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The Association Between Antibiotic Resistance and Biofilm Production in *Klebsiella pneumoniae* Isolated from Urinary Tract Infections.

Safa'a Mohammed M Darwiesh [1] , Hassan Abdulwahab Al-Shamahy [1,3,*], Dekra A El-Aghbary [1]
Khaled Abdulkarim Al-Moyed [1,] , Anas A. AL-Mabashi [4] , Aldualraoof M. Al-Shawkany [5]

1. Department of Medical Microbiology and Clinical Immunology, Faculty of Medicine and Health Sciences, Sana'a University, Sana'a, Yemen.
2. University of 21 September for Medical and Applied Sciences, Sana'a, Yemen.
3. Basic Sciences, Faculty of Dentistry, Sana'a University, Sana'a, Yemen.
4. Department of Biological Science, Faculty of Science, Sana'a University, Sana'a, Yemen.
5. Department of Molecular Genetic, Fcculty of Veterinary Medicine, Sana'a University, Sana'a, Yemen.

***Corresponding author:** *shmahe@yemen.net.ye*

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ABSTRACT

Background and aims This research investigates the link between biofilm formation and antibiotic resistance among *Klebsiella pneumoniae* bacteria in Sana'a city, Yemen, a common cause of hospital-acquired infections and community-acquired infections.

Methods: A 6-month study investigated 300 urinary tract infections samples, with 241 culture-positive. Standard methods were used for isolation and identification, including biofilm production and antibiogram susceptibility patterns of *Klebsiella pneumoniae*.

Results: The study reveals that penicillin resistance is linked to *Klebsiella pneumoniae's* ability to form biofilms. Ampicillin-resistant isolates formed biofilms 97.5% of the time, while non-biofilm-producing isolates had a lower rate of resistance. Carbapenem group resistance was also significant, with Meropenem-resistant isolates forming biofilms 42.5% of the time. Cephalosporin resistance ranged from 80-95% for Biofilm-Forming Isolates, while Quinolones resistance ranged from 65-82%. Aminoglycoside resistance was also significant, with gentamicin and amikacin resistance rates being 42.5% and 42.5%, respectively. These findings highlight the importance of understanding the phenotypes of *Klebsiella pneumoniae* in forming biofilms.

Conclusion: The study found a strong correlation between the biofilm production of *Klebsiella pneumoniae* and antibiotic resistance, with quinolones and cephalosporins being the least active, while carbapenems and aminoglycosides showed slight sensitivity.

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1. Introduction:

Bacterial resistance to antibiotics has been steadily rising as a result of the extensive usage of antibiotics worldwide. *Klebsiella pneumoniae* (*K. pneumoniae*) is becoming more virulent and resistant to drugs as a result of the increasing frequency of functional gene acquisition through mobile components [1–5]. *Klebsiella pneumoniae* is a common Gram-negative *Enterobacteriaceae* family bacterium that can coexist with its host as a symbiotic partner in the intestinal mucosa, skin, and nasopharynx [6]. Numerous infections, including those of the Respiratory Tract System (RTIs), Blood Stream (BSIs), liver abscess, and Urinary Tract System (UTIs), might be brought on by it [7, 8]. The development of innovative therapies is particularly important due to the major health danger posed by Antimicrobial-Resistant *Enterobacter* species, *Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter faecium*. Because *K. pneumoniae* can evade the inhibitory effects of antibiotics, it belongs to the ESKAPE pathogen group [7, 8]. The production of biofilms further increases this bacterium's resistance to antibiotics [3]. A structured population of bacteria encased in Extracellular Polymeric Substance (EPS) is referred to as a biofilm [3]. 90% of the biofilm is made up of EPS, which is mostly made up of proteins, deoxyribonucleic acid (DNA), and polysaccharides [3]. Compared to planktonic cells, bacteria within the biofilms are 1000 times more resistant to antibiotics due to the presence of thick EPS layers, enhanced expression of efflux pumps, and the presence of persistent cells [3,9].

Currently, biofilm development is linked to 60–80% of bacterial illnesses [3]. Biofilms can enhance antibiotic resistance and bacterial survivability by shielding the pathogen from host immune responses and the antipathogenic effects of drugs. They can also make treating the resulting disease more challenging. Clinicians have a tough time treating infected biomaterials and tissue surfaces because of the great resistance of hospital surfaces to desiccation, Benzalkonium Chloride disinfection, and Ultra Violet (UV) radiation. Nunez *et al.* discovered that biofilm development plays a role in the survival of these bacteria [10]. Therefore, identifying the processes that regulate the production of biofilms in bacteria and their detection is crucial for identifying novel targets for the prevention and management of illnesses linked to biofilms [9].

2. Subjects and methods

In Sana'a City, this descriptive cross-sectional study was carried out at the University of Science and Technology Hospital and Al-48 Modern General Hospital. The work was done in the lab for a year, beginning in April 2021 and finishing in April 2022, at the National Centre of Public Health Laboratories (NCPHL), Sana'a city, Yemen, in the department of microbiology and molecular biology. About 10 to 20 milliliters of midstream urine (MSU) were collected from 300 individuals with UTIs using a sterile screw-capped wide-mouth container.

Isolation and identification of *Klebsiella pneumoniae*

A standard calibrated loop was used to grow the urine sample with leukocyte counts more than 10 WBCs/HPF, and 0.01 ml of urine was placed on Cysteine-Lactose-Electrolyte-Deficient (CLED) agar media. For 18 to 24 hours, inoculated plates were incubated aerobically at 37°C. Following incubation, growth features were observed and the plates were analyzed. To lower the possibility of sample contamination, samples were often inoculated prior to cell content analysis. The bacterial count was calculated by counting colonies on the inoculation medium and multiplying the result by the loop volume (significant growth > 10^5 CFU/mL). The automated Vitek2 system was

used to identify bacteria in accordance with the manufacturer's instructions (BioMarieux, France).

Biofilm Detection

The isolated bacteria were tested for biofilm production by a tissue culture plate.

Determination of the Antibiogram Susceptibility Pattern

Using a Modified Disk Diffusion approach as outlined by Kirby-Bauer [11], the antibiogram susceptibility pattern of uro-pathogens to several antibacterial drugs was ascertained in vitro. The process consisted of determining the diameter of the zone of inhibition that develops when the antibiotic agent diffuses into the media around the disc.

3. Results

The current study found that the penicillin resistance phenotype was significantly associated with the biofilm-forming ability of *K. pneumoniae*, and 97.5% of ampicillin-resistant isolates formed a biofilm compared to 59% of non-biofilm-Producing isolates. The rate of resistance to ampicillin-sulbactam was 85% for biofilm-producing isolates versus 15.4% for non-biofilm strains, and 42.5% for piperacillin-tazobactam for biofilm-producing isolates versus 0.0% for non-biofilm strains. As for non-biofilm strains, the rate of resistance to amoxicillin-clavulanic was. 80% for biofilm-producing strains compared to 20.5% for non-biofilm-producing strains, and finally for biofilm-producing isolates, the Aztreonam resistance rate was 67.5% compared to 12.8% for non-biofilm-Producing isolates. The current study found that the Carbapenems group resistance phenotype was significantly associated with the biofilm-forming ability of *K. pneumoniae*, and 42.5% of Meropenem-resistant isolates formed a biofilm compared to 5.1% of non-biofilm-Producing isolates. The rate of resistance to Imipenem was 20% for Biofilm-Producing Isolates versus 7.7% for Non-Biofilm Strains, and 52.5% for Etrapenem for biofilm-producing isolates versus 10.3% for Non-Biofilm Strains. The current study found that the Cephalosporin Resistance Phenotype was significantly associated with the ability of *K. pneumoniae* to form biofilms, and resistance ranged from 80–95% for biofilm-forming isolates versus 23.1%–41% for non-biofilm-Producing isolates. For example, the resistance rate to Cefazolin was 90% for Biofilm-Producing Isolates versus 33.3% for Non-Biofilm-Producing Strains, and 80% for Cefepime (a third-generation Cephalosporin) for Biofilm-Producing Isolates versus 23.1% for Non-Biofilm-Producing Strains. The current study found that the Quinolones resistance phenotype was significantly associated with the ability of *K. pneumoniae* to form biofilm-producing isolates, and resistance ranged from 65–82% for biofilm-forming isolates versus 7%–15.4% for non-biofilm-Producing isolates. For example, the resistance rate to Nalidixic acid was 80% for biofilm-producing isolates versus 15.4% for non-biofilm-producing strains, and 65% for levofloxacin for biofilm-producing isolates versus 15.4% for non-biofilm-Producing strains.

The current study found that the aminoglycoside resistance phenotype was significantly associated with the ability of *K. pneumoniae* to form biofilm-producing isolates, and the resistance rate for Gentamicin was 42.5% for biofilm-forming isolates versus 7.7% for non-biofilm-Producing isolates. Also, the resistance rate to Amikacin was 42.5% for Biofilm-Producing Isolates versus 7.7% for Non-Biofilm-Producing Strains.

Table 1: The association of antibiotic resistant with biofilm production of isolated *K. pneumoniae* for Penicillins group and Aztreonam.

Antibiotics		Biofilm producing- *K. pneumoniae*						Non-Biofilm producing- *K. pneumoniae*					
		Sensitive		Moderate		Resistance		Sensitive		Moderate		Resistance	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Penicillin	Ampicillin	1	2.5	0	0.0	39	97.5	13	33.3	3	7.7	23	59.0
Antibiotic with β-lactamase inhibitors	Ampicillin-sulbactam	5	12.5	1	2.5	34	85.0	28	71.8	5	12.8	6	15.4
	Piperacillin-tazobactam	21	52.5	2	5.0	17	42.5	35	89.7	4	10.3	0	0.0
	Amoxicillin-clavulanic acid	6	15.0	2	5.0	32	80.0	24	61.5	7	17.9	8	20.5
Aztreonam		8	20.0	5	12.5	27	67.5	33	84.6	1	2.6	5	12.8
Total		40						39					

Table 2: The association of antibiotic resistant with Biofilm Production of isolated *K. pneumoniae* for Carbapenems group.

Antibiotics		Biofilm producing- *K. pneumonia*						Non- biofilm producing- *Klebsiella pneumoniae*					
		Sensitive		Moderate		Resistance		Sensitive		Moderate		Resistance	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Carbapenems	Meropenem	22	55.0	1	2.5	17	42.5	36	92.3	1	2.6	2	5.1
	Imipenem	26	65.0	6	15.0	8	20.0	33	84.6	3	7.7	3	7.7
	Etrapenem	15	37.5	4	10.0	21	52.5	32	82.1	3	7.7	4	10.3
Total		40						39					

Table 3: The association of antibiotic resistant with biofilm production of isolated *K. pneumoniae* for Cephalosporins group.

Antibiotics (Cephalosporins)		Biofilm producing- *K. pneumoniae*						Non-Biofilm producing- *K. pneumoniae*					
		Sensitive		Moderate		Resistance		Sensitive		Moderate		Resistance	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
First generation	Cefazolin	3	7.5	1	2.5	36	90.0	23	59.0	3	7.7	13	33.3
	Cefadroxil	6	15.0	0	0.0	34	85.0	23	59.0	3	7.7	13	33.3
Second generation	Cefaclor	5	12.5	2	5.0	33	82.5	27	69.2	3	7.7	9	23.1
	Cefuroxime	5	12.5	2	5.0	33	82.5	25	64.1	2	5.1	12	30.8
	Cefoxitin	6	15.0	2	5.0	32	80.0	22	56.4	7	17.9	10	25.6
Third generation	Cefataxime	2	5.0	0	0.0	38	95.0	17	43.6	6	15.4	16	41.0

	Ceftazidme	**4**	**10.0**	**1**	**2.5**	**35**	**87.5**	**23**	**59.0**	**2**	**5.1**	**14**	**35.9**
	Ceftriaxone	**6**	**15.0**	**1**	**2.5**	**33**	**82.5**	**23**	**59.0**	**3**	**7.7**	**13**	**33.3**
	Cefpodoxime	**3**	**7.5**	**3**	**7.5**	**34**	**85.0**	**27**	**69.2**	**3**	**7.7**	**9**	**23.1**
Fourth generation	**Cefepime**	**7**	**17.5**	**1**	**2.5**	**32**	**80.0**	**28**	**71.8**	**2**	**5.1**	**9**	**23.1**
Total		**40**						**39**					

Table 4: The association of antibiotic resistant with biofilm production of isolated *K. pneumoniae* for Quinolones

Antibiotics		**Biofilm producing- *K. pneumoniae***						**Non-Biofilm producing- *K. pneumoniae***					
		Sensitive		**Moderate**		**Resistance**		**Sensitive**		**Moderate**		**Resistance**	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No**	**%**
Quinolones	**Nalidixic acid**	**3**	**7.5**	**5**	**12.5**	**32**	**80.0**	**30**	**76.9**	**3**	**7.7**	**6**	**15.4**
	Levofloxacin	**10**	**25.0**	**4**	**10.0**	**26**	**65.0**	**32**	**82.1**	**1**	**2.6**	**6**	**15.4**
	Lomefloxacin	**5**	**12.5**	**2**	**5.0**	**33**	**82.5**	**31**	**79.5**	**4**	**10.3**	**3**	**7.7**
	Ciprofloxacin	**10**	**25.5**	**1**	**2.5**	**29**	**72.5**	**32**	**82.1**	**1**	**2.6**	**6**	**15.4**
	Moxifloxacin	**7**	**17.5**	**3**	**7.5**	**30**	**75.0**	**33**	**84.6**	**2**	**5.1**	**4**	**10.3**
	Norfloxacin	**5**	**12.5**	**3**	**7.5**	**32**	**80.0**	**31**	**79.5**	**2**	**5.1**	**6**	**15.4**
Total		**40**						**39**					

Table 5: The association of antibiotic resistant with biofilm production of isolated *K. pneumoniae* for Aminoglycosides

Antibiotics		**Biofilm producing- *K. pneumoniae***						**Non-Biofilm producing- *K. pneumoniae***					
		Sensitive		**Moderate**		**Resistance**		**Sensitive**		**Moderate**		**Resistance**	
		No.	**%**	**No.**	**%.**	**No.**	**%**	**No.**	**%**	**No.**	**%.**	**No**	**%**
Aminoglycosides	**Gentamycin**	**16**	**40.0**	**7**	**17.5**	**17**	**42.5**	**34**	**87.2**	**2**	**5.1**	**3**	**7.7**
	Amikacin	**15**	**37.5**	**8**	**20.0**	**17**	**42.5**	**28**	**71.8**	**8**	**20.5**	**3**	**7.7**
Total		**40**						**39**					

4. Discussion

Given the rising incidence of carbapenemase-producing strains and broad-spectrum b-lactamases (ESBLs), the proliferation of antibiotic-resistant *K. pneumoniae* strains both locally and globally has become a serious issue [12–17]. In comparison to planktonic bacteria, biofilm bacteria had increased antibiotic resistance to ampicillin, ciprofloxacin, gentamicin, and cefotaxime [3, 18]. Varied research produced varied conclusions about the correlation between biofilm formation and infection sites. Shadkam *et al.* examined the biofilm formation and antibiotic resistance of 100 non-duplicative *K. pneumoniae* that were obtained from sputum, blood, urine, wound exudates, and intra-tracheal tubes (ITTs). According to the findings, 75% of *K. pneumoniae* isolates were biofilm-producing, and the ability of Multi-Drug Resistant (MDR) isolates to form biofilms was significantly higher than that of non-MDR isolates ($P < 0.05$). Additionally, the sputum isolates' capacity to form biofilms was significantly higher than that of other isolates ($P < 0.001$) [19]. According to Ashwath *et al.* [20], isolates of *K. pneumoniae* that were clinically Multi-Drug Resistant (MDR) produced biofilms in

97.1% of the cases, and these biofilms were more likely to form in blood, pus, and trachea secretions. When Tuncer *et al*. [21] looked into the biofilm-forming characteristics of pan-resistant (agent-resistant) *K. pneumoniae*, they discovered that all of the isolates formed high-level biofilms, while isolates from various samples (blood, sputum, and wound) did not significantly differ from one another [21]. Variations among research can be ascribed to geographical locations, types and quantities of samples, and antibiotic resistance in microorganisms. Antibiotic resistance and biofilm formation are significantly correlated with the intensities of biofilm formation of drug-resistant bacteria. An analysis of biofilm formation among 137 *K. pneumoniae* strains from urine and sputum showed that, in contrast to biofilm negative strains, which had a rate of 11.7% (9/77), 85.0% (51/60) of biofilm-positive bacteria were able to manufacture Extended-Spectrum beta-lactamases (ESBLs) [22].

According to Subramanian *et al.*, of 100 urine isolates, 83.3% and 73.3% of the isolates that formed biofilms were resistant to ampicillin and cefotaxime, respectively, while only 60% and 35% of the isolates that did not form biofilms were [23]. According to Rahdar *et al.* [24], who also observed a substantial correlation between the Carbapenem resistance phenotype and *K. pneumoniae's* capacity to create biofilms, 99.9% of isolates resistant to Carbapenem were able to generate medium- and high-strength biofilms. When compared to MDR and sensitive strains, the *K. pneumoniae* isolates from the current investigation also showed a higher capacity for biofilm formation, suggesting a positive link between the two traits. In the current investigation, there was a noteworthy correlation between robust biofilm production and antibiotic resistance to beta lactams. 59% of *K. pneumoniae* that does not produce biofilm is resistant, compared to 97% of Biofilm-Producing *K. pneumoniae*. According to Khodadadian *et al.* [25], this may suggest the presence of the VIM gene (Verona Integron-Encoded Metallo-b-Lactamase gene).

Antibiotic-sensitive isolates are not the only strains that can develop biofilm; resistant bacteria can also create it. 250 *K. pneumoniae* bacteremia isolates were subjected to biofilm formation analysis by Zheng *et al.* [26], however, no meaningful correlation between biofilm development and antibiotic resistance was discovered. 139 clinically isolated *K. pneumoniae* strains were examined by Cusumano *et al*. [27], who discovered that XDR (n = 25) isolates were similar amongst the groups and that multi-drug resistance isolates (n = 81) more frequently developed weak biofilms.

Carbapenem-resistant *K. pneumoniae* (CRKP) in this study were more likely to generate a robust biofilm. For instance, the percentage of resistance to Meropenem for *K. pneumoniae* that produced biofilms was 42.5%, compared to 5.1% for *K. pneumoniae* that did not produce biofilms. Additionally, the rate of Imipenem resistance in *K. pneumoniae* that produced biofilm was 20% VS to 7.7% for *K. pneumoniae* that did not produce biofilm, and the rate of Etrapenem resistance in *K. pneumoniae* that produced biofilm was 52.5% VS to 10.3% for *K. pneumoniae* that did not produce biofilm. This finding contrasts with that of Fang *et al.* [28], who found that Carbapenem-Resistant *K. pneumoniae* (CRKP) had a lower propensity to build a robust biofilm.

By analyzing 40 imipenem-resistant bacteria and 40 imipenem-sensitive strains, Fang *et al.* [28] also observed that CRKP was more likely to develop weak biofilms than Carbapenem-sensitive strains. The mrkH gene, which is more commonly found in carbapenem-sensitive strains than in carbapenem-resistant strains, may be the reason for the strong biofilm development observed in carbapenem-sensitive strains. When Sabenca *et al*. [29] examined the biofilm formation of KPC- and ESBL-producing *K. pneumoniae*, they discovered that

the majority of these isolates had weak biofilm formation rates (40.0% and 60.0%), respectively. The capacity to build more robust biofilms was unrelated to the presence of ESBL and KPC enzymes [29]. Therefore, more research is needed to determine the relationship between biofilm production and antibiotic resistance.

5. Conclusion

There was a significant correlation between the ability of biofilm-producing *K. pneumoniae* and the antibiotic resistance. The biofilm is difficult to treat with antimicrobial agents in this study, in which a very high rate of antibiotic resistance of isolated *K. pneumoniae* was found. Quinolones and cephalosporins appeared to be the least active drugs on the studied biofilm producing *K. pneumoniae*, and a slight sensitivity was shown to carbapenems and aminoglycosides.

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A dispute of interest

Regarding this project, there is no conflict of interest.

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