive Care	Urinary catheter	7 days	Amp, CAZ, CTX, OFX, CF, Gn,TB, Cip
Kp6	Intensive Care	Urinary catheter	7 days	Amp, CAZ,CTX,CF,GN, C, Cip
Kp7	Intensive Care	Endotracheal tube	5 days	Amp, CAZ, CTX
Kp8	Intensive Care	Urinary catheter	12 days	Amp, CAZ,CTX
Kp9	Intensive Care	Urinary catheter	7 days	Amp, CAZ,CTX,OFX, CF GN,TB, Na, Cip
Kp3	Intensive Care	Urinary catheter	7 days	Amp, CAZ, CTX, OFX,CF, Gn, Tb, Na
Kp11	Intensive Care	Urinary catheter	15 days	Amp, CAZ, CTX, CF, GN, C, Na
Kp12	Intensive Care	Urinary catheter	14 days	Amp, CAZ, CTX, CF
Kp13	Intensive Care	Urinary catheter	7 days	Amp,CAZ, CTX
Kp14	Urology	Urinary catheter	7 days	Amp,CAZ, CTX, OFX, CF, GN, TB, Na, Cip
Kp2	Urology	Urinary catheter	13 days	Amp, CAZ, CTX
Kp16	Urology	Urinary catheter	15 days	AmpCAZ, CTX, OFX, CF, GN, Tb, Na
Kp17	Urology	Urinary catheter	14 days	Amp, CAZ, CTX, CF
Kp18	Urology	Urinary catheter	7 days	Amp,CAZ, CTX, CF
Kp19	Urology	Urinary catheter	15 days	AmpCAZ,CTX, CF
Kp20	Urology	Urinary catheter	15 days	Amp, CAZ, CTX, CF
Kp21	Neurology	Urinary catheter	20 days	Amp, CAZ, CTX, CF, GN, TB, C, Na, Cip
Kp22	Neurology	Urinary catheter	8 days	Amp, CAZ, CTX, CF
Kp23	Neurology	Urinary catheter	8 days	Amp,CAZ, CTX, CF OFX GN, TB. Na, Cip
Kp24	Neurology	Urinary catheter	7 days	Amp, CAZCTX, CF, OFX, GN, TB, Na, Cip

Kp, K. pneumoniae; Amp, Ampicillin; CAZ, ceftazidime; CTX, cefotaxime; OFX, Ofloxacine; CF, Cefalotine; TB, Tobramycine; GN, Gentamycin; Cip, Ciprofloxacin; C, Chloramphenicol; Na, Nalidixic acid.

DNA for PCR amplification. PCR amplifications were performed using the Expand Long Template PCR system (Roche, Mannheim, Germany). In a final volume of 50 µM, 5 µL of template DNA was added to the amplification solution containing 5 µl of 10 x kit buffer, 0.5 µl of *Taq* polymerase, each deoxynucleoside triphosphate at a final volume of 0.1 µl, and each primer at a final concentration of 25 µM. Two primers were used: MrkD F: 5'-CCACCAACTATTCCCTCGAA-3' and MrkD R: 5'-ATGGAACCCACATCGACATT-3' (GenBank accession number for MrkD: AY225462) (Hennequin and Forestier, 2007). Cycling conditions were as follows: one denaturation step of 2 min at 94°C and 10 initial cycles of 10 s at 94°C, 30 s at 63°C, and 15 min at 68°C, followed by 20 iterative cycles of 10 s at 94°C, 30 s at 63°C, and 15 min plus 20 s for each new cycle at 72°C. A final elongation step of 7 min at 72°C was added. Amplified products were examined by 1.3 % agarose gel electrophoresis in 0.5 x TBE buffer.

Characterization of the strains studied

In this study, we collected urinary catheters and endotracheal tube from patients catheterized for longer than one week of hospitalization. Four strains were isolated from endotracheal tubes (*Kp*1, *Kp*4, *Kp*10, and *Kp*15) and 20 from urinary catheters (Table 1). The 24 clinical isolates from the hospital clustered into three API biotype profiles: 5215773, 5205773, 52155573. Antimicrobial profiles demonstrated that all the isolates were resistant to ceftazidime, amoxicillin/clavulanic acid, cefotaxime, and cefalotine; nine isolates were resistant to ofloxacine, six isolates were resistant to cipro-floxacin, three were resistant to chloramphenicol, 11 were resistant to gentamycin, eight were resistant to nalidixic acid, and all strains were susceptible to imipenem. Eight of them expressed the phenotype ESBL resulting in a synergy image between a disc containing an antibiotic inhibitor of ß lactamases (amoxicillin/clavulanic acid) and a disc of 3rdgeneration cephalosporin (ceftazidime).

Analysis of biofilm formation

The bacterial mass accumulated within the biofilms was quantified by staining of adherent bacteria and measuring the OD. In PVC titer plates, 12 isolates were high biofilm producers whereas 10 were moderate biofilm producers and two isolates were biofilm non-producers (Figure 1). In the microfermenter model, six isolates showed a strong adhesion to glass slides, 16 had a weak adhesion whereas two isolates did not adhere (Figure 2). An example of the levels of adhesion of *K. pneumoniae* to glass slides in a micro-fermenter is shown in Figure 3.

RESULT

Result of determination of MICs and Biofilm susceptibility

The MICs of the planktonic forms and antibiotic suscepti-

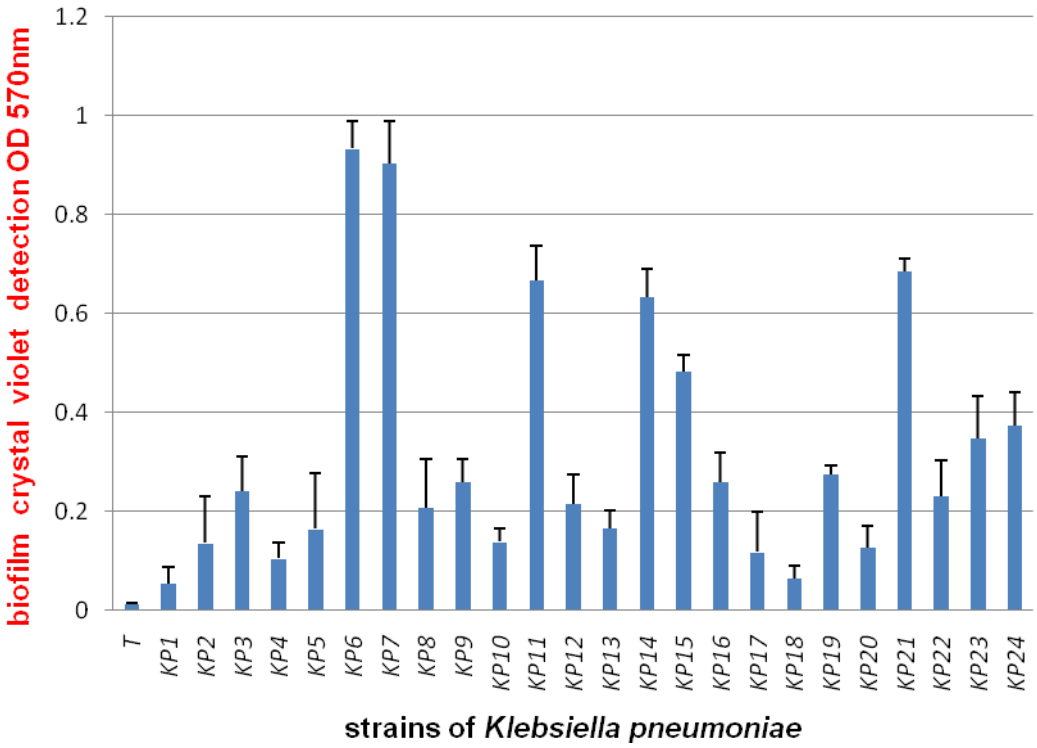

Figure 1. Quantification by measuring OD at 570 nm of crystal violet-stained biofilms formed by strains of *K .pneumoniae* in PVC microplates for 8 h in M36B1 glucose + 0. 4%. Values are means ± SD of three independent experiments. *KP3, KP6, KP14, KP15, KP21, KP5,* high biofilm producers; *KP2, KP6, KP7, KP11, KP14, KP15, KP21, KP23, KP24, KP9, KP16, KP19, KP3:* high biofilm producers*; KP2, KP4, KP5, KP17, KP18, KP20, KP10, KP8, KP3, KP13*, moderate biofilm producers; *KP1, KP18:* biofilm non-producers.

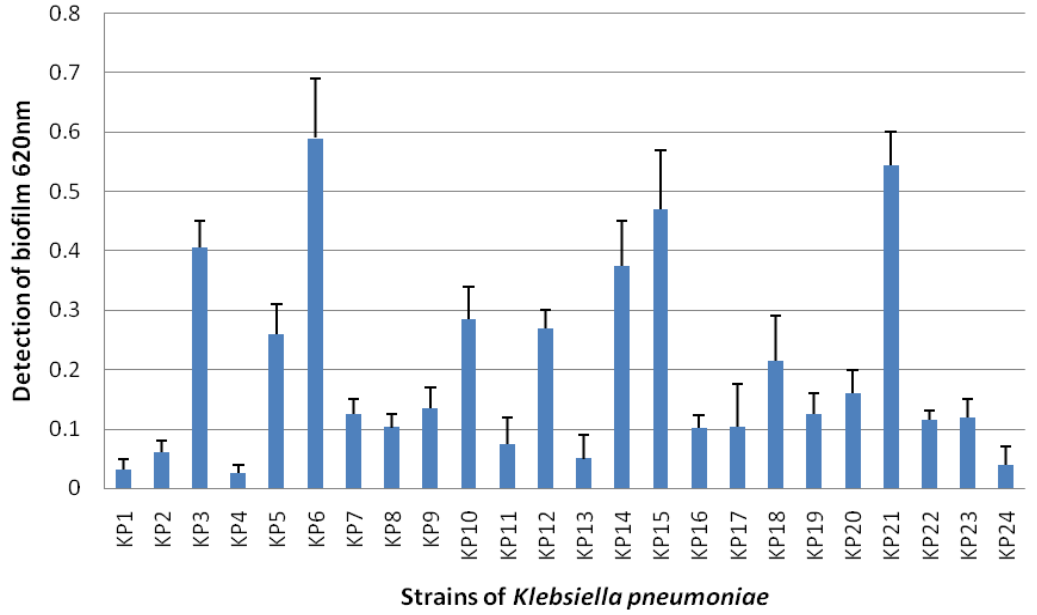

Figure 2. Quantification by measuring OD at 620 nm of biofilms formed by strains of *K. pneumoniae* on glass slides following incubation for 24 h in a microfermenter in M36B1 media supplemented with 0.4 % glucose. *KP3, KP6, KP14, KP15, KP21, KP5,* high biofilm producers; *KP2, KP7, KP8, KP9, KP11, KP16, KP17, KP19, KP20, KP22, KP23, KP10, KP12, KP13, KP18, KP24*, moderate biofilm producers; *KP1, KP4,* biofilm non-producers.

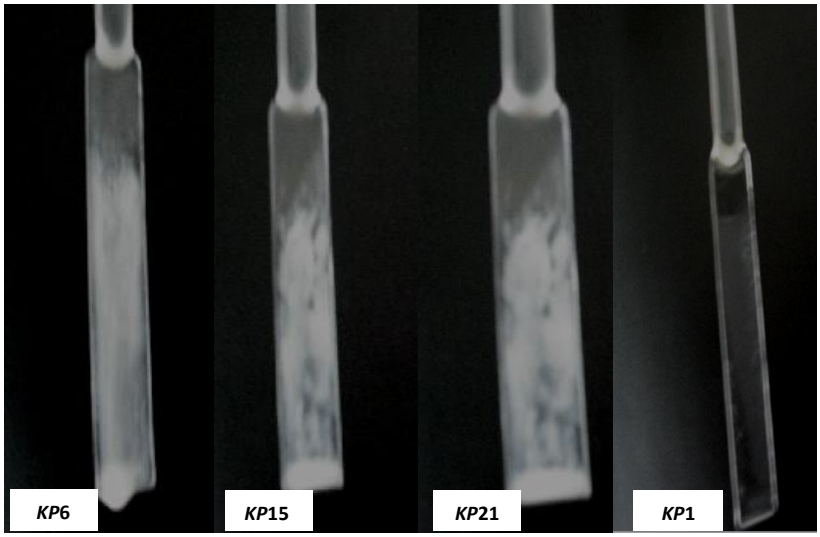

Figure 3. An example demonstrating the adhesion of *K. pneumoniae* to glass slides of microfermenteur after 24 h of incubation. *KP*6, *KP15, KP21*, strong adhesion. *KP1*, weak adhesion.

bility of biofilms for all strains of *K. pneumoniae* are shown in Table 2. The present results demonstrate that the concentration of gentamycin required to inhibit the biofilm form was on average 10 times higher than the MIC of the planktonic form and at least 25 times higher for cefo-taxime. As regards the ciprofloxacin three samples require double the amount of antibiotic and the remaining sam-ples show a slight difference.

Detection of type 3 pili-encoding genes

The PCR technique was applied to the 24 clinical isolates and one reference strains Kp Lm 21. A fragment of 500 bp specific to the type 3 fimbriae-encoding gene *mrkD* was detected in 22 out of 24 strains.

DISCUSSION

Over the past few decades, *K. pneumoniae* has emerged as a significant cause of severe nosocomial infections due to it being difficult to treat. The persistence of this species in hospitals is partly due to its ability to adhere and multiply on inanimate surfaces. Little is known about the bacterial factors involved in *K. pneumoniae* adherence to abiotic surfaces (Podschun and Ullmann, 1998). It has been previously reported that quorum sensing is involved in biofilm formation by *K. pneumoniae* (Balestrino et al., 2005). Other studies demonstrated the influence of capsule and extended-spectrum beta-lactamase encoding plasmids upon *K. pneumoniae* adhesion (Hennequin and Forestier, 2007). A high prevalence of type 1 and type 3 pili has been previously observed in clinical and environmental *K. pneumoniae* isolates (Livrelli et al., 1996; Martynenko et al., 1992; Podschun et al., 2001; Schurtz et al., 1994). Type 3 pili, mostly found in *K. pneumoniae*, are also involved in biofilm formation by this bacterial species (Di Martino et al., 2003) and are generally considered as a virulence factor. In this study, 24 strains of *K. pneumoniae* were isolated from urinary catheters and endotracheal tubes at the University Hospital of Tlemcen. Between 15 and 25% of patients in general hospitals had a urinary catheter in place sometime during their stay (Warren, 2001). Patients studied with a long term urinary catheterization (average length of stay of 10 days) had a nosocomial urinary tract infection. These results emphasize previous observations showing that the duration of the device implantation significantly influenced biofilm formation (Domka et al., 2007). This can be explained by several risk factors, the most important is the open drainage system that all the studied patients had, which remains a major cause of nosocomial urinary tract infections. In our study, the majority of biofilm producing bacteria were isolated from urinary catheter tips

Similarly Hennequin and Forestier (2007) reported in a previous study the association of biofilm producing bacteria with colonization of urinary catheters. Long term catheters become colonized by extensive biofilms, which can have profound effects on the health of the patient. Urinary tract infections in catheterized patients can occur in several ways. Organisms that colonize the periurethral skin can migrate into the bladder through the mucoid film that forms between the epithelial surface of the urethra and the catheter. In addition, contamination of the urine in the drainage bag can allow organisms to access the bladder through the drainage tube and the catheter lumen (Stamm, 1991; Tambyah et al., 1999). The reason why biofilms are so prevalent on urinary catheters is that they convey a survival advantage to the microorganisms for the same reason; urinary catheter biofilm is difficult to eradicate. In hospitals and other medical institutions, *K.*

Table 2. Susceptibility of *K. pneumoniae* isolates to three antibiotics of both the planktonic (MICs) and the biofilm forms.

Strain	Gentamycin (mg/L)		Cefotaxime (mg/L)		Ciprofloxacin (mg/L)	
	MIC	Biofilm susceptibility	MIC	Biofilm susceptibility	MIC	Biofilm susceptibility
Kp5	0.128	2.56	1.024	10	0.5	1
Kp14	0.512	2.56	1.024	10	0.5	1.05
Kp15	0.512	10	0.512	5	1	1.05
Kp21	1.024	10	0.512	5	1	1
Kp23	0.128	10	1.024	10	1	1.05
Kp24	0.512	10	1.024	10	0.5	1
Kp3	0.512	10	0.128	10		
Kp6	0.256	10	0.128	10		
Kp9	0.256	10	0.256	3		
Kp11	1.024	10	0.256	3		
Kp16	0.512	10	1.024	10		
Kp2	0.512	10	1.024	10		
Kp7			2.048	15		
Kp8			2.048	10		
Kp10			2.048	15		
Kp12			0.256	1.5		
Kp13			0.256	1.5		
Kp17			0.512	1.5		
Kp18			1.024	10		
Kp19			0.256	1.5		
Kp20			0.128	1.5		
Kp22			0.256	3		
KP1			1.024	1.5		
KP4			0.128	1.5		

pneumoniae has emerged as a significant and problematic human pathogen. This bacterium has a capacity to quickly acquire antimicrobial resistance. The resistance profile in this study revealed a remarkable resistance to most of the antibiotic agents tested, however, a significant susceptibility to ciprofloxacin was observed. The capacities of our isolates to form biofilm were assessed using PVC substrate (microplates) and glass slides in a microfermenter system. Most strains had a capacity to form biofilm on PVC and six strains were highly forming biofilm. These six strains also adhered highly to the glass slide whereas two isolates did not adhere to slides of the microfermenter. For the remaining strains, the biofilm formation on PVC is more important than glass.

One of the most commonly studied properties of biofilms is their increased resistance to the effects of antibiotics (Liaqat et al., 2009). We demonstrated a clear difference in antibiotic susceptibility between planktonic and biofilm populations, similar to results previously reported with the Calgary Biofilm Device (Ceri et al., 1999). The reasons for the higher resistance of cells embedded in biofilms may include limited diffusion of antibiotics into the biofilm or decreased bacterial growth. Some antibiotics can react with the biofilm matrix and, on the other hand, the cells in biofilms can adapt and form protected phenotypes (Amorena et al., 1999; Costerton et al., 1999; Hoyle and Costerton, 1991; Monzon et al., 2001; Stewart and Costerton, 2001; Watnick and Kolter, 2000). The present results demonstrate that the CMIs of biofilm form for gentamycin and cefotaxime was at least 10 times higher than the CMIs of planktonic form. As regards the ciprofloxacin, we did not find a large difference between the MIC and biofilm susceptibility compared with other antibiotics. Agents that penetrate the biofilm matrix, such as rifampin and the fluoroquinolones have been shown to be effective (Abdi-Ali et al., 2006). These results would indicate that the biofilm formation should be considered in antimicrobial therapy.

Previous studies have revealed that type 3 fimbriae are important in *K. pneumoniae* biofilm formation (Allen et al., 1991; Jagnow and Clegg, 2003; Langstraat et al., 2001). Therefore, the MrkD may play significant roles in forming biofilms on medically inserted devices. *K. pneumoniae* is an opportunistic pathogen frequently associated with infections in hospitalized patients with indwelling urinary catheters or endotracheal tubes leading to nosocomially acquired urinary and respiratory tract infections, respectively (Johnson and Clegg, 2010). For this reason in the

present study, we looked for the presence of the *mrkD* gene in our isolates genome. Twenty two (22) strains that harbored the gene had the capacity to form biofilm on the two types of materials (PVC and glass). Our results support a role for type 3 pili in the *K. pneumoniae* interaction with abiotic surfaces.

In summary, we demonstrated that most strains with high adherence to PVC cling strongly to glass slides and that the biofilm forms of the isolates were at least 10 times more resistant than their planktonic counterparts. In addition, we showed that the presence of type 3-encoding gene *mrkD* was associated with high adhesion indexes.

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REFERENCES

Abdi-Ali A, Mohammadi-Mehr M, Alaei YA (2006). Bactericidal activity of various antibiotics against biofilm-producing *Pseudomonas aeruginosa*. Int. J. Antimicrob. Agents 27:196-200.

Allen BL, Gerlach GF, Clegg S (1991). Nucleotide sequence and functions of mrk determinants necessary for expression of type 3 fimbriae in *Klebsiella pneumoniae*. J. Bacterial. 173(2):916-20.

Amorena B, Gracia E, Monzon M, Leiva J, Oteiza C, Perez M, Alabart JL, Hernandez-Yago J (1999). Antibiotic susceptibility assay for *Staphylococcus aureus* in biofilms developed *in vitro*. J. Antimicrob. Chemother. 44:43-55.

Balestrino D, Haagensen JA, Rich C, Forestier C (2005). Characterization of type 2 quorum sensing in *Klebsiella pneumoniae* and relationship with biofilm formation. J. Bacteriol.187:2870-2880.

Beloin C, Valle J, Latour-Lambert P, Faure P, Kzreminski M, Balestrino D, Haagensen JA, Molin S (2004). Global impact of mature biofilm lifestyle on *Escherichia coli* K-12 gene expression. Mol. Microbiol. 51:659-674.

Ceri H, Olson M, Stremick C, Read RR, Morck D, Buret A (1999). The Calgary Biofilm Device: a new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J. Clin. Microbiol. 37:1771-1776.

CLSI (2006). Clinical and Laboratory Standards Institute (CLSI): Performance Standard for Antimicrobial Susceptibility Testing. 16th Informational supplement. CLSI document M100-S16. Wayne, PA.

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scottn HM (1995). Microbial biofilms, Annu. Rev. Microbiol. 49:711-745

Costerton JW, Stewart PS, Grenberg EP (1999). Bacterial biofilms: a common cause of persistent infections. Science 284:1318-1322.

De Araujo C, Balestrino D, Roth L, Charbonnel N, Forestier C (2010). Quorum sensing affects biofilm formation through lipopolysaccharide synthesis in *Klebsiella pneumoniae*. Res. Microbiol. 161:595-603

Di Martino P, Cafferini N, Joly B, Darfeuille-Michaud A (2003). *Klebsiella pneumoniae* type 3 pili facilitate adherence and biofilm formation on abiotic surfaces. Res. Microbiol. 154:9-16.

Domka J, Lee J, Bansal T, Wood TK (2007). Temporal gene-expression in *Escherichia coli K-12* biofilms. Environ. Microbiol. 9:332-346.

Donlan RM (2001). Biofilms and device-associated infections. Emerg Infect Dis. 7(2):277-281.

Duguid JP (1959). Fimbriae and adhesive properties in *Klebsiella* strains. J Gen Microbiol. 21:271-286.

Farber BF, Wolff AG (1993). Salicilyc acid prevents the adherence of bacteria and yeast to silastic catheters. J. Biomed. Mater. Res. 27:599-602.

Galdiero F, Cotrufo M, Catalanotti PG, De Luca TS, Ianniello R, Galdiero E (1987). Adherence of bacteria to cardiac valve prostheses. Eur. J. Epidemiol. 3:216-221.

Hennequin C, Forestier C (2007). Influence of capsule and extended-spectrum beta-lactamases encoding plasmids upon *Klebsiella pneumoniae* adhesion. Res. Microbiol. 158:339-347

Hoyle BD, Costerton JW (1991). Bacterial resistance to antibiotics: the role of biofilms. Progr. Drug Res. 37: 91-105.

Jagnow J, Clegg S (2003). *Klebsiella pneumoniae* MrkD-mediated biofilm formation on extracellular matrix- and collagen-coated surfaces. Microbiology 149:2397-2405.

Jarlier V, Nicolas MH, Fournier G, Philippon A (1988). Extended broad-spectrum b-lactamases conferring transferable resistance to newer b-lactam agents in enterobacteriaceae, hospital prevalence and susceptibility patterns. Rev Infect Dis. 10:867-878.

Johnson JG, Clegg S (2010). Role of MrkJ, a phosphodiesterase, in type 3 fimbrial expression and biofilm formation in J.bacteriol. 192(15):3944-3950.

Kim L (2001). Riddle of biofilm resistance. Antimic Ag Chemother. 45(4):999-1007.

Langstraat J, Bohse M, Clegg S (2001). Type 3 fimbrial shafts (MrkA) of *Klebsiella pneumoniae*, but not the fimbrial adhesin (MrkD), facilitates biofilm formation. Infect. Immun. 69:5805-5812.

Liaqat I, Sumbal F, Sabri AN (2009). Tetracycline and chloramphenicol efficiency against selected biofilm forming bacteria. Curr. Microbiol. 59(2):212-220.

Liu WK, Tebbs SE, Byrne PO, Elliott TS (1993).The effects of electric current on bacteria colonizing intravenous catheters. J. Infect. 27: 261-269.

Livrelli V, De Champs C, Di Martino P, Darfeuille-Michaud A, Forestier C, Joly B (1996). Adhesive properties and antibiotic resistance of *Klebsiella pneumoniae*, *Enterobacter*, and *Serratia* clinical isolates involved in nosocomial infections. J. Clin. Microbiol. 34:1963- 1969

Martynenko LD, Zemliankina LP,Vilko-Baturo AP (1992). The surface structures and biological properties of *Klebsiella* strains, Mikrobiol. Zh. 54:14-18.

Monzon M, Oteiza C, Leiva J, Amorena B (2001). Synergy of different antibiotic combinations in biofilms of *Staphylococcus epidermidis*. J. Antimicrob. Chemoter. 48:793-801.

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

Podschun R, Pietsch S, Holler C, Ullmann U (2001). Incidence of *Klebsiella* species in surface waters and their expression of virulence factors. Appl. Environ. Microbiol. 67:3325-3327.

Podschun R, Ullmann U (1998). *Klebsiella* spp as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin. Microbiol. Rev. 11: 589-603.

Schurtz TA, Hornick, DB, Korhonen, TK, Clegg S (1994).The type 3 fimbrial adhesin gene (*mrkD*) of *Klebsiella* species is not conserved among all fimbriate strains. Infect. Immun. 62:4186-4191.

Schroll C, Barken K, Krogfelt K, Struve C (2010). Role of type 1 and type 3 fimbriae in *Klebsiella pneumoniae* biofilm formation. BMC Microbiology 10(1):179.

Stamm WE (1991). Catheter-associated urinary tract infections: epidemiology, pathogenesis, and prevention. Am. J. Med. 91: 65-71

Stewart PS, Costerton JW (2001). Antibiotic resistance of bacteria in biofilms. Lancet 358:135-138.

Struve C, Bojer M, Krogfelt KA (2009). Identification of a conserved chromosomal region encoding *Klebsiella pneumoniae* type 1 and type 3 fimbriae and assessment of the role of fimbriae in pathogenicity. Infect. Immun. 77(11):5016-5024.

Tambyah PA, Halvorson KT, Maki DG (1999). A prospective study of pathogenesis of catheter-associated urinary tract infections. Mayo Clin. Proc. 74:131-136.

Tarkkanen AM, Allen BL, Westerlund B, Holthofer H, Kuusela P, Risteli L, Clegg S, Korhonen TK (1990). Type V collagen as the target fortype-3 fimbriae, enterobacterial adherence organelles. Mol. Microbiol. 4:1353-1361.

Warren JW (2001). Catheter-associated urinary tract infections. Int. J. Antimicrob. Agents 17:299-303.

Watnick P, Kolter R (2000). Biofilm, city of microbes. J. Bacteriol. 182:2675-2679.

Williams P, Tomas JM (1990). The pathogenicity of *Klebsiella pneumoniae*. Med. Microbiol. 21:271-286.