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Research Article

Correlation between *Klebsiella* Species' Biofilm Formation and Antibiotic Resistance from Erbil Hospital Patients

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ABSTRACT

The primary cause of nosocomial infections is *Klebsiella pneumoniae*, an environmental Gram-negative, encapsulated, non-motile bacterium. Antibiotic resistance and biofilm formation are two crucial characteristics that contribute to the pathogenicity of this bacterium. This research aimed to examine the relationship between antibiotic resistance and the capability of *Klebsiella* species isolated from Erbil hospital patients to produce biofilms. Therefore, in this study, 38 samples were collected from different laboratories and hospitals. At first, the samples were rejuvenated in Tryptic soy broth and then cultured on MacConkey agar. Gram staining and urease test were performed to identify the bacterial isolates while VITEK 2 system was performed to determine both the bacterial isolates and the antibiotic susceptibility. At last, biofilm detection was assessed by the microtiter plate technique and Congo red agar method. The results revealed that positive *Klebsiella* samples did not significantly correlate with the formation of biofilm despite being resistant to some antibiotics and biofilm producers.

Keywords: *Klebsiella* species, Antibiotic resistance, Biofilm formation, Extended-spectrum β-lactamase, *Klebsiella pneumoniae* carbapenemase

INTRODUCTION

Klebsiella Species

Klebsiella species are a type of bacteria that are Gram-negative, non-motile, encapsulated, lactose-fermenting, and facultative anaerobic. They are known to cause various illnesses, particularly in regions with inadequate health-care systems.[1] The pathogens that take advantage of favorable conditions, *Klebsiella* species are commonly present in the nasal passages, throat, skin, and intestinal flora of individuals in good health. However, they can also give rise to a range of illnesses, such as pneumonia, infections of soft tissues and surgical wounds, urinary tract infections (UTIs), bloodstream infections, and sepsis. On a global scale, a multitude of *Klebsiella* strains has emerged as a significant concern in both clinical and public health settings. The *Klebsiella* family comprises an extensive variety of species, including the *Klebsiella pneumoniae* species complex as well as other more distantly related species.[2] All *Klebsiella* species, but especially *K. pneumoniae*, *Klebsiella oxytoca*, *Klebsiella ozaena*, and *Klebsiella rhinocerotic*, are significant sources of human infections.[3] As well such as nosocomial infections of the respiratory tract, urinary tract, wounds, and bloodstream, *K. pneumoniae* is a major contributor to serious communitarian outbreaks, such as necrotizing pneumonia, hepatic abscess, and endogenous endophthalmitis.[4] Friedlander first documented this bacterium in 1882, after isolating it from the lungs of patients with pneumonia. Initial indications of pneumonia encompass elevated body temperatures, shivering, symptoms resembling influenza, expectorating yellow or bloody phlegm, difficulty in breathing, and discomfort in the chest region. Antibiotic-resistant pneumonia brought on by *K. pneumoniae* can have a 50% fatality rate.[5] The outer membrane proteins (OMPs), type 1 and type 3 fimbriae, lipopolysaccharide (LPS), capsule polysaccharide, and determinants for getting iron and utilizing the nitrogen source are virulence factors present in *Klebsiella*. These virulence elements helped *K. pneumoniae* survive an infection, evade the immune system, and create biofilms.[6] *K. pneumoniae* infections are associated with community-acquired illnesses and nosocomial infections and

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can be transmitted by contaminated food or water in situations involving interaction between humans and animals.[7]

K. pneumoniae isolation reports from various sources are increasing, and many of these isolates show signs of multidrug resistance (MDR).[8] The bacterium's virulence is influenced by a number of many factors, and it can result in infection and antibiotic resistance, for example, as a result of alterations in the organism's fundamental DNA.[9] There are limited therapeutic interventions for the treatment of infections such as pneumonia, liver abscess, meningitis, bloodstream infections, and UTIs due to the presence of an MDR pathogen.[10]

Antibiotic Susceptibility and Biofilm Formation

Antibiotic susceptibility testing (AST) is a procedure used to ascertain the optimal dosage of antibiotics required to effectively treat a patient's medical condition and aids in formulating a treatment strategy for the management of severe infections. Consequently, prompt diagnosis plays a vital role in achieving successful treatment of bacterial infections. Although all AST methods offer qualitative assessments using susceptible, intermediate, or resistant classifications, some methods also provide quantitative evaluations, such as minimum inhibitory concentration, to establish the optimal antibiotic dosage. These methods establish a profile of empirical therapy to effectively manage each patient's health against life-threatening infections.[11] Antibiotic resistance is the ability of microorganisms to withstand exposure to antibiotics that would typically eliminate them or stop their development. Antibiotic misuse in people and animals, antibiotic use in the livestock and food sectors, a lack of quick diagnosis methods, and the presence of antibiotics in the environment are some of the major causes of antibiotic resistance. Due to different genetic processes, antibiotic resistance can either be intrinsic or acquired.[12] *K. pneumoniae* developed a variety of defense mechanisms against antimicrobial agents. Only a limited number of virulence factors have been discovered thus far that are capable of circumventing the host's first defense mechanisms. The virulence factors consist of fimbriae, LPS, and capsule formation, primarily associated with the highly virulent strain. Additional virulence factors have been subsequently discovered, although their complete characterization necessitates additional investigation. Pathogenic factors include OMPs, porins, efflux pumps, and transporters. The capsule, composed mainly of a polysaccharide matrix, is the most extensively researched pathogenicity component of *K. pneumoniae*.[13] Gram-negative bacteria, such as *K. pneumoniae*, have evolved diverse mechanisms to withstand the effects of routinely employed antibiotics. The presence of certain antibiotic-resistance genes is a major contributing factor to the occurrence of antibiotic resistance. A significant health-care issue related to the worsening condition of hospital infections is the prevalence of resistance to different types of antibiotics, along with the coexistence of certain virulence factors that promote disease severity and genes that confer resistance.[14] *K. pneumoniae* AMR high-risk clones pose a significant public health threat and have been pivotal in the AMR epidemic's worldwide spread.[15] A major global public health concern is the emergence of drug resistance among infectious disease-causing microbes like *K. pneumoniae.* Not only does it raise patient illness and death rates, but it also elongates hospital stays and increases the cost of care.

Most studies on resistance genes have identified genes that encode for beta-lactams, which are believed to be responsible for the increasing antibiotic resistance in the beta-lactam class. This high prevalence number is concerning because MDR *K. pneumoniae* therapy is challenging and costly.[16] MDR infections are commonly being brought on by *K. pneumoniae* carbapenemases (KPCs), which are capable of producing these enzymes. Plasmid-borne enzymes identified as KPCs have the ability to build up and spread antibiotic resistance to other drug classes.[17] Extended-spectrum beta-lactamases (ESBLs), which are bacterial enzymes that impart resistance to numerous B-lactam antibiotics that are commonly prescribed.[18] Global health is seriously threatened by the rapid spread of *K. pneumoniae* ESBL producers, the emergence of antimicrobial resistance, and the production of KPC and cefotaxime's enzymes. This is compounded by the implementation of local and national guidelines and the establishment of innovative therapeutic approaches.[19] The development of biofilms is a process that begins when planktonic (free-living) organisms develop aggregation and/or change to a lifestyle associated with surfaces. It is completed when cells disperse from the biofilm structure to restore their single-cell, planktonic mode of growth. Notably, compared to their planktonic counterparts, biofilms have been found to be 10–1000 times more resistant to different antibiotics. In both medicinal and industrial settings, these characteristics have bad impacts in both medicinal and industrial settings.[20]

K. pneumoniae can generate surface-attached microbial communities, or "biofilms," which are multicellular, sessile microorganisms that agglomerate their cells within a self-produced matrix of extracellular polymeric substance, as opposed to being dispersed and free-floating. This enables them to successfully spread and endure in the hospital setting.[21]

Extracellular carbohydrates, eDNA, and proteins make up the majority of *K. pneumoniae* biofilm aggregates. In addition, biofilms can adhere to medical equipment, such as catheters and endotracheal tubes, but they are most harmful when they are on body surfaces.[13] Compared to infections caused by other strains, the management of infections caused by *K. pneumoniae* strains that form biofilms presents greater challenges.[22] Biofilm-associated cells usually exhibit increased antibiotic resistance and considerably contribute to therapeutic failure.[23]

MATERIALS AND METHODS

Sample Collection

The samples were collected from patients at several hospitals and laboratories in Erbil city, as well as students at our university, Cihan University-Erbil. Urine, Vaginal swab, and sputum samples were collected regardless of the patient's age, which provided an overall of 38 samples. Samples were suspended in Tryptic soy broth and incubated for 24 h at 37°C after collection.

Bacterial Isolation and Identification

Manual methods for identification

The manual methods that were used for the identification of *Klebsiella* include culturing the samples on MacConkey agar, Gram staining, and urease test.

Culturing on MacConkey agar

After the sample collection, they were rejuvenated and proliferated in Tryptic soy broth (Accumix), as well as cultivated on MacConkey agar (Accumix) and then incubated at 37°C for 24 h.

Gram staining

A colony was taken from the cultured samples in terms of making a smear and performing gram staining, identifying the *Klebsiella* under the microscope based on their morphological properties.

Urease test

The urease test is a diagnostic technique employed to differentiate between urease-positive and urease-negative organisms by detecting the presence of cytosolic urease enzyme. A urease test was performed to confirm that the samples were *Klebsiella* urease positive. For the preparation of the urea base agar, 1.5 g of urea base agar (Acumedia) was dissolved in 95 mL of distilled water, and 0.12 g of urea was mixed with 5 ml of distilled water to obtain the urea solution. Both solutions were mixed and added into tubes. They were left to cool down, cultured, and then incubated at 37°C for 24 h.

Automated method

Identification and antibiotic susceptibility by VIETIK 2

In clinical microbiology laboratories, the VITEK 2 automated system is one of the most extensively used instruments for identifying and assessing the susceptibility profiles of bacteria, including the detection of ESBLs generated by *Klebsiella* species. The source of these data was Global CLSI-based.

The antibiotics that were utilized and the interpretations according to the MIC are listed in Table 1.

Table 1: The utilized antimicrobial agents

Antimicrobial agents	Resistant	Sensitive	Intermediate
Amoxicillin/ Clavulanic Acid	≥32/16	≤8/4	16/8 ^
Piperacillin/ Tazobactam	≥128	≤16	32–64 ^
Cefazolin	≥8	≤2	4
Cefuroxime	≥32	≤8	16 ^
Ceftazidime	≥16	≤4	8 ^
Ceftriaxone	≥4	≤1	2 ^
Aztreonam	≥16	≤4	8 ^
Ertapenem	≥2	≤0.5	1 ^
Imipenem	≥4	≤1	2 ^
Meropenem	≥4	≤1	2 ^
Amikacin	≥64	≤16	32 ^
Gentamicin	≥16	≤4	8 ^
Ciprofloxacin	≥1	≤0.25	0.5 ^
Fosfomycin	≥256	≤64	128
Nitrofurantoin	≥128	≤32	64
Trimethoprim/ sulfamethoxazole	≥4/76	≤2/38	/
Cefepime	≥16	≤2	4–8
Tigecycline	≥16	/	/

Detection of Biofilm Formation Assay

Congo red agar (CRA) method

The qualitative CRA assay is utilized to identify biofilm-forming *Klebsiella* samples. This screening approach relies on observing changes in the color of colonies cultivated on CRA medium. This medium was prepared by dissolving 5 g of agar, 2.5 g sucrose, and 18.5 g brain–heart infusion broth (HIMEDIA) in 400 mL of distilled water and then autoclaved at 121°C for at least 30 min alone. 0.4 g of conger red to 100 mL of distilled water was prepared and then it was autoclaved at 121°C for at least 30 min as well. Later on, the two solutions were mixed to obtain a 500 mL solution. At last, it was poured into the petri dish and after cooling, it was inoculated with the isolated cultures and incubated for 24 h at 37°C.

Microtiter plate (MTP) method and enzyme-linked immunosorbent assay (ELISA)

Bacterial colonies from the fresh cultures of all isolates on MacConkey agar were inoculated with tubes containing tryptic soy broth (Accumix) with 0.25% glucose and incubated overnight at 37°C. 190 μL of tryptic soy broth containing inoculated bacteria, and 10 μL of tryptic soy broth containing glucose were mixed, then this bacterial suspension was diluted to 1/20 with tryptic soy broth. 200 μL from the diluted bacterial suspension was dispensed into each 96-well flat-bottomed microplate. C5 well was the negative control with only broth containing glucose. The MTP was incubated for 24 h at 37°C. The contents of the wells were emptied and the plates were rinsed three times with 200 μL of sterile distillate water while shaking and inverted to eliminate any non-stick bacteria. After fixating the bacteria that had adhered to the wells for 15 min with 200 μL of 99% methanol, they were subsequently rinsed 3 times with 200 μL of sterile distilled water, shaken, and inverted. Following a 5-min staining period with 200 μL of crystal violet, the wells were rinsed to eliminate any residual stain. Finally, the wells were air-dried. The dye bound to the wells was solubilized with 200 μL of glacial acetic acid. The optical density (OD) of each well was measured at 490 nm using an ELISA auto reader.

RESULTS

Collected Samples

Regardless of the patient's age, urine, vaginal swab, and sputum samples were obtained, making up to 38 samples. The most collected sample regardless of the patient's age was urine samples that made up to 83% of the total samples. While 10% of the samples were sputum, vaginal swabs were the least collected samples making up to 7%. The collected samples are displayed in Figure 1.

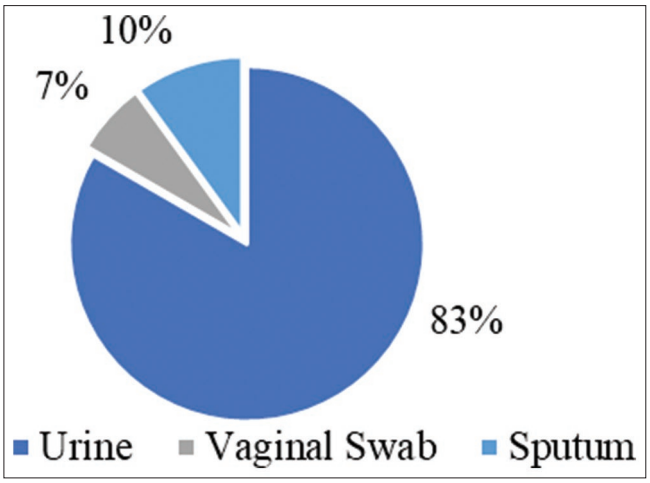

Figure 1: Displays the total 38 samples collected from the hospitals and laboratories

Most of the collected urine samples were obtained from females, while most of the sputum samples were obtained from males than females [Figure 2]. Demonstrates the distinction in the number and type of samples according to sex group.

Isolation of *Klebsiella* on Culture Media

Bacterial examination on MacConkey agar

After incubating the samples at 37°C for 24 h the colonies that developed on the MacConkey agar were pink, and some of them appeared to be mucoid lactose fermenters which indicate *Klebsiella* species characteristics as shown in Figure 3.

Bacterial examination by gram-staining

Gram staining was conducted to confirm the presence of *Klebsiella* species depending on their morphological properties by microscopical examination that revealed pink rod-shaped encapsulated bacteria.

Bacterial Identification by urease test

After incubating the samples at 37°C for 24 h samples with a bright pink slant were considered as *Klebsiella* urease positive, whereas the samples without any color change were considered as a negative result for urease. The urease test is demonstrated in Figure 4.

Automated Method for Bacterial Identification and Antibiotic Susceptibility

VIETIK 2 system for bacterial identification and antibiotic susceptibility

It was determined by VIETIK2 that out of the total of 38 samples, only 30 samples were *Klebsiella* and the remaining 8 were *Escherichia coli*. The samples varied in their antibiotic susceptibility, although they were in general susceptible and not resistant to antibiotics.

Tables 2 and 3 show the antibiotic susceptibility among *Klebsiella* and *E. coli* samples.

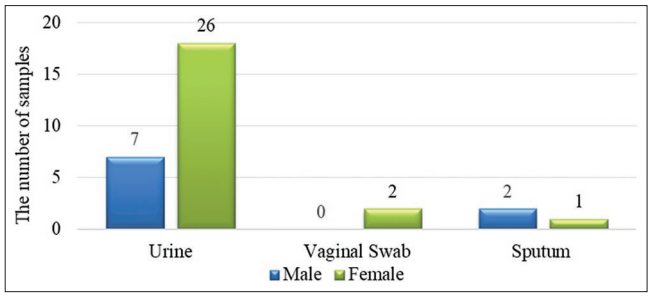

Figure 2: Demonstrates the differences in the number and type of samples according to sex group

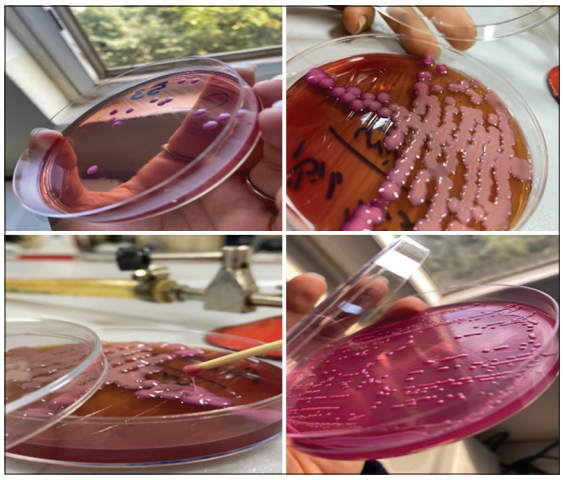

Figure 3: Samples cultured on MacConkey agar showing the formation of mucoid, pink colonies

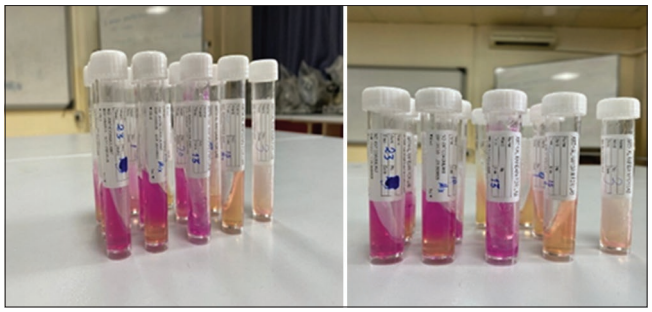

Figure 4: Demonstrates urease test, in which the bright pink color slants are *Klebsiella* urease-positive samples while the non-color changed samples are *Klebsiella* urease negative

Figures 5 and 6 show an overall antibiotic susceptibility among *Klebsiella* and *E. coli* samples.

Figure 7 shows the antibiotic susceptibility variation between males and females.

Examination of the Biofilm Formation

Biofilm examination on CRA

After incubating the 30 *Klebsiella* samples for 24 h at 37°C, the black colonies that developed were considered as biofilm formers, whereas the white/pink colonies were non-biofilm formers as shown in Figure 8.

Table 2: Antibiotic susceptibility among *Klebsiella* samples. Most of the samples were resistant to amoxicillin/clavulanic acid, trimethoprim/sulfamethoxazole as well as ceftriaxone. While being sensitive to amikacin, ertapenem, imipenem, meropenem, ciprofloxacin, piperacillin/tazobactam, and gentamicin

Antimicrobial agent	Resistant	Sensitive	Intermediate
Amoxicillin/ Clavulanic Acid	13 (43.33)	16 (53.33)	1 (3.33)
Piperacillin/ Tazobactam	1 (3.33)	29 (96.66)	
Ceftriaxone	8 (26.66)	20 (66.66)	2 (6.66)
Amikacin		30 (100)	
Ertapenem		29 (96.66)	1 (3.33)
Imipenem	1 (3.33)	29 (96.66)	
Meropenem	1 (3.33)	29 (96.66)	
Ciprofloxacin	2 (6.66)	28 (93.33)	
Gentamicin	1 (3.33)	29 (96.66)	
Trimethoprim/ Sulfamethoxazole	14 (46.66)	16 (53.33)	

MTP Assay and ELISA for biofilm examination

The MTP method is a quantitative method that was used to detect biofilm, and it was quantified using an ELISA reader. The OD was measured at 490 nm and the assay was repeated 3 times to reduce the errors.

Table 4 shows the mean ± standard deviation and the *P*-value.

Table 5 shows the number of strong, moderate, and weak samples.

DISCUSSION

The major hospital and laboratory *Klebsiella*-positive samples collected were urine samples (83%), while (10%) were sputum samples, vaginal swabs were the least collected samples making up to (7%) as Naqid *et al*. revealed that *K. pneumoniae* infection was the most common bacteria found in the patient's urine samples (66.2%), followed by blood samples (12.3%) and wound swabs (10%).[24]

Whereas a study's findings revealed that the majority of *K. pneumoniae* samples were obtained from throat cultures as they stated that 32 (38.5%) of the 83 *K. pneumoniae* clinical isolates were found in the throat culture, 18 (21.6%) in the urine, 17 (20.4%) in the trachea, 6 (7.25%) in the blood culture, 6 (7.25%) in the wound, 2 (2.5%) in the sputum, and 2 (2.5%) in the samples from the abscess drain.[25]

Positive urine samples with *Klebsiella* were more prevalent in females (68.4%) in comparison to males (18.4%) due to the difference in the genital tract which makes females more susceptible as Jafari-Sales *et al*. mentioned that the urethra's shortness is one of many potential causes. In contrast to the various anatomical systems of the male urinary tract, the prostate secretions contain bactericidal compounds. These all play an important role in preventing the invasion of pathogenic bacteria.[26]

Table 3: Antibiotic susceptibility among *Escherichia coli* samples. The samples were sensitive to amikacin, ertapenem, imipenem, meropenem, piperacillin/tazobactam, gentamicin, fosfomycin, nitrofurantoin, ceftazidime, ciprofloxacin, and cefepime. While showing resistance mostly to cefazolin as well as cefuroxime

Antimicrobial agent	Resistant	Sensitive	Intermediate
Amoxicillin/Clavulanic Acid	1 (12.5)	4 (50)	3 (37.5)
Piperacillin/ Tazobactam	1 (12.5)	6 (75)	1 (12.5)
Cefazolin	4 (50)	4 (50)	/
Cefuroxime	4 (50)	4 (50)	/
Ceftazidime	2 (25)	5 (6.25)	1 (12.5)
Ceftriaxone	4 (50)	4 (50)	/
Ertapenem	/	8 (100)	/
Imipenem	/	8 (100)	/
Meropenem	/	8 (100)	/
Amikacin	/	8 (100)	/
Gentamicin	/	8 (100)	/
Ciprofloxacin	3 (37.5)	5 (6.25)	/
Fosfomycin	/	8 (100)	/
Nitrofurantoin	/	8 (100)	/
Trimethoprim/ Sulfamethoxazole	2 (25)	6 (75)	/
Cefepime	1 (12.5)	5 (62.5)	/
Amoxicillin/ Clavulanic Acid	13 (43.33)	16 (53.33)	1 (3.33)
Piperacillin/ Tazobactam	1 (3.33)	29 (96.66)	/
Ceftriaxone	8 (26.66)	20 (66.66)	2 (6.66)
Amikacin	/	30 (100)	/
Ertapenem	/	29 (96.66)	1 (3.33)
Imipenem	1 (3.33)	29 (96.66)	/
Meropenem	1 (3.33)	29 (96.66)	/
Ciprofloxacin	2 (6.66)	28 (93.33)	/
Gentamicin	1 (3.33)	29 (96.66)	/
Trimethoprim/ Sulfamethoxazole	14 (46.66)	16 (53.33)	/

While in contrast to a study carried out in Southern Brazil, whose findings showed that male patients were more susceptible to *Klebsiella* infection as they mentioned that the risk for the presence of ESBL was significantly higher in males (26.4%) than females (8%), from hospital-acquired infections (29.1%) than community-acquired infections (7.0%), and in *Klebsiella* species (27.4%) than in *E. coli* (7.7%). It's somewhat demanding to pinpoint the cause of this variance, which may be related to differences in sample collection, research design, environmental factors, and personal hygiene as well.[27]

Identification and the presence of *Klebsiella* were determined by performing different tests. The samples were cultured to detect the presence of *K. pneumoniae*, as it was cultured on MacConkey agar because of being selective for

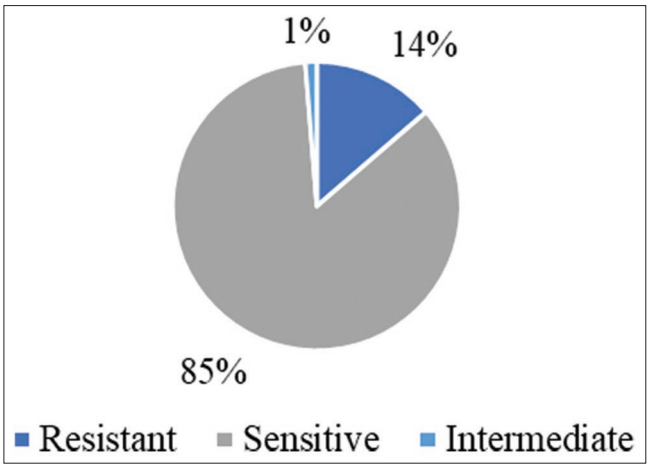

Figure 5: Overall antibiotic susceptibility among *Klebsiella* samples. Most of the samples, up to 85% were sensitive to the antibiotics while only 14% showed resistance

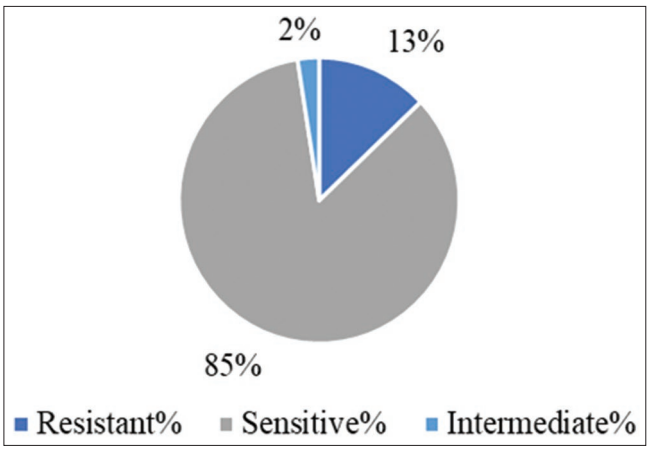

Figure 6: Overall antibiotic susceptibility among *Escherichia coli* samples. About 85% of the samples were sensitive to the antibiotics, while 13% being resistant

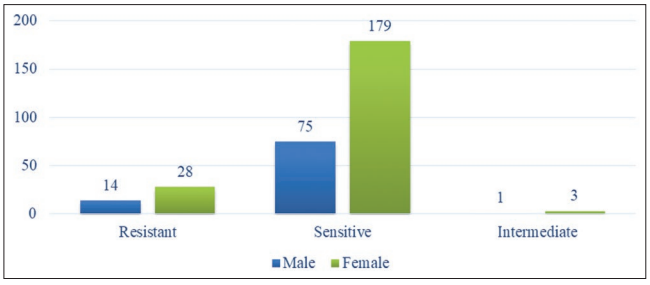

Figure 7: Antibiotic susceptibility variation in *Klebsiella* between male and female samples

Gram-negative bacteria and from its characteristics as it appeared to be mucoid lactose fermenter, forming mucoid pink colonies on the MacConkey.

As Osman *et al*. suggested in their work that the hospital laboratories identify colonies that are lactose-fermenting mucoid colonies on MacConkey's, CLED agar, and blood agar as *K. pneumoniae*. Under a light microscope, these colonies also show up as Gram-negative rods after staining.[28]

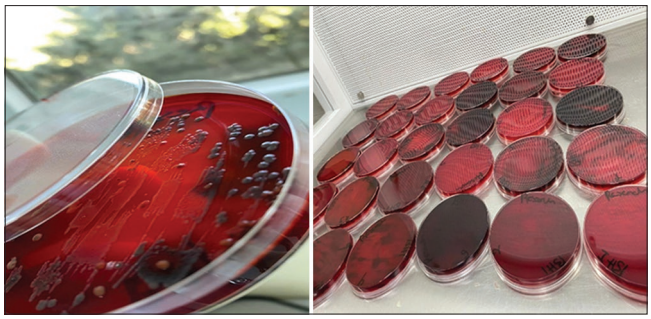

Figure 8: Demonstrates the formation of black and red colonies on the Congo red agar. The black colonies are due to strong biofilm producers, while the red colonies being moderate biofilm producers

Table 4: The mean±standard deviation and the *P*-value of the *t*-test, which showed significance as the *P*-value being <0.05

Concentration mg/mL OD at 490 nm	Mean±standard deviation	*P*-value
200	0.280±0.0519	0.001
Control	0.148±0.0125	

Table 5: Number of strong, moderate, and weak biofilm samples as well as the *P*-value of the Chi-squared test. Most of the samples were strong biofilm producers, while six of them were moderate and only four were weak biofilm producers. The work showed significance as the *P*-value being <0.05

Biofilm	Number	Percentage	*P*-value
Strong	20	66.6	
Moderate	6	20	0.0005
Weak	4	13.3	

Urease test which is a biochemical test was also performed for the identification of *K. pneumoniae*. The samples that changed the slant color to pink were considered as a positive result for urease in *Klebsiella* as Kumar Arya *et al*. suggested from their study that *K. pneumoniae* has the following biochemical properties, in addition to forming a pink, mucoid colony that ferments lactose on MacConkey agar: The bacterium is indole negative, citrate positive, TSI acid/acid with gas, a mannitol fermenter, non-motile, and also tests positive for urease.[29]

However, research in contrast revealed that a considerable percentage of *K. pneumoniae* isolates lack the enzyme urease due to the insertion of IS5075 or other related ISs, such as IS4321, into the ureC gene, rendering the operon inactive. Both carriage isolates and isolates linked to clinical symptoms can have urease inactivation capability.[30]

The antibiotic susceptibility test in this research was performed by VITEK2 with *K. pneumoniae* being sensitive to some antibiotics, amoxicillin/clavulanic acid (53.33%), ceftriaxone (66.66%), and trimethoprim/sulfamethoxazole (53.33%) being the least effective while amikacin (100%), piperacillin-tazobactam (96.66%), gentamicin (96.66%), meropenem (96.66%), and ertapenem (96.66%) had the

most favorable profile. As Alzahrani *et al*. showed from their work that amikacin, aztreonam, cefepime, ciprofloxacin, gentamicin, imipenem, and levofloxacin were the most effective antibiotics against *K. pneumoniae* (100% sensitivity), followed by meropenem, nitrofurantoin, and norfloxacin (80% sensitivity), ceftriaxone (66.67% sensitivity), tazocin (60% sensitivity), and (50% sensitivity) of amoxicillin/clavulanic acid. *K. pneumoniae* was discovered to be completely resistant to tobramycin, ampicillin, cefoxitin, cefuroxime, as well as cephalothin. Sulfamethoxazole/trimethoprim displayed a lower susceptibility pattern of 63.64% simultaneously.[31]

Ghenea *et al*. proposed in their work that they have observed an increase in *K. pneumoniae* isolates in recent years, particularly antibiotic-resistant strains, in high-risk ward such as ICUs. The proliferation of hospital-acquired infections within the population can give rise to significant public health concerns that must not be overlooked.[32] Furthermore, Abdelwahed *et al*. mentioned that in their study, different levels of drug resistance have been observed in *K. pneumoniae*. This research found greater resistance rates to ceftriaxone, ceftazidime, cefepime, piperacillin-tazobactam, ciprofloxacin, imipenem, and cefuroxime when compared to Bangladesh. However, there was less resistance to colistin and amikacin. Greater resistance to amoxicillin-clavulanic acid, ceftazidime, imipenem, and sulfamethoxazole-trimethoprim was observed in urine samples of *K. pneumoniae* than in Grenada. However, in their research, gentamycin, cefuroxime, and ciprofloxacin resistance levels were reduced.[33]

Our findings revealed as the samples were mostly obtained from females, both the resistance to certain antibiotics as well as sensitivity were more in females than in males. Females are more likely than males to acquire *Klebsiella* infections. As in this research, we noticed that the infection rates in females (61.7%) were higher than in males (38.3%).[34]

There are different methods that can be used for screening the biofilm formation, CRA is a qualitative test that was performed to determine whether the samples were biofilm producers, and it was confirmed by the black and red colony formation on the CRA. According to our findings, out of the thirty *Klebsiella* samples, twenty (66.6%) of them produced biofilm at a strong level, six (20%) at a moderate level, and only four (13.3%) at a weak level.

The biofilm producers develop black colonies (strong), red colonies (moderate), and non-biofilm producers develop pink/white colonies on CRA medium.[34]

Naji and Awadh also suggested in their work that out of the total number of isolates, 57 (38%) exhibited weak biofilm formation and were characterized by dark red (maroon) colonies. On the other hand, 24 isolates (16%) did not form any biofilm and were identified by pale red colonies. Out of the total isolates, 34 of them, accounting for (22.67%), exhibited moderate biofilm formation. These isolates were characterized by the presence of black-dark gray colonies, sometimes accompanied by a clear zone around the colonies, which were similar in color to pink.[35]

MTP assay was performed as a quantitative method for biofilm detection and an ELISA auto reader was utilized to measure the OD at 490 nm. Depending on the OD findings in the current study, it was determined that most of the samples were strong biofilm producers and also that the *P*-values were significant for both the *t*-test and Chi-squared test. The strong biofilm samples were also determined depending on the references which validated the results as Naji and Awadh stated from their study that an OD of 490 nm >0.12 was regarded as a sample that would produce a biofilm after measuring the absorbency at this wavelength. and no/weak biofilm OD <0.120, biofilm OD = 0.120–0.240, and Heavy biofilm OD >0.240.[35]

As opposed to a study conducted by Haghighifar *et al*. which demonstrated that among *K. pneumoniae* isolates, 20% exhibited high biofilm formation, 30% showed moderate biofilm formation, and 50% displayed low biofilm formation. These isolates had OD values that varied from more than 0.38 but <0.74.[36]

Furthermore, a study's findings of the MTP analysis revealed that 86 (67.2%) isolates had weak biofilms, 24 (18.8%) had moderate biofilms, and 18 (14.1%) had strong biofilms. Out of 128 samples, 57 (44.5%) were identified as MDR.[37]

The findings of this study revealed that most of the collected *Klebsiella* samples were strong biofilm producers while sensitive to most antibiotics, as Siddique *et al*. revealed that according to one of the studies conducted by the National Institutes of Health and the Centre for Disease Control, biofilm-forming microorganisms are responsible for 65–80% of infections and have the capacity to contribute to a variety of diseases.[38] Furthermore, Wang *et al*. mentioned that particularly in individuals with compromised immune systems, *K. pneumoniae* biofilm can cause invasion of the respiratory, gastrointestinal, and urinary tracts as well as the rise of invasive infections.[39]

While, research revealed that weak biofilm development was independently correlated with patient clinical outcomes, pathogenicity, and resistance. The most resistant organism and the one that produced the most biofilms was *K. pneumoniae*. Antibiotic resistance and biofilm development did not statistically correlate, which may be due to the fact that the majority of isolates were poor biofilm producers.[40]

The *Klebsiella* samples that were obtained from the hospitals and laboratories demonstrated resistance to some antibiotics as a result of their strong biofilm formation in agreement with Shadkam *et al*. The majority of *K. pneumoniae* isolates that have been isolated from hospitalized patients are able to produce biofilms. In addition, MDR-*K. pneumoniae* genotypes tend to produce stronger biofilms than the non-MDR strains in their study.[22]

Whereas, a study revealed that *K. pneumoniae* isolates resistant to the antibiotic carbapenem were 91% less likely to be strong biofilm producers, making it the first study to their knowledge to demonstrate an antagonistic correlation between *K. pneumoniae* antimicrobial resistance and biofilm production.[41]

However, Nirwati *et al*. suggested according to this research, biofilm-producing *K. pneumoniae* had higher levels of drug resistance than non-biofilm-producing *K. pneumoniae*.[6]

CONCLUSION

The prevalence of antibiotic resistance in *Klebsiella* is rising which is why samples were obtained from hospitals and laboratories. In this research, we wanted to investigate if the resistance is correlated to biofilm formation. Several tests were performed, to identify and confirm the presence of *Klebsiella*. Antibiotic resistance was evaluated using VITEK 2 and the capability of biofilm formation was assessed by both qualitative and quantitative techniques. Although there is not a significant correlation between antibiotic susceptibility and biofilm formation, the samples demonstrated the capability of strong biofilm formation as well as resistance to certain antibiotics.

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