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Original article

A Study On The Antimicrobial Resistance Of Klebsiella Pneumoniae Isolated From Patients With Urinary Tract Infections And Its Correlation With Tendency Of Biofilm Formation.

NaglaaAbd El-MoneimRadi [a], Ekram Adham Abd El-kader [a], Ossama Mahmoud Mohamad [b]and Ahmed Mohamed Gamal El-DinWahba [a]

[a]*MedicalMicrobiology and Immunology department, Faculty of Medicine, Beni-suef University, Egypt*

[b]*Urology department, Faculty of Medicine, Beni-Suef University, Egypt*

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Corresponding Author:

Ekram Adham Abd El-kader

akramadhm207@gmail.com

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Abstract

Background: *Klebsiella pneumoniae* is one of the common causes of nosocomial infection. Antibiotic resistance in addition to the capability of forming biofilm, as 2 essential virulence factors of *K. pneumoniae*, are responsible for the persistent infections. The aim of the present study was to assess the correlation between antimicrobial resistance and the ability to form biofilm in *K. pneumoniae* strains isolated from hospitalized cases at urology department with their tendency to form biofilm. **Methods**: Over a 6-month duration, a total of 25 *K. pneumonia* isolates were collected. Antibiotic susceptibility was detected using Kirby–Bauer disk diffusion approach based on CLSI. Biofilm formation was evaluated using tissue culture plate method. Lastly, isolates were tested for ESBL production. **Results:** All the identified *klebsiella* isolates (100%) showed resistance to cefepime, 92 percent of the isolates showed resistance to ceftazidime, 80% showed resistance to both

cefotaxime and ceftriaxone and 72% were resistant to ciprofloxacin, 28% showed multidrug resistance, 20% were extensive drug resistant and 8% were pan drug resistant. The results of biofilm formation in the tissue culture plate assay indicated that 15 (60 %) strains might produce biofilm and only 10 (40 %) isolates weren't capable of forming biofilm. **Conclusion:** The current study findings supported the importance of biofilm formation in resistance to antimicrobial agents.Further studies concerning the mechanism of biofilm production in *K. pneumoniae* may finally help treating biofilm-mediated infections.

1. Introduction:

Klebsiella pneumonia is actually determined clinically as essential opportunistic pathogens account for healthcare accompanying infections, such as septicemia, soft tissue infection, pneumonia and urinary tract infections (UTI) (1).

Indwelling urinary tract catheterization (IUTC) is considered one of the frequent intervention protocol needed in hospitalized cases and each day it remains, a patient has a 3 - 7 percent increase in the risk of acquiring a catheter-associated urinary tract infection (CA-UTI) (2).

*Klebsiella pneumoniae*is is a multidrug resistant (MDR) microrganism proved to be an urgent threat to our health by the WHO, and the US Centers for Disease Control and Prevention (3).

Biofilms are the microbial communities of the surface-attached cells which are embedded in aself-produced extracellular polymeric matrix.Bacterial biofilms have been associated with greater than sixty percent of nosocomial infections and eighty percemn of all microbial infections occurring as chronic and persistent infections due to relapse with increasing the cost of treatment, time of morbidity and burden of infections with serious public health significance (4).

Many of the *K. pneumonia* strains are capable of forming biofilms, where bacteria are enclosed in an extracellular polysaccharide (EPS) matrix, that leads to high antibiotic impermeability (5)

In addition, the production of biofilms can protect the bacteria from being eradicated by phagocytic cells (6).

2. Patients and Methods:

This was a cross sectional study among in-patients of the Urology ward at Beni-Suif University Hospital, between July and December, 2021. A total of 98 urine specimens were obtained from catheterized

and non-catheterized patients who met the criteria of study (Developed ≥ 2 symptoms of UTI after ≥ 2 days of hospitalization).

- ***Sample collection***

Mid-stream morning urine specimens were collected aseptically into a sterile container from non-catheterized patients and from catheterized patients directly from catheter valve not from drainage bag, then taken to the Laboratory for immediate processing.

- ***Isolation and Identification of Bacteria:***

Urine samples were cultured on conventional culture media. Bacterial pathogens were identified on the basis of gram staining, cultural and morphological features in combination with biochemical reactions.

Bacterial counts $<10^5$ CFU were excluded(4).

- ***Antibiotic Susceptibility Testing of the Isolates :***

The disc diffusion approach of Bauer Kirby was to confirm the sensitivity of isolates to different antibiotic agents.

The resulting diameter of inhibition zone was assessed in millimeter and interpreted based on CLSI guidelines (7).

- ***Phenotypic detection of ESBL:***

Phenotypic detection of ESBL was carried out by Phenotypic confirmatory disc diffusion method and interpreted according to CLSI guidelines. (7).

- ***Biofilm assay:***

Biofilm assay was done using microtitre plate adherence method (8).

- ***Ethical considerations***

Ethical clearance of the study was obtained from Local Research Ethical Committee(REC) at Beni-Suef University, Faculty of medicine. The objective of the study was clarified to the patients in Arabic and their right to say no to participate in this study was explained to them. Once the patients showed agreement to participate in our study they were signed a consent form.

Approval No: FMBSUREC/09052021/abd El-kader.

- **Statistical methodology**

Data were collected then entered and coded into the SPSS version 25 for windows.

3. Results:

The present study was performed on 25 *klebsiella* isolates obtained from 98 patients who were admitted to urology department at Beni-Suif University Hospital in the period betweenJuly 2021 till December 2021 and were diagnosed as hospital acquired urinary tract infection.

In our study the commonest isolated organisms were *E-coli* (51%) followed by *k. pneumonaie (25.5%),proteus (10.2%)*, and the least prevalent isolates were *staph.aureus* (4.1%) and *enterococci* (4.1%).

All the identified *klebsiella pneumonaie* isolates in our study, were resistant to cefepime, 92% of theisolates were resistant to ceftazidime, 80% were resistant to both cefotaxime andceftriaxoneand 72% were resistant tociprofloxacin while 80% of isolates weresensitive to both imipenem and meropenem, and72% were sensitive to gentamicin.

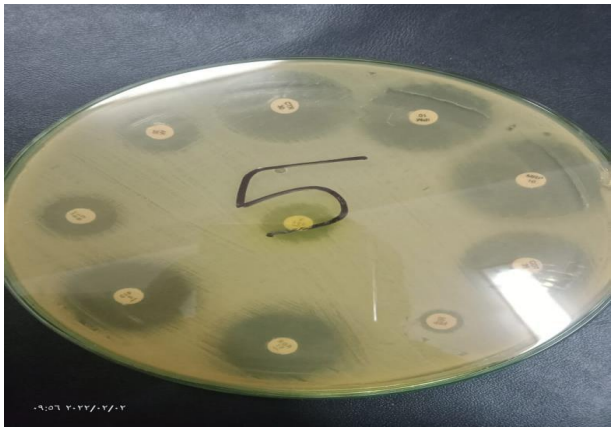


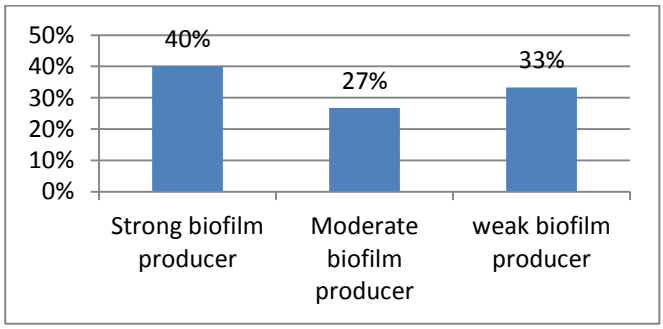
Fig (1)Antibiotic sensitivity test by disk diffusion approach.

In the current study, There were 10(40.0%) of the *Klebsiella pneumonia* isolates with extended spectrum β-lactamase production and 15 (60%) were non-extended spectrum β-lactamase producers.

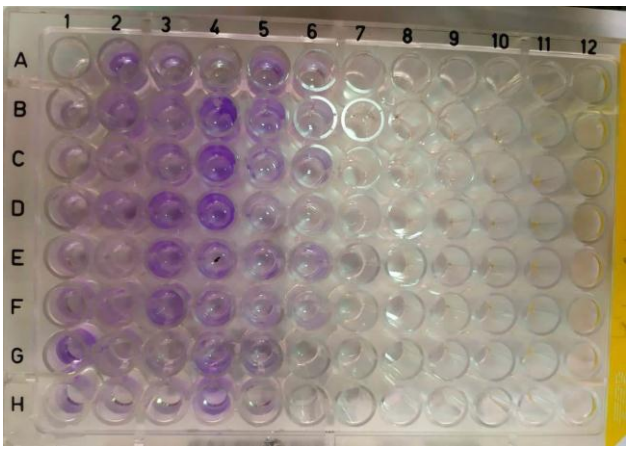
In our study,10 (40%) isolates were non-biofilm producers, and 15(60%) isolates were biofilm producers as shown in figure (2)

Fig (2) screening of ESBL using PCDDT.

Fig (3) Biofilm pattern among biofilm producing *klebsiella pneumonia* isolates.



We found that 6 (40%)among biofilm producers were strong biofilm producers, 4 (26.7%) were moderate while 5(33.3%) were weak biofilm producers as shown in figure (2).



Fig(4)Biofilm screening using tissue culture plate assay.

Table (1) Association between biofilm production and catheterization:

Catheter	BIOFILM		Total
	Biofilm producer	non-biofilm producer	
+Catheterized	4	1	5
	80.0%	20.0%	100.0%
non catheterized	11	9	20
	55.0%	35.0%	100.0%
Total	15	10	25
	100.0%	100.0%	100.0%
P-value	0.716		

In our study, we found that there is insignificant relation between catheterization and biofilm formation by bacteria as shown in table (1).

In our study, there is a statistically significant difference between strong, moderate, weak biofilm producers and non-biofilm producers regarding the distribution of sensitivity& resistance to ceftriaxone, imipenem, meropenem, gentamicin, amikacin, and nitrofurantoin.

Table (2) Correlation between the strength of biofilm formation and the strength of resistance to each antibiotic:

Antibiotic resistance		Degree of strength of biofilm
ceftriaxone	Correlation Coefficient (r)	.218
	P-value	.295
imipenem	Correlation Coefficient (r)	.626**
	P-value	.001
meropenm	Correlation Coefficient (r)	.626**
	P-value	.001
gentamicin	Correlation Coefficient (r)	.788**
	P-value	.000
amikacin	Correlation Coefficient (r)	.803**
	P-value	.000
ciprofloxacin	Correlation Coefficient (r)	.283
	P-value	.170
norfloxacin	Correlation Coefficient (r)	.458*
	P-value	.021
sulph-trim	Correlation Coefficient (r)	.104
	P-value	.620
nitrofurantoin	Correlation Coefficient (r)	.437*
	P-value	.029
cefotaxime	Correlation Coefficient (r)	.339
	P-value	.098
ceftazidime	Correlation Coefficient (r)	.321
	P-value	.117

***P-value is significant**

In the current, we found that a significant positive linear correlation was determined between the strength of biofilm formation and the strength of resistance to imipenem meropenem, gentamicin, amikacin, ciprofloxacin, norfloxacin and nitrofurantoin as shown in table (2).

4. Discussion:

Nosocomial *Klebsiella* infection is still a heavy burden on the economy as well as on the life expectancy of diseased individuals in developed countries. Therefore, further progress in preventing hospital-acquired infections necessitates novel modalities to control infections. The increased evidence regarding the capability of *K. pneumonia* to produces biofilms, predominantly on medical equipment and the novel data that support the correlation of such a behavior with acquiring resistance to the antibiotics should alert even more as regards the hazards of such pathogen in hospital setting. (9)

The current study aimed at evaluation the correlation between antibiotic resistance and the capability of *klebsiella pneumonaie* to form biofilm.The present study is cross sectional study, it was done at the urology department at Beni-Suef University Hospital over a period of six months (July 2021 till December 2021). It included 98 patientswho have hospital acquired urinary tract infections.

In this study, we found that the commonest isolated uropathogens were *E.Coli* (51%) followed by *Klebsiella pneumonaie* (25.5%), *Proteus (*10.2%), and *Pseudomonas* (5.1%) with the least isolated uropathogens were *Enterococci* (4.1%) and *Staph aureus* (4.1%)**.** ***Bitew and Abdeta, 2022***(10)reported that *E.coli* was the commonest bacterium responsible for 42.9 percent (21/49) of the total bacterial isolates while *K. pneumoniae* comes after it representing 34.6 percent (17/49) of the bacterial isolates.

The majority of *K. pneumonia* was resistant to several antibiotics, with cefepime, ceftriaxone, ciprofloxacin, cefotaxime, ceftazidime and sulpha-trimethoprim showing high resistance. This agreed with a study by ***Ahmed et al., 2022***(11) who documented high resistance to cephalosporins. Also ***Shadkam et al., 2021***(1) reported high resistance pattern to cefotaxime, cefepime, and ceftriaxone. Furthermore, ***Azim et al., 2019***(12)reported that 100% of the isolates showed resistance to cefotaxime, ceftazidime, ceftriaxone, cefepime and ampicillin.

In our study, we found thatimepenem, meropenem, gentamicin, amikacin and nitrofurantoin showed high sensitivity 80%, 80%, 72%, 56% and 56% respectively .***Azim et al., 2019***(12)stated that 91.3% of the isolates were sensitive to imepenem and amikacin, and 65% were sensitive to getamicin.***Abd El-Mongy et al., 2018*** (13)also reported high susceptibility to amikacin68.75% and gentamycin 73.75%, in Egypt.

In our study, 40% of isolates were ESBL producers. A study in Egypt identified eighty (53.3%) *K. pneumoniae* isolates out of one hundred fifty different clinical specimens (13).***Aitta et al., 2013***(14) revealed high prevelance of ESBL producing *klebsiella pneumoniae*(50%). A previous study in Egypt

concluded the isolation of 21percent (30/138) ESBL-producing *K. pneumonia*e from various clinical samples that included urine and blood in cases with suspected nosocomial infection (15).

In this study, 60% of isolates were biofilm producers and 40% were non-biofilm producers. Among the biofilm producers, 40% were strong, 27% were moderate and 33% were weak biofilm producers. This is supported by ***Shadkam et al., 2021***(1)who revealed that 75% of *klebsiella* isolates were biofilm producers.A similar study documented by ***Hassan &Khider, 2019***(16) exhibited that out of 110 *K. pneumonia* tested, 70 isolates (64.7%) were identified as high or moderate biofilm producers and 40 isolates (35.3%) were determined as weak biofilm producers. A study carried out by ***Cepas et al., 2019***(17) revealed that 37.6% of *K. pneumoniae* strains were biofilm producers. .

When considering the strength of the biofilm formation, the antimicrobial resistance among strong, moderate, and weak biofilm producing *K. pneumonia* strains was proved to be significantly elevated in comparison with that of non-biofilm producing *K. pneumonia* strains ($p < 0.05$). The correlation between the strength of biofilm production and antimicrobial resistance was proved to be statistically significant ($p < 0.05$) in many antibiotics from various classes; ceftriaxone, imipenem, meropenem, gentamicin, amikacin, and nitrofurantoin. However, the correlation wasn't significant in relation to ciprofloxacin, norfloxacin, sulpha-trimethoprim, cefotaxime and ceftazidime.

Nirwati et al., 2019(5) carried out a study to detect the drug resistance profile and biofilm-producing ability of *K.pneumoniae* isolated from clinical samples. In line with our study, a remarkablerate of their isolates were biofilm producers. They concluded that drug resistance was higher in *K.pneumoniae* which are biofilm producers, in comparison with non-biofilmproducer. Also, ***Yang et al.,2008***(18) found a significant association between antimicrobial resistance and biofilm production in clinical isolates of *K. pneumoniae*.

Nevertheless, on the contrary of our results, ***Hassan &Khider, 2019***(16) revealed that the susceptible isolates to antibiotic agents tend to produce stronger biofilms when compared to the resistant strains.

5. Conclusion and Recommendations:

The findings of the current study confirmed the significance of biofilmformation in resistance toantimicrobial agents. The vast majority of the *K. pneumonia* strains isolated from hospitalized cases can form biofilms. Further research concerning the mechanism of biofilm formation in *K. pneumoniae* can finally assist in treating biofilm-mediated infections and in reducing mortality as well as

the morbidity in cases having life-threatening nosocomial infections.

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