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Detection of mrkD and fimH Biofilm Genes and Antibiotic Susceptibility of Uropathogenic Biofilm-Producing Klebsiella pneumoniae in Sana'a City, Yemen

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Short Report

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Abstract

Background

Urinary tract infections (UTIs) caused by antibiotic-resistant strains of *Klebsiella pneumoniae* (*K. pneumoniae*) are one of the most important causes of morbidity and health care spending affecting people in Yemen, including young women, children, and the elderly. Biofilm formation has become a major factor among those uropathogenic strains.

Objectives

This study aimed to investigate the biofilm-forming ability of *K. pneumoniae* isolated from patients with UTIs, to determine the antibiotic susceptibility of those biofilm producers, and to assess the association between fimbrial virulence genes (*fimH* and *mrkD*) and biofilm strength.

Methods

Standard phenotypic microbiological procedures were utilized for the isolation and identification of *K. pneumoniae* from symptomatic UTI patients. Quantitative biofilm production was determined using the standard tissue culture plate (TCP) assay. Antimicrobial susceptibility testing was performed via the Kirby-Bauer disk diffusion technique in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. The presence of fimbriae-encoding virulence genes (*fimH* and *mrkD*) among biofilm-forming isolates was detected using conventional polymerase chain reaction (PCR).

Results

Out of 241 urine samples processed, *K. pneumoniae* was verified in 79 cases (32.8%). Biofilm production was phenotypically confirmed in 40 isolates (50.6%), whereas 39 isolates (49.4%) were classified as non-biofilm formers. Among the 40 biofilm-producing strains, 19 (47.5%) displayed a strong biofilm phenotype and 21 (52.5%) exhibited a moderate biofilm phenotype. Regarding resistance patterns, extensively drug-resistant (XDR) phenotypes predominated among biofilm producers at47.50% (n = 19), followed by multidrug-resistant (MDR) phenotypes at 32.5% (n = 13). Pandrug-resistant (PDR) strains represented the most resistant phenotype but were observed at the lowest frequency of 5.0% (n = 2). Complete antibiotic susceptibility was preserved in only 15.0% (n = 6) of biofilm formers. Molecular screening revealed that the *mrkD* gene was present in 100.0% (n = 40) of the biofilm-producing isolates. The *fimH* gene was detected in 87.5% (n = 35) of the strains and demonstrated a statistically significant correlation with the strong biofilm phenotype (Prevalence Ratio [PR]: 2.19; 95% Confidence Interval [CI]: 1.5–3.1; p = 0.031).

Conclusion

The fimbrial virulence genes *mrkD* and *fimH* were highly prevalent among biofilm-producing uropathogenic *K. pneumoniae* isolates in Sana'a City. The presence of *fimH* was statistically associated with the strong biofilm phenotype; however, the cross-sectional design does not establish causality. The frequent MDR and XDR phenotypes observed among biofilm producers underscore the need for continued antimicrobial-resistance and molecular surveillance.

Key Message

What is already known on this topic

Klebsiella pneumoniae is a major cause of urinary tract infections (UTIs) and is known for its ability to form biofilms, which enhance its resistance to antibiotics and contribute to persistent infections. The fimbrial genes mrkD (type 3) and fimH (type 1) are established virulence factors that facilitate adhesion and biofilm formation in various clinical settings.

What this study adds

This study provides the first baseline data from Sana'a City, Yemen, revealing that 50.6% of uropathogenic K. pneumoniae isolates are biofilm producers, with a striking 80% of these exhibiting multidrug-resistant (MDR) or extensively drug-resistant (XDR) phenotypes. It further demonstrates that while mrkD is universally present (100%), the presence of fimH is significantly associated with the strong biofilm phenotype ($p = 0.031$), highlighting its potential as a key marker for more virulent strains in this region**.**

How this study might affect research, practice, or policy

The high prevalence of XDR and MDR phenotypes among biofilm-forming isolates underscores the urgent need for enhanced antimicrobial stewardship and infection control protocols in Yemeni healthcare facilities. The strong association of fimH with robust biofilm formation suggests that this gene could be a promising target for future diagnostic screening or novel therapeutic interventions. These findings advocate for the implementation of routine molecular surveillance to monitor virulence and resistance genes, which is critical for guiding empirical therapy and combating the spread of highly resistant uropathogens.

Introduction

Klebsiella pneumoniae, a common Gram-negative bacterium in clinical settings, can lead to severe infections such as pneumonia, wound infections, and urinary tract infections [1, 2, 3]. Due to the high death rate from *K. pneumoniae* infections, particularly in children and people with weak immune systems, a considerable lot of study has focused on the development of antibiotic resistance [4]. In fact, it has

been shown that *K. pneumoniae* frequently causes infections in patients with indwelling urinary catheters [5, 6, 7]. These bacteria can develop antibiotic resistance through a number of mechanisms, which results in the emergence of multidrug-resistant (MDR) strains that seriously jeopardize patient safety and medical facility design. One important factor in the start and spread of infection is the formation of biofilms [8].

Biofilm development is the ability of bacteria to survive under adverse conditions [9]. The bacteria within are extremely resistant to antibiotic treatments because this biofilm protects them from both the human immune system and medications [10]. Because biofilms provide an environment that is resistant to antibiotic penetration and permits the horizontal transfer of virulence genes, multidrug-resistant organisms (MDRO) can proliferate more readily in them [11]. Approximately 80% of urinary tract infections (UTIs), a serious health risk, are caused by biofilm-forming bacteria. Furthermore, biofilm plays a major role in catheter-associated UTIs and recurrent UTIs [12]. Since biofilm formation by pathogenic bacteria may be a virulence factor in individual attacks, it is currently of interest. This emphasizes the need for ongoing assessment of pathogen development [13].

The type 1 or type 3 fimbriae, the capsule, and the lipopolysaccharides are the main virulence factors that enable *K. pneumoniae* to form a biofilm. It has been established that type 3 fimbriae are the primary appendages that mediate biofilm formation on abiotic surfaces and adherence to endothelium and bladder epithelial cell lines [14]. In particular, the type 3 fimbrial adhesin *mrkD* is required for development on surfaces coated with human extracellular matrix, whereas the type 3 fimbrial protein mrkA encourages *K. pneumoniae* growth on abiotic surfaces [15].

After *E. coli*, *K. pneumoniae* is the second reported bacterial cause of UTIs in Yemen [6, 7, 16, 17]. Although UTIs represent an important health problem in Yemen, data on fimbrial virulence genes among biofilm-forming uropathogenic *K. pneumoniae* and their relationship with antimicrobial resistance remain limited. Therefore, this study aimed to determine the biofilm-forming capacity and antimicrobial susceptibility patterns of uropathogenic *K. pneumoniae* isolates in Sana'a City, assess the prevalence of *fimH* and *mrkD* among biofilm-producing isolates, and evaluate their association with biofilm strength.

Subjects and Methods

Study Design and Ethical Clearance

This descriptive, cross-sectional, hospital-based investigation was conducted across two major medical centers: Al-48 Modern General Hospital and the University of Science and Technology Hospital in Sana'a City, Yemen. Laboratory processing and molecular assays were centralized at the Department of Microbiology and Molecular Biology within the National Center of Public Health Laboratories (NCPHL) in Sana’a. The study spanned a 12-month period, from February 2024 to February 2025.

Ethical clearance for this protocol was formally reviewed and granted by the Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences at Sana'a University (Approval No. 287),

strictly adhering to the tenets of the Declaration of Helsinki. Prior to specimen collection, written informed consent was acquired from all adult participants or legal guardians in the case of pediatric patients.

Clinical Specimen Collection and Processing

Symptomatic male and female patients presenting with clinical manifestations of UTI and significant pyuria (≥ 10 WBCs/HPF) were eligible for inclusion. Patients with reported antimicrobial use during the preceding two weeks and duplicate isolates from the same patient were excluded. Samples were collected by consecutive non-probability sampling from inpatient and outpatient departments of the participating hospitals. Clean-catch midstream urine samples (approximately 10–20 mL) were collected in sterile, wide-mouth, screw-capped containers and processed within two hours of collection.

Isolation and Identification of *K. pneumoniae*

Initial screening for pyuria was performed via microscopic urinalysis; samples displaying significant inflammatory responses (≥ 10 white blood cells per high-power field [WBCs/HPF]) were prioritized for culture. Quantitative bacteriuria assessment was executed by inoculating 0.01 mL of uncentrifuged urine onto CLED (Cysteine-Lactose-Electrolyte-Deficient) agar and MacConkey agar plates using standard calibrated loops. Inoculated plates were incubated aerobically at 37°C for 18–24 hours. A threshold of ≥ 105 colony-forming units per milliliter (CFU/mL) was defined as significant monobacterial growth. Primary identification was based on colonial morphology (mucoid, lactose-fermenting colonies) and conventional Gram staining. Definitive species-level identification and biochemical verification were systematically achieved using the automated VITEK 2 compact system (bioMérieux, Marcy-l'Étoile, France) with specialized GN cards according to the manufacturer’s instructions.

Quantitative Biofilm Detection via Tissue Culture Plate (TCP) Assay

Phenotypic biofilm quantification was executed utilizing the standard 96-well polystyrene tissue culture plate method as originally described by Christensen et al. [36], with minor modifications. Briefly, individual clinical isolates were inoculated into Trypticase Soy Broth (TSB) supplemented with 1% glucose to promote biofilm formation and grown overnight at 37°C. The cultures were subsequently diluted 1:100 with fresh TSB medium. Aliquots of 200 \μL of the bacterial suspension were transferred into parallel wells of a sterile, flat-bottomed 96-well microtiter plate.

To ensure the quality, validity, and reproducibility of the assay, standard reference strains were included in each microtiter plate as controls. Sterile TSB medium supplemented with 1% glucose (without bacterial inoculation) was utilized as the negative control to determine background absorbance. Conversely, *Klebsiella pneumoniae* ATCC 700603 was employed as the positive control strain due to its well-documented, robust biofilm-forming capacity.

Plates were incubated statically under aerobic conditions at 37°C for 24 hours. Following incubation, the planktonic bacterial suspension was aspirated, and the wells were gently washed three times with 250 \µL of sterile phosphate-buffered saline (PBS, pH 7.2) to remove non-adherent cells. Adherent biofilms were fixed with 99% methanol for 15 minutes, air-dried, and stained with 0.1% crystal violet for 15 minutes. Excess stain was rinsed under running tap water, and the plates were dried. The biofilm-bound crystal violet was solubilized by adding 200 \µL of 33% glacial acetic acid per well. The optical density (OD) of each well was measured at 570 nm (OD_{570}) using an automated microplate ELISA reader. Biofilm production was stratified into categorized adherence levels based on calculated mathematical cut-off boundaries relative to the negative controls (OD_C).

Formula

$\overline{OD}$ ODc = Mean OD of the negative control + (3 × SD of the negative control)

(where Mean OD of the negative control represents the mean optical density of the negative-control wells, and SD represents their standard deviation).

$\overline{OD}$ $_{\text{negative control}}$ represents the mean optical density of the negative control wells, and $SD_{\text{negative control}}$ represents the standard deviation of the negative control wells).

Based on the derived mathematical boundaries, the phenotypic biofilm-forming capacities of the *K. pneumoniae* isolates were systematically stratified into the following distinct categories:

Non-biofilm producer: OD ≤ ODc

Weak biofilm producer: ODc < OD ≤ 2 × ODc

Moderate biofilm producer: 2 × ODc < OD ≤ 4 × ODc

Strong biofilm producer: OD > 4 × ODc

(Fig. 1).

Determination of the antibiogram susceptibility pattern

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method in accordance with CLSI 2021 recommendations [19]. A *K. pneumoniae* suspension was adjusted to a 0.5 McFarland standard and inoculated onto Mueller-Hinton agar [18]. The following antimicrobial discs (HiMedia, India) were tested: imipenem (10 µg), meropenem (10 µg), cefuroxime sodium (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefoxitin (30 µg), gentamicin (10 µg), amikacin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ampicillin (10 µg), piperacillin-tazobactam (110 µg), and co-trimoxazole (1.25/23.75 µg). Plates were incubated at 37°C for

24 h. Inhibition-zone diameters were measured in millimetres and interpreted as susceptible, intermediate, or resistant according to CLSI 2021 criteria [19].

Isolates were categorized according to the international definitions proposed by Magiorakos et al. [37]:

Multidrug-resistant (MDR): non-susceptible to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR): non-susceptible to at least one agent in all but two or fewer antimicrobial categories (i.e., susceptible to only one or two categories). Pandrug-resistant (PDR): non-susceptible to all agents in all antimicrobial categories tested. In this study, “complete susceptibility” denotes isolates categorized as susceptible to all antimicrobial agents included in the testing panel.

Molecular Detection of Virulence Genes (mrkD and fimH) via PCR

To isolate bacterial genomic DNA, phenotypically verified biofilm-producing *K. pneumoniae* isolates (n = 40) were cultivated overnight in nutrient broth. Extraction was subsequently executed employing a commercial DNA extraction kit, strictly adhering to the guidelines provided by the manufacturer. The identification of the type 1 fimbrial tip adhesin gene (*fimH*) along with the type 3 fimbrial adhesin gene (*mrkD*) was accomplished through conventional polymerase chain reaction (PCR) amplification. This molecular screening utilized specific oligonucleotide primer pairs synthesized in accordance with validated configurations previously documented by Shah et al. [20] (Table 1).

Table 1
Primers used for detection of the *fimH* and *mrkD* genes in this study

Gene	Primer Sequence (5–3)	Expected size (bp)
FimH	F: 5´- ATG AAC GCC TGG TCC TTT GC -3´	688
	R:5´-GCT GAA CGC CTA TCC CCT GC -3´	
mrkD	F: 5´- CCA CCA ACT ATT CCC TCG AA -3´	240
	R: 5´- ATG GAA CCC ACA TCG ACA TT -3´	

The specific reaction configuration used for compounding the total 25 \muL PCR mixture for gene amplification is outlined in Table 2. The thermal cycling profiles were set according to the protocol optimized for each target locus (Table 3). The amplification routine was initiated with an initial denaturation phase at 94°C for 6 minutes, followed by 35 continuous cycles consisting of denaturation at 94°C for 30 seconds, primer annealing at 58°C for 30 seconds, and a chain extension step at 72°C for 30 seconds. A final elongation step was completed at 72°C for 5 minutes, followed by a final hold at 4°C for 3 minutes. To ensure assay validity, a negative control containing sterile distilled water and a positive control using *K. pneumoniae* ATCC 13883 were run concurrently. The resulting amplification products were separated via horizontal gel electrophoresis on a 1.5% agarose matrix impregnated with ethidium bromide, and the specific amplicon bands were subsequently visualized under ultraviolet illumination using a ChemiDoc XRS imaging system (Bio-Rad).

Table 2
The PCR reaction components (25µl) for genes amplification

Component	Volume (µl)
DNA Template	4 µl
Forward Primer (10 picomoles)	1 µl
Reverse Primer (10 picomoles)	1 µl
Nuclease-Free Water	6.5 µl
Master Mix (2X)	12.5 µl
Total volume	25 µl

Table 3
Program for amplification of the *fimH* and *mrkD* genes

Stage	°C	Time	Cycle number
Initial denaturation	94	6 mins	1
Denaturation	94	30 sec	35
Annealing	58	30 sec	
Extension	72	30 sec	
Final extension	72	5 mins	1
Hold	4	3 mins	1

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Categorical data are presented as frequencies (n) and percentages (%). Associations between fimbrial-gene detection and biofilm strength (moderate versus strong) were assessed using Pearson's chi-square test; Fisher's exact test was used when expected cell counts were < 5. Prevalence ratios (PRs) with 95% confidence intervals (CIs) were used to quantify binary associations. Because only the two prespecified fimbrial genes were evaluated, no correction for multiple comparisons was applied. All tests were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Prevalence of K. pneumoniae and Phenotypic Biofilm Profiles

A total of 241 non-duplicate clinical urine specimens were processed. *K. pneumoniae* was confirmed in 79 specimens (32.8%). TCP screening of these 79 isolates identified 40 biofilm-producing isolates (50.6%) and 39 non-biofilm producers (49.4%). Among the 40 biofilm-producing isolates, 19 (47.5%) were classified as strong and 21 (52.5%) as moderate biofilm producers; no isolates met the threshold for weak biofilm production (Figs. 3 and 4).

Phenotypic biofilm screening of the 79 isolates via the quantitative TCP assay demonstrated that 40 isolates (50.6%) were capable of establishing functional biofilms, whereas the remaining 39 isolates (49.4%) were non-biofilm formers. Further stratification of the 40 biofilm-positive strains based on calculated optical density thresholds revealed that 19 isolates (47.5%) were strong biofilm producers and 21 isolates (52.5%) developed moderate biofilms; no isolates were classified as weak biofilm formers (Fig. 4).

Intersection of Biofilm Production with Antimicrobial Resistance Categories

The distribution of multi-resistance classifications among the 40 biofilm-forming uropathogenic strains was heavily skewed toward advanced resistance profiles (Fig. 5). Extensively drug-resistant (XDR) phenotypes constituted the absolute majority, detected in 19 isolates (47.50%). Multidrug-resistant (MDR) phenotypes were verified in 13 isolates (32.50%). Pandrug-resistant (PDR) isolates—representing the maximum possible phenotypic resistance profile—were documented at the lowest overall frequency, accounting for 2 isolates (5.0%). Conversely, total baseline susceptibility to the basic testing matrix was maintained in only 6 isolates (15.0%) of the biofilm-producing cohort.

MDR: multidrug-resistant, XDR: extensively drug-resistant, PDR: pan-drug-resistant

Figure 5: The antibiogram susceptibility pattern among the isolated biofilm-producing *K. pneumoniae*

Distribution of Virulence Genotypes and Association with Biofilm Intensity

Molecular screening of the 40 biofilm-producing *K. pneumoniae* isolates demonstrated that the type 3 fimbrial adhesin gene *mrkD* was universally present in 100.0% (n = 40) of the cohort. Within this total distribution, the *mrkD* gene was carried by 19 strong biofilm formers (47.5%) and 21 moderate biofilm formers (52.5%). Because the *mrkD* gene exhibited zero variance within the biofilm-forming population (present in 100%), statistical correlation modeling or chi-square evaluation could not be calculated for this marker (denoted as Undefined).

In contrast, *fimH* was detected in 35 of the 40 biofilm-producing isolates (87.5%). All 19 strong biofilm producers were *fimH*-positive, whereas 16 of 21 moderate biofilm producers were *fimH*-positive. Because of the small cell counts, the association was evaluated using Fisher's exact test. A statistically

significant association was observed between *fimH* detection and the strong biofilm phenotype (p = 0.031). The reported prevalence ratio was 2.19 (95% CI: 1.5–3.1). These observational findings indicate an association and should not be interpreted as evidence that *fimH* causes increased biofilm strength.

Table 4
The prevalence of *fimH* and *mrkD* genes among the isolated biofilm-producing *K. pneumoniae* and their associations with biofilm strength.

Fimbriae virulence genes	**Biofilm producer**				**PR (CI 95%)**	***p*-value**
	Moderate		**Strong**			
No.	**%**	**No.**	**%**			
fimH (n = 35)	16	45.7	19	54.3	2.19(1.5–3.1)	0.031
mrkD (n = 40)	21	52.5	19	47.5	UD	UD

CI: confidence interval; PR: prevalence ratio; UD: Undefined, as statistical correlation could not be calculated due to 100% prevalence (zero variance) in the biofilm-producing cohort.

Discussion

The clinical complications associated with uropathogenic *K. pneumoniae* are significantly amplified by its propensity to synthesize complex biofilms. This matrix-encapsulated lifestyle serves as a critical mechanism for long-term bacterial persistence within the urinary tract, directly impairing standard empirical treatments and favoring colonization on indwelling medical hardware [20]. The phenotypic analysis conducted in this study revealed that 50.6% of the uropathogenic *K. pneumoniae* strains isolated from patients in Sana'a City were capable of establishing functional biofilms. Among these positive isolates, a high-intensity adherence profile (strong biofilms) was verified in 47.5%, while the remaining 52.5% displayed a moderate colonization capacity.

When benchmarking these findings against international epidemiological surveys, regional variations in biofilm-forming rates become evident. For instance, a total baseline production rate of 100% was reported by Makhrmash et al., who documented strong adherence in 57% of clinical *K. pneumoniae* strains [21]. Similarly, Hamam et al. noted a phenotypic biofilm prevalence of 81% among their studied isolates [22]. Epidemiological assessments from other regions, including Iran and Nigeria, indicated positive biofilm profiles in 79.6% and 68.18% of urinary *K. pneumoniae* samples, respectively [23, 24]. These contrasting geographical patterns are likely multi-factorial, stemming from discrepancies in localized empirical antibiotic choices, differing demographic distributions of patient cohorts, specific clonal lineages circulating within healthcare systems, or subtle variations in microtiter plate assay parameters such as nutritional enrichment and staining times [21, 25, 26].

A notable finding was the high frequency of advanced antimicrobial-resistance phenotypes among biofilm-producing isolates: XDR and MDR phenotypes accounted for 47.5% and 32.5%, respectively, while PDR isolates represented 5.0%. Only 15.0% of biofilm-producing isolates were susceptible to all agents in the tested panel. The higher observed proportion of XDR than MDR isolates should be interpreted descriptively and may reflect the clinical setting and local antimicrobial exposure patterns; however, the present cross-sectional study did not directly measure the mechanisms or determinants responsible for this distribution.

The coexistence of biofilm production and antimicrobial resistance is consistent with reports from other settings, including the study by Musa et al. [24], which evaluated antibiotic susceptibility and biofilm formation among urinary *K. pneumoniae* isolates in Nigeria. Biofilm-associated antimicrobial tolerance may involve restricted antibiotic penetration through the extracellular polymeric substance, altered bacterial metabolic activity, efflux mechanisms, and enhanced opportunities for horizontal transfer of resistance determinants [27–29].

These findings reinforce the importance of local antimicrobial stewardship and continued molecular surveillance. Because the present study did not characterize carbapenemase genes or experimentally evaluate alternative antimicrobial compounds, conclusions regarding specific resistance determinants or novel anti-biofilm therapies are beyond the scope of the current data.

Molecular profiling of the fimbrial determinants showed that the type 3 fimbrial adhesin gene *mrkD* was universally carried by 100.0% of the biofilm-forming strains. The absolute conservation of this gene (carried by all 19 strong and 21 moderate formers) suggests that *mrkD* constitutes a fundamental element of the genetic architecture of uropathogenic *K. pneumoniae* in Yemen. This aligns with molecular surveillance from Iraq, which similarly noted a 100% carriage rate of *mrkD* among urinary isolates [14, 33]. Detailed functional studies have validated that the type 3 fimbria, primarily through the *mrkD* tip adhesin, orchestrates the initial attachment phase to host matrix proteins and steers the maturation of the biofilm structure [15, 34]. This central role is corroborated by gene-knockout investigations proving that mutant strains lacking functional mrk exhibit severely crippled biofilm development on surfaces coated with human matrix components [35].

In contrast, *fimH* was detected in 87.5% of the biofilm-producing isolates and was statistically associated with the strong biofilm phenotype ($p = 0.031$). The reported PR was 2.19 (95% CI: 1.5–3.1). This finding supports an association between *fimH* detection and biofilm intensity in the studied isolates, but the cross-sectional observational design does not establish a causal effect. Previous studies have described roles for type 1 fimbriae in adherence and biofilm development [21, 22, 30–32].

Several limitations should be considered. First, the descriptive cross-sectional design precludes causal inference and long-term assessment of clinical outcomes. Second, the study was conducted in two hospitals in Sana'a, which limits generalizability beyond the sampled population. Third, the antimicrobial panel did not encompass all advanced therapeutic categories. Finally, molecular screening was restricted to the fimbrial loci *mrkD* and *fimH*; additional virulence and hypervirulence determinants,

including *rmpA* and *magA*, were not investigated. Thus, the study should be interpreted primarily as local baseline epidemiological evidence rather than a comprehensive molecular characterization of uropathogenic *K. pneumoniae*.

Conclusion

Among biofilm-producing uropathogenic *K. pneumoniae* isolates from Sana'a City, *mrkD* was universally detected and *fimH* was highly prevalent. *fimH* detection was statistically associated with the strong biofilm phenotype, while MDR and XDR phenotypes were frequent among biofilm-producing isolates. These findings provide local baseline data and support the need for broader multicenter studies incorporating expanded virulence and resistance gene panels.

Declarations

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Availability of data and materials

The data will be available to anyone upon request from the corresponding author.

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Ethics declarations

Ethics approval and consent to participate

The ethical approval of this study was formally reviewed and granted by the Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences at Sana'a University (Approval No. 287), in strict accordance with the standards outlined in the Declaration of Helsinki. Written informed consent was obtained from all adult participants or legal guardians prior to performing any research procedure.

Competing interests

The authors declare that there is no conflict of interest.

Authors' contributions

HAA supervision and revision, and editing of the manuscript. SMMD collected data and samples from all patients and performed the laboratory procedures for the samples. DAE and KAA wrote of introduction

and methodology. HAA and ANMG analyzed the data, prepared tables and figures, and discussed the results. All authors arranged the referencing style, read, and made the final revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

References

1. Abinaya M, et al. Evaluation of in-vitro biological and antibiofilm activities of various leaf extracts of Aerva lanata. Int J Pharm Sci Rev Res. 2016;38:65–72.
2. Al-Shehari MM, et al. Distribution and antibacterial resistance of wound pathogenic bacteria in patients of Sana'a hospitals, Yemen. Univers J Pharm Res. 2023;8(3):1–8. https://doi.org/10.22270/ujpr.v8i3.942.
3. Al-Yosaffi EA, et al. Antibiotic sensitivity of bacterial bloodstream infections in the intensive care unit patients of university hospitals in Sana'a city, Yemen. Univers J Pharm Res. 2023;8(5):1–8. https://doi.org/10.22270/ujpr.v8i5.1004.
4. Abdelmegeed ES, et al. Comparative study on prevalence and association of some virulence factors with extended spectrum beta-lactamases and AmpC producing *Escherichia coli*. Afr J Microbiol Res. 2015;9:1165. https://doi.org/10.5897/AJMR2015.7463.
5. Frank DN, et al. Culture-independent microbiological analysis of Foley urinary catheter biofilms. PLoS ONE. 2009;4:e7811. https://doi.org/10.1371/journal.pone.0007811.
6. Al-Haifi AY, et al. Urinary tract infections in post operative patients: prevalence rate, bacterial profile, antibiotic sensitivity and specific risk factors. Univers J Pharm Res. 2020;5(3):1–8. https://doi.org/10.22270/ujpr.v5i3.411.
7. Saleh AAM, et al. Biofilm formation and antibiotic susceptibility of uropathogens in patients with catheter-associated urinary tract infections in Ibb city. Yemen Univers J Pharm Res. 2020;4(6):1–8. https://doi.org/10.22270/ujpr.v4i6.329.
8. Rana S, et al. Prevalence and antibiogram of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* and Proteus mirabilis. Res J Pharm Technol. 2015;8:1465–8. https://doi.org/10.5958/0974-360X.2015.00262.0.
9. Sadiq FA, et al. Community-wide changes reflecting bacterial interspecific interactions in multispecies biofilms. Crit Rev Microbiol. 2021;47:338–58. https://doi.org/10.1080/1040841X.2021.1887079.
10. Chhibber S, et al. Inhibiting biofilm formation by *Klebsiella pneumoniae* B5055 using an iron antagonizing molecule and a bacteriophage. BMC Microbiol. 2013;13:174. https://doi.org/10.1186/1471-2180-13-174.
11. Katongole P, et al. Biofilm formation, antimicrobial susceptibility, and virulence genes of uropathogenic *Escherichia coli* isolated from clinical isolates in Uganda. BMC Infect Dis. 2020;20:453. https://doi.org/10.1186/s12879-020-05186-1.

12. Agarwal J, Radera S. Biofilm-mediated urinary tract infections. In: Biofilms Hum Diseases: Treatment Control 2019;177–213.
13. Mohamed SH, et al. A combination of essential oil and ciprofloxacin to inhibit/eradicate biofilms in multidrug-resistant *Klebsiella pneumoniae*. J Appl Microbiol. 2018;125:84–95. https://doi.org/10.1111/jam.13755.
14. Mahmood MT, Abdullah BA. The relationship between biofilm formation and the presence of *fimH* and *mrkD* genes among *E. coli* and *K. pneumoniae* isolated from patients in Mosul. Mosul Nurs. J. 2015;1:1–6. https://doi.org/10.33899/MJN.2015.160018
15. Jagnow J, Clegg S. *Klebsiella pneumoniae* MrkD-mediated biofilm formation on extracellular matrix- and collagen-coated surfaces. Microbiology. 2003;149:2397–405. https://doi.org/10.1099/mic.0.26434-0.
16. Nasher MA, Nasher TM, Gunaid AA. Etiologies of the urinary tract infections in a Yemeni city. Saudi Med J. 2001;22:599–602.
17. Ishak AA, et al. Childhood urinary tract infection: clinical signs, bacterial causes and antibiotic susceptibility. Univers J Pharm Res. 2021;6(4):1–6. https://doi.org/10.22270/ujpr.v6i4.643.
18. Chesebrough M. District Laboratory Practice in Tropical Countries. Cambridge University Press; 2010.
19. Clinical and Laboratory Standards Institute (CLSI). In: Supplement CLSI M100, editor. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2021.
20. Shah RK, et al. The determination and correlation of various virulence genes, ESBL, serum bactericidal effect, and biofilm formation of clinically isolated classical *Klebsiella pneumoniae* and hypervirulent *Klebsiella pneumoniae* from respiratory tract infected patients. Pol J Microbiol. 2017;66:501–8.
21. Makhrmash JH, et al. Investigation of biofilm virulence genes prevalence in *Klebsiella pneumoniae* isolated from urinary tract infections. Arch Razi Inst. 2022;77:1421. https://doi.org/10.22092/ARI.2022.357626.2076.
22. Hamam SS et al. Study of various virulence genes, biofilm formation, and extended-spectrum β-lactamase resistance in *E. coli* isolated from urinary tract infections. Open Microbiol. J. 2019;13.
23. Seifi K, et al. Evaluation of biofilm formation among *Klebsiella pneumoniae* isolates and molecular characterization by ERIC-PCR. Jundishapur J Microbiol. 2016;9:e30682.
24. Musa KS, et al. Isolation, antibiotic susceptibility patterns, and biofilm formation of *Klebsiella pneumoniae* from urine samples of patients with suspected urinary tract infections at Dutse General Hospital, Jigawa State, Nigeria. J Appl Sci Environ Manage. 2025;29:3222–6.
25. Vuotto C, et al. Antibiotic resistance related to biofilm formation in *Klebsiella pneumoniae*. Pathogens. 2014;3:743–58. https://doi.org/10.3390/pathogens3030743.
26. Wang G, et al. The characteristics of virulence, biofilm, and antibiotic resistance of *Klebsiella pneumoniae*. Int J Environ Res Public Health. 2020;17:6278.

27. Nirwati H et al. Biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. BMC Proc. 2019;13:20. https://doi.org/10.1186/s12919-019-0176-7
28. Ballén V, et al. Antibiotic resistance and virulence profiles of *Klebsiella pneumoniae* strains isolated from different clinical sources. Front Cell Infect Microbiol. 2021;11:738223. https://doi.org/10.3389/fcimb.2021.738223.
29. Atray D, Atray M. Correlation between biofilm production and antibiotic resistance pattern in uropathogenic *Escherichia coli* in a tertiary care hospital in Southern Rajasthan, India. Int J Curr Microbiol Appl Sci. 2015;4:640–6.
30. Johnson JG, Clegg S. Role of MrkJ, a phosphodiesterase, in type 3 fimbrial expression and biofilm formation in *Klebsiella pneumoniae*. J Bacteriol. 2010;192:3944–50.
31. Nepal HP, et al. Biofilm formation and antimicrobial resistance in *Klebsiella pneumoniae* isolated from patients visiting a tertiary care center of Nepal. Asian Pac J Trop Dis. 2017;7:347–51.
32. Alwan AM, et al. Molecular analysis of the FimH gene in biofilm-producing and multidrug-resistant *Klebsiella pneumoniae*. Hilla Univ Coll J Med Sci. 2024;2:25–31.
33. Razooqi Al-Aajem BM et al. Detection of extended-spectrum beta-lactamase genes (bla-CTX and bla-SHV) and the same virulent genes in *K. pneumoniae* isolated from urinary tract infection. Biochem Cell Arch. 2021;21.
34. Ong CL, et al. Molecular analysis of type 3 fimbrial genes from *Escherichia coli*, Klebsiella, and Citrobacter species. BMC Microbiol. 2010;10:183. https://doi.org/10.1186/1471-2180-10-183.
35. Willsey GG, et al. Pulmonary surfactant promotes virulence gene expression and biofilm formation in *Klebsiella pneumoniae*. Infect Immun. 2018;86:e00135–18. https://doi.org/10.1128/IAI.00135-18.

Figures

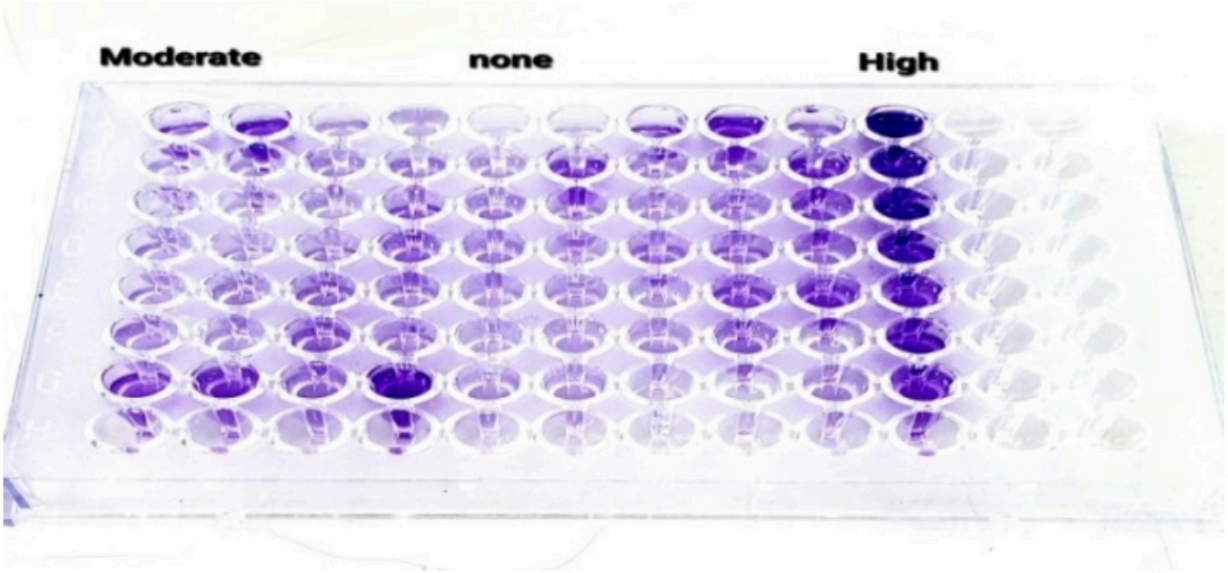


Figure 1

Tissue culture plate

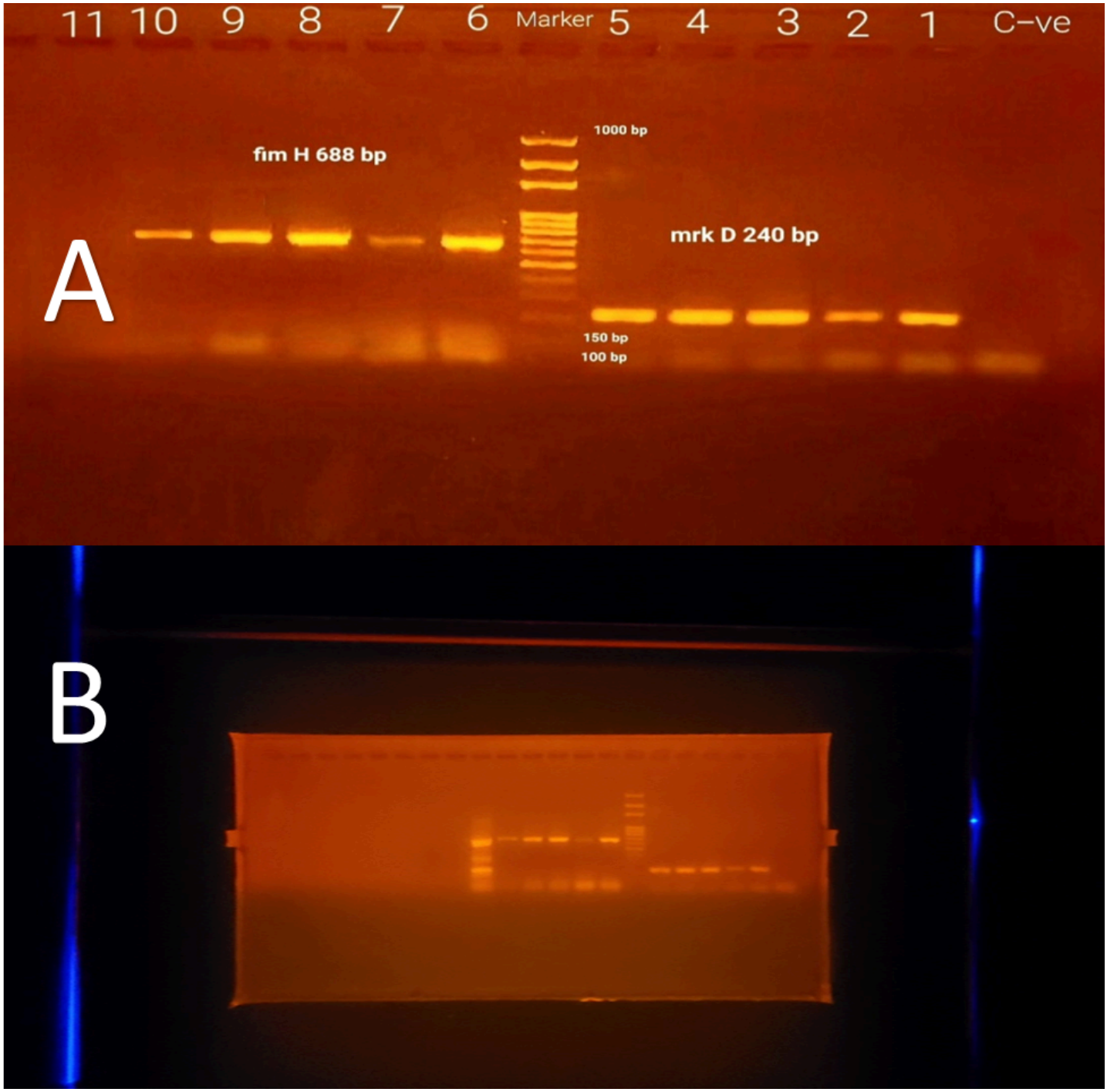


Figure 2

A) Agarose gel electrophoresis for the PCR amplified products of *Klebsiella pneumoniae fimH* and *mrkD* genes . Lane marker: DNA molecular size marker (1000 bp). Lane C-ve: negative control. Lanes 1, 2, 3, 4 and 5: represent positive *mrkD* gene (240 bp) and lanes 6, 7, 8, 9 and 10: represent positive *fimH* gene (688 bp). Lane 11: represents negative sample. B) full-length original unprocessed version gel with clearly marked edges.

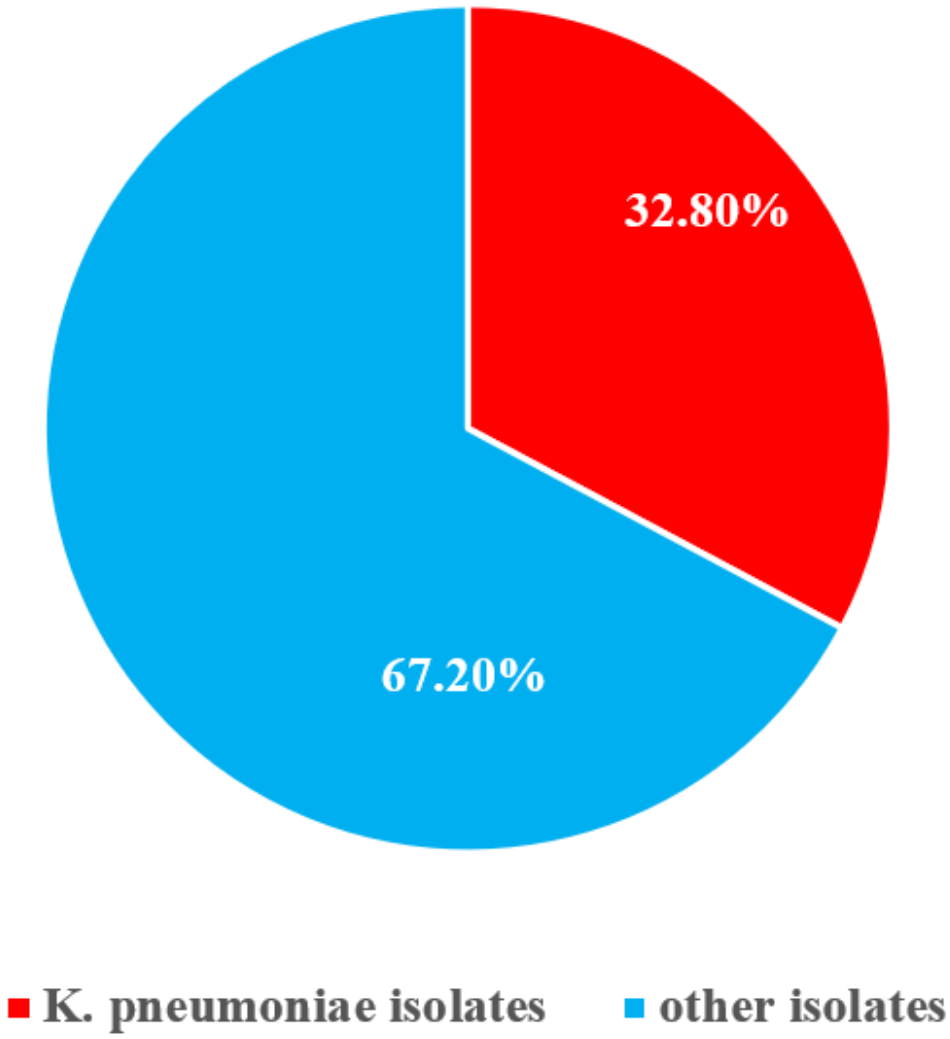


Figure 3

The rate of *K. pneumoniae* isolates from UTI cases (n=241).

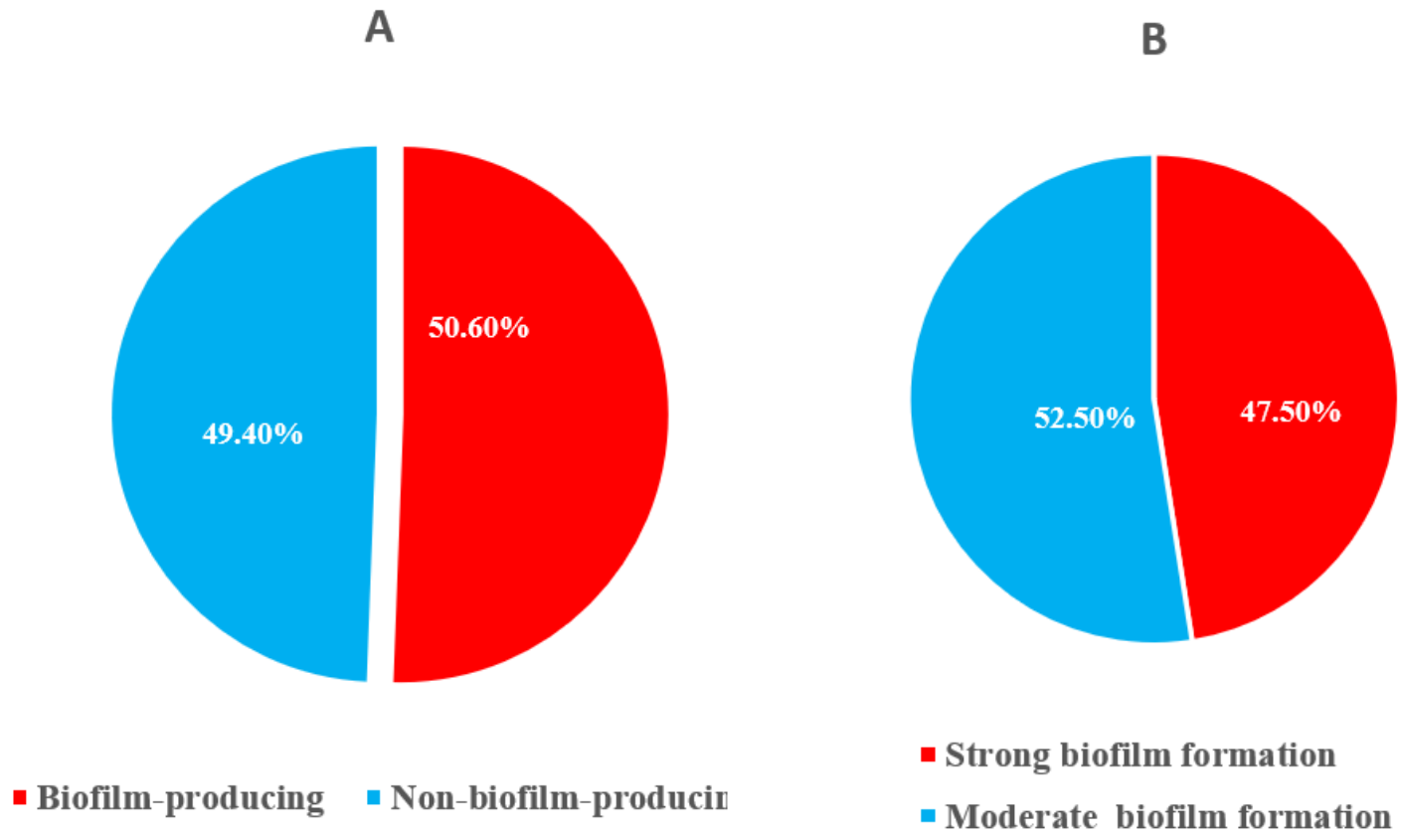
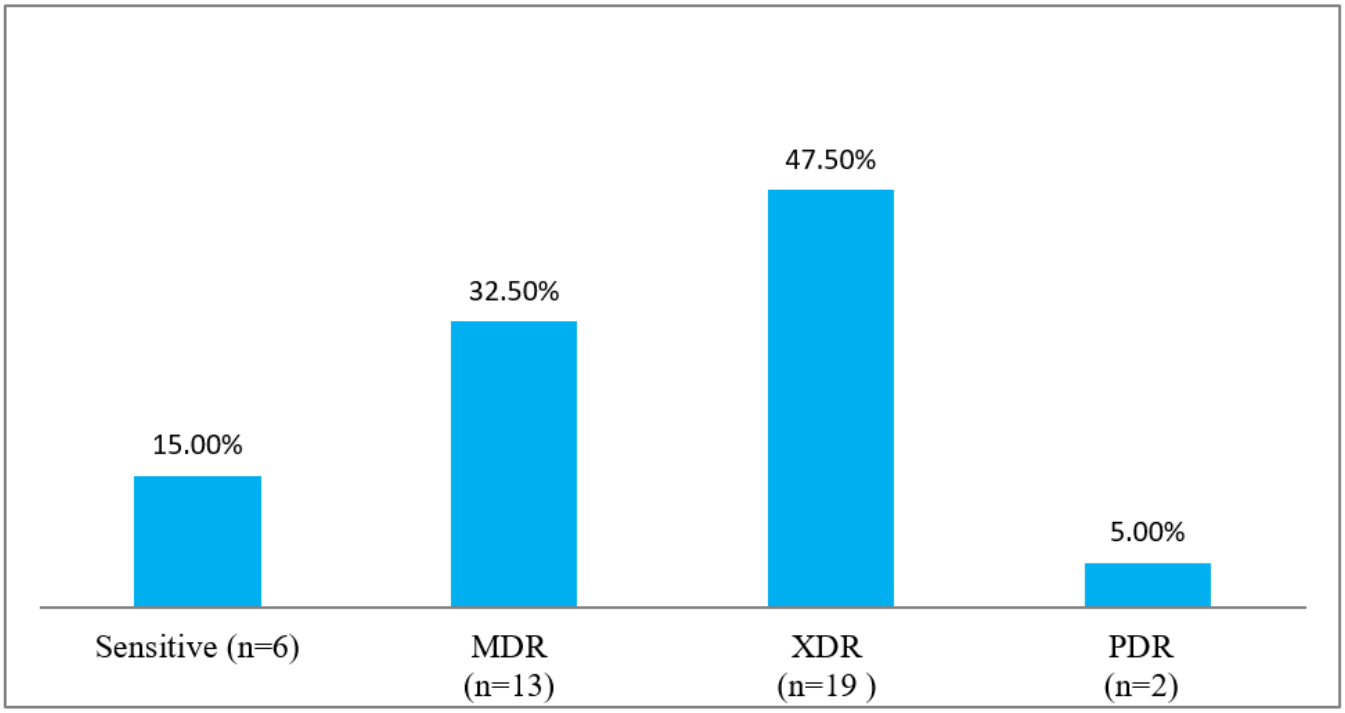


Figure 4

A) The rate of biofilm-producing *K. pneumoniae*, B) The strength of biofilm among biofilm-producing *K. pneumoniae*



MDR: multidrug-resistant, XDR: extensively drug-resistant, PDR: pan-drug-resistant

Figure 5

The antibiogram susceptibility pattern among the isolated biofilm-producing *K. pneumoniae*

Supplementary Files

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