Journal of Communicable Diseases
Volume 57, Issue 1 - 2025, Pg. No. 222-230
Peer Reviewed & Open Access Journal

Research Article

Detection of Virulence Genes in *Klebsiella pneumoniae* and *Staphylococcus aureus* Isolated from Urinary Catheterised Patients

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DOI: 10.24321/0019.5138.202529

I N F O

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How to cite this article:
Khuder H S, Khudhur S S. Detection of Virulence Genes in Klebsiella pneumoniae and Staphylococcus aureus Isolated from Urinary Catheterised Patients. J Commun Dis. 2025;57(1):222-230.

Date of Submission: 2024-09-10
Date of Acceptance: 2024-10-12

A B S T R A C T

Background: One of the most predominant health-related illnesses universally is catheter-associated urinary tract infection (CAUTI). CAUTIs constitute over 50% of all hospital-acquired infections. Majority of the healthcare-acquired urinary tract infections result from catheter tubes implantation.

Objective: To reveal the existence and frequency of the *fimH, Ycfm, clfA,* and *Cap5* genes in two species isolated from patients with urinary catheters.

Methods: During the period from November 2023 to February 2024, a total of 50 samples of urine were collected from patients with urinary catheters at Tikrit Teaching Hospital and private clinics in Salah Al-Deen province, Iraq. Different antibiotics were used to examine the antibiotics sensitivity test for bacterial isolates.

Results: Among the participants, 30 (60%) were female and 20 (40%) were male, with ages ranging between 18 to 80 years and a mean age of 42.06± 14.6 years. Sensitivity for isolates of *S. aureus* to antibiotics was 100% for amoxicillin, 66.6% for trimethoprim, and 44.4% for gentamicin and nitrofurantoin. 11.1% of isolates showed resistance to ciprofloxacin. Among the *K. pneumoniae* isolates, 66.6 % exhibited resistance to amoxicillin and trimethoprim, while 11.1% showed resistance to ciprofloxacin. Molecular analysis was done for 2 isolates of *S. aureus* and 2 isolates of *K. pneumoniae* based on their high resistance to antibiotics. The results revealed that the clfA gene was found in 100% of *S.aureus* isolates, while the *Cap5* gene was found in 50% of *S.aureus* isolates. Also, the result unveiled that 100% of *K. pneumoniae* isolates produced *FimH-1* and *Ycfm* genes.

Conclusion: S. aureus and *K. pneumoniae* are common causes of urinary tract infections in patients with urinary catheters. Virulence genes have an important role in the pathogenicity of *S. aureus* and *K.pneumoniae* in patients with urinary catheters.

Keywords: Catheter-Associated Urinary Tract Infection, fimH-1, Ycfm, clfA, Cap5 Genes

Introduction

Urinary tract infections (UTIs) associated with catheters (CAUTIs) is a major concern in healthcare settings. UTIs are among the most prevalent hospital-acquired infections, with CAUTIs being the most common type.[1,2] Several factors increase the risk of developing CAUTIs, including advanced age, female gender, diabetes, and prolonged catheterisation.[3] The presence of a catheter creates a unique environment for bacterial growth and biofilm formation, increasing the risk of colonisation by non-enteric, hospital-acquired bacteria.[4] Gram-negative bacteria, such as Escherichia coli, Proteus mirabilis, Klebsiella species and Pseudomonas aeruginosa are the primary cause of UTIs, accounting for 90% of cases, while gram-positive bacteria are responsible for the remaining 10%. The most frequently identified causative organisms, following Uropathogenic Escherichia coli (UPEC), are Enterococcus spp. (11%), *K. pneumoniae* (8%), Candida spp. (7%), S.aureus (3%), P.mirabilis (2%), P.aeruginosa (2%), and Group B Streptococcus (2%).[5]

Klebsiella pneumoniae is a common opportunistic pathogen that infects about 10% of urinary catheterised patients. The catheter insertion site gives an optimal environment for bacterial attachment, especially for gram-negative Enterobacteriaceae.[6, 7, 8] Fimbriae are coded by the Fim gene cluster, which contains all genes necessary for their formation. These genes encode for repeated FimA subunits, with an adhesin molecule FimH at the apex.[9] Recent research indicates that S. aureus is increasingly recognised as a cause of UTIs in specific patient groups, like pregnant women and patients with complex UTIs. Complicated S. aureus UTIs are mostly associated with the existence of foreign objects (urinary catheters or kidney stones).[10]

Investigating the Presence of Virulence Genes in Klebsiella pneumoniae

Genes Responsible for Adhesions and Biofilm Formation

Microorganisms like *K. pneumoniae* have remarkable adaptability, allowing them to survive in varied environments, both externally and during host colonisation. The diverse structures of *K. pneumoniae* proteins release a wide range of molecular designs, including several virulence factors. These factors, such as fimbriae encoded by the fimH gene and outer membrane lipoprotein encoded by the Ycfm gene, enable the bacteria to attach to and form biofilm on host cells. Bacterial DNA is able to express the Ycfm gene, which codes for an outer membrane lipoprotein.[11]

Ycfm Gene

Ycfm belongs to the YhcN family, which includes proteins with nine similar structures and low molecular weights. The Ycfm family is believed to have originated from a prominent ancestor that played crucial roles in cell-to-cell attachment, colony formation, and self-recognition. It is also known to respond to cellular stress, particularly in unusual conditions such as pH variation ranging from 3 to 8.[12]

FimH Gene

The fimH-1 gene encodes an important virulence factor known as fimbriae, which plays a significant role in the first stages of *Klebsiella pneumoniae* infection by smoothing the attachment to epithelial cells. These fimbriae are also required for tissue colonisation and subsequent infection. *Klebsiella pneumoniae* has attachment factors, including type 1 fimbriae and outer membrane lipoprotein; this feature facilitates the bacteria to colonise host tissues and is implicated in the first stages of adhesion and biofilm formation.[11]

Genes Responsible for the Virulence of Staphylococcus aureus

Clumping Factor A (clfA)

Clumping factor A (clfA) is a cell surface-associated protein that facilitates its binding to both soluble and immobilised fibrinogen. This protein appears as the original example of a group of staphylococcal surface proteins known as the Sdr protein family characterised by the presence of a region called the serine-aspartate dipeptide repeat region (R region).[13] clfA binds to fibrinogen which is produced by S. aureus bacteria, inducing attachment to the surface of the cells.[14] Clumping factor is significant for S.aureus virulence, and is considered to be crucial for colonisation and progression of infection; nearly all S aureus strains possess the clfA gene.[14,15]

Capsular Polysaccharides (Cap5)

Capsular polysaccharides (CPs) produced by *Staphylococcus aureus* play an essential role in its colonisation, pathogenesis, and capability to resist phagocytosis by masking of proteins surface.[16] Over 90% of S. aureus strains produce CPs. A total of 11 distinct serotypes (CP1to CP11) have been detected, which can be divided into two morphological categories, thick macrocapsules, which producing by strains express CP1 and CP2, While 70–80% of S.aureus clinical isolates express CAP5 or CAP8 generate thinner microcapsules.[17] The aim of this research is to reveal the existence and frequency of the fimH, Ycfm, clfA, and Cap5 genes in two species isolated from patients with urinary catheters.

Methods

Study Design and Sample Selection

In this descriptive cross-sectional study conducted during the period from November 2023 to January 2024, 50 samples were taken from urinary catheterised patients at

ISSN: 0019-5138
DOI: https://doi.org/

Tikrit Teaching Hospital and private clinics in Salah Al-Deen province. The patients' ages ranged from 18 to 80 years.

Ethical Considerations

The research ethics committee approval was obtained from the Scientific and Ethical Committee at the College of Pharmacy, Tikrit University (approval number: SREC7).

Isolation and Identification of Bacteria

Urine samples were grown on two types of agar plates: MacConkey agar, blood agar (HI Media, India), and incubated in the presence of oxygen at 37 °C for 24 hours.[18] Bacteria identified as *Klebsiella pneumoniae* were confirmed based on their appearance under a microscope (gram stain), their shape, and their reactions observed in various chemical tests. *Staphylococcus aureus* bacteria were identified based on their appearance on the culture media and their reactions in various chemical tests which were performed according to the Bergeys manual.[19]

Antibiotic Sensitivity Tests

To determine which antibiotics would be effective against the isolated bacteria, the "disk diffusion method" was used. This method follows the "Kirby-Bauer" protocol and involves placing commercially available antibiotic discs (Oxoid Ltd, UK) onto Mueller Hinton medium plates. The size of the clear zone around each disc, where the bacteria were unable to grow, was measured and interpreted according to the guidelines set by the Clinical Laboratory Standards Institute (CLSI).[20]

DNA Extraction

All isolated bacteria were grown in a nutrient-rich liquid called Brain Heart Infusion Broth (Hi Media, India) for 18–24 hours at 37 °C. To extract the genetic material (DNA) from the *Staphylococcus aureus* bacteria, a specific kit called the Genomic DNA Extraction Kit (Geneaid, Korea) was used. For the *Klebsiella pneumoniae* bacteria, a different DNA extraction kit was used called the Genomic DNA Purification Kit (Favorgen).

Polymerase Chain Reaction Protocol

The protocol utilized relies on Promega manufacture instructions, as displayed in Table 1.

Detection of Virulence Genes

To identify specific genes associated with virulence (the ability to cause disease) in the bacteria, a technique called polymerase chain reaction (PCR) was used. This technique employs specially designed primers that target specific genes: clfA and Cap5 for Staphylococcus aureus, and Ycfm and fimH1 for Klebsiella pneumoniae. The presence of these genes was confirmed by running the PCR products on a gel (electrophoresis) and visualising the resulting bands. This analysis was performed using a 1.5% agarose gel and an electrical voltage of 80 volts for 95 minutes (Table 2).

Table 1.PCR Protocol Reaction Mixture Volumes

Master mix	8.0 µL
DNA template	5.0 µL
Forward primers	1.5 µL
Reverse primers	1.5 µL
Deionised water	4.0 µL
Final volume	20.0 µL

Table 2.Gene Characteristics and PCR Condition

Name of Bacteria	Gene Name	Annealing (ºC)	Product Size (bp)
Klebsiella pneumoniae	*fimH-1*	57	180
Klebsiella pneumoniae	*Ycfm*	57	160 (168)
Staphylococcus aureus	*clfA*	61	292 (288)
Staphylococcus aureus	*Cap5*	53	454 (448)

Statistical Analysis

Microsoft Excel was used for descriptive analysis of percentage and frequency.

Results

A total of 50 samples of urine were collected from patients who utilise urinary catheters. Of these patients, 60% were female and 40% were male, with ages ranging from 18 to 80 years. The mean age was 42.06 years with a standard deviation of 14.6 years (Table 3). Out of the 50 samples, 18% (9 samples) were positive for *Klebsiella pneumoniae* and another 18% (9 samples) were positive for Staphylococcus aureus. The distribution of these bacterial isolates is shown in Table 4.

Table 3.Demographic Data of Urinary Catheterised Patients

N=50

Parameters	Results
Age (years) (mean ± SD)	42.06 ± 14.6
Gender n (%)	
Female	30 (60)
Male	20 (40)
BMI (kg/m^2) (mean ± SD)	25.066 ± 5.759
Catheterisation duration (week) n (%)	
< 1	35 (70)
≥ 1	15 (30)

ISSN: 0019-5138
DOI: https://doi.org/

Table 4.Distribution of Bacterial Isolates

Bacteria		No. of Isolates	Perce-ntage
Gram -negative bacteria	*Klebsiella pneumoniae*	9	18
Gram -positive bacteria	*Staphylococcus aureus*	9	18
Total		18	36

Based on the identification tests, *Klebsiella pneumoniae* appeared as large, round, lactose-fermenting mucoid colonies on MacConkey agar. On Eosin Methylene Blue medium, *K. pneumoniae* grew as brown, dark- centered, mucoid colonies, indicating lactose fermentation and acid production.

Staphylococcus aureus isolates were confirmed via their positive catalase reaction, coccus (spherical to ovoid) shape, growth on mannitol salt agar, along with fermentation of mannitol, production of coagulase, and production of DNase.

Antimicrobial Susceptibility of *Staphylococcus aureus*

Antimicrobial susceptibility tests revealed that 4 (44.4%) isolates of S.aureus were resistant to Nitrofurantoin. Resistance to amikacin and ceftriaxone was observed in 33.3% (n = 3) and 22.2% (n = 2) isolates, respectively. All isolates were resistant to amoxicillin, while 66.6% (n = 6) of isolates showed resistance to trimethoprim. Furthermore, 44.4% (n = 4) of isolates were resistant to cefalexin and gentamicin and only one isolate (11.1%) of S.aureus showed resistance to ciprofloxacin.Table 5.

Antimicrobial Susceptibility of Klebsiella pneumoniae

Results revealed that amongst 9 samples, only 2 (22.2%) isolates of *K. pneumoniae* were resistant to Nitrofurantoin and amikacin, 66.6% (n = 6) of isolates were resistant to amoxicillin and trimethoprim While 44.4% (n = 4) of isolates were resistant to cefalexin and gentamicin, resistance to ceftriaxone and ciprofloxacin was seen in 33.3% (n = 3) and 11.1% (n = 1) of isolates respectively, Table 6.

Table 5.Antimicrobial Susceptibility of *Staphylococcus aureus*

Antibiotics Bacteria	N	TMP	AMC	CIP	CEF	GN	CET	AK
Staphylococcus aureus 1	S	R	R	S	S	R	S	S
Staphylococcus aureus 2	R	R	R	S	S	S	S	R
Staphylococcus aureus 3	S	R	R	S	S	R	S	S
Staphylococcus aureus 4	S	R	R	S	R	S	S	S
Staphylococcus aureus 5	S	S	R	S	R	S	S	S
Staphylococcus aureus 6	R	R	R	S	R	R	R	R
Staphylococcus aureus 7	R	S	R	S	S	S	S	S
Staphylococcus aureus 8	S	R	R	S	S	R	S	S
Staphylococcus aureus 9	R	S	R	R	R	S	R	R

S: Sensitive, R: Resistant, N: Nitrofurantoin, TMP: Trimethoprim, AMC: Amoxicillin, CIP: Ciprofloxacin, CEF: Cefalexin, GN: Gentamicin, CET: Ceftriaxone, AK: Amikacin

Table 6.Antimicrobial Susceptibility of *Klebsiella pneumoniae*

Antibiotics Bacteria	N	TMP	AMC	CIP	CEF	GN	CET	AK
Klebsiella pneumoniae 10	S	R	R	S	R	R	R	R
Klebsiella pneumoniae 11	S	R	S	S	S	R	S	S
Klebsiella pneumoniae 12	S	S	R	S	S	S	S	S
Klebsiella pneumoniae 13	S	R	S	S	S	S	R	S
Klebsiella pneumoniae 14	R	R	R	R	S	R	S	R

ISSN: 0019-5138
DOI: https://doi.org/

Klebsiella pneumoniae 15	S	S	S	S	R	S	S	S
Klebsiella pneumoniae 16	S	S	R	S	R	S	S	S
Klebsiella pneumoniae17	R	R	R	S	S	R	R	S
Klebsiella pneumoniae 18	S	R	R	S	R	S	S	S

S: Sensitive, R: Resistant, N: Nitrofurantoin, TMP: Trimethoprim, AMC: Amoxicillin, CIP: Ciprofloxacin, CEF: Cefalexin, GN: Gentamicin, CET: Ceftriaxone, AK: Amikacin

DNA Extraction

The genomic DNA extraction of 9 isolates of S. aureus and 9 isolates of *K. pneumoniae* was successfully performed as shown in Figure 1. DNA extracted were assured and analysed by gel electrophoresis.

Detection of Virulence Genes

Two isolates each of Klebsiella pneumonia and *Staphylococcus aureus* were selected for further analysis based on their high resistance to antibiotics. These isolates were examined for the existence of specific virulence genes: FimH-1 and Ycfm for *K. pneumoniae*, and clfA and Cap5 for S. aureus.

Molecular analysis revealed that both S. aureus isolates (100%) carried the clfA gene, which was approximately 292 bp in size. The Cap5 gene was amplified at 448 bp, but one isolate (50%) did not show any positive result for this gene.

For *K. pneumoniae*, both isolates (100%) produced amplicons of 180 bp and 160 bp, confirming the presence of the FimH-1 and Ycfm genes, respectively. These genes are associated with adhesion and biofilm formation, which contribute to the bacteria's ability to cause infections Figure 2.

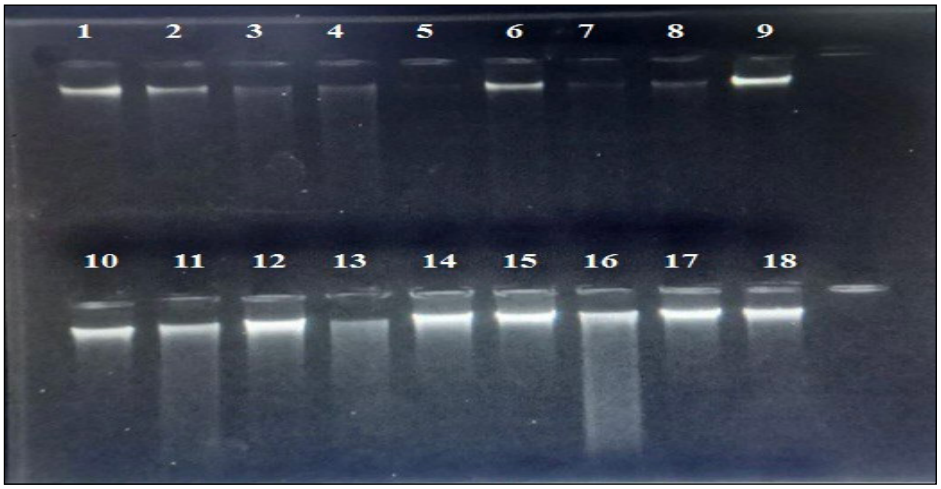

Figure 1.Agarose Gel Electrophoresis of Genomic DNA Extraction of Isolates; 1–9: Isolates of *S. aureus*, 10–18: Isolates of *K. pneumoniae*

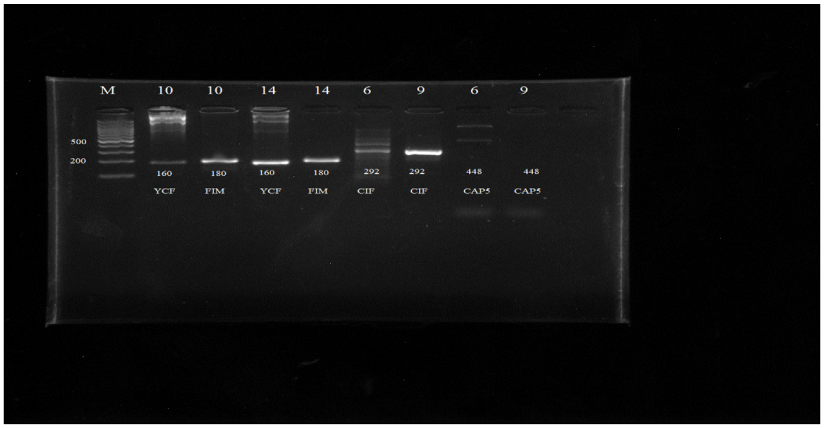

Figure 2.Agarose Gel Electrophoresis of *Ycfm*, *FimH-1*, *clfA* and *Cap5* Genes Amplification, M: 100–1500 bp DNA ladder, 10, 14: Isolates of *K. pneumoniae*, 6, 9: Isolates of *S. aureus*

ISSN: 0019-5138
DOI: https://doi.org/

Discussion

Urinary tract infections linked to catheters are in the midst of infections related to medical care. These infections typically arise from bacteria entering the body during catheter insertion, prolonged/unnecessary catheter use, or disruptions in the closed drainage system. In this study, most of the patients (60%) were female, with an average age of 42.06 years. This finding aligns with that of a study by Alshomrani et al., who reported a higher percentage of female patients (62%) with an average age of 32.9 years.[21]the current study found that 18% of isolates were Klebsiella pneumoniae. This finding differs from the results reported by Alwan and Khwen, who isolated *K. pneumoniae* in 88% of cases.[22] Similarly, Al-Kraety et al. reported positive results for *K. pneumoniae* in all 40 isolates obtained from urinary catheterised patients.[9] This study identified *Staphylococcus aureus* in 18% of clinical specimens. This finding is higher than the 1.08% reported by Al shomrani et al. in their study of 8,322 clinical isolates.[21] Similarly, another study conducted in France found S. aureus in only 1.3% of urine specimens.[23]

It is important to note that the S. aureus isolates of urine are often incidental through urinary catheterisation. This is supported by research conducted by Muder et al., who found that 82% of the 102 sufferers had undergone recent urinary catheterisation. Additionally, 33% of these patients had UTIs during the period of initial S. aureus isolation.[24]

Antimicrobial Susceptibility of *Staphylococcus aureus*

In the present study, for amoxicillin, the sensitivity of S. aureus isolates was100%; it was 66.6% for trimethoprim and 44.4% for gentamicin and nitrofurantoin. About 11.1% of isolates showed resistance to ciprofloxacin. Distinct studies have outlined diverse susceptibility patterns of S. aureus to various antibiotics. This might be attributable to the study cohort and the selection criteria for S.aureus isolates. Yousefi et al. observed that the overall susceptibility of S.aureus was 76.9% for trimethoprim and around 40% for both nitrofurantoin and gentamicin; while for ciprofloxacin was 35.9 % .[25] Another study accomplished by Akortha et al. revealed that the susceptibility of S.aureus was 63.5%, 58.9%, and 50.2% to nitrofurantoin, amoxicillin, and gentamicin, respectively, while 87.3% of S. aureus isolates were resistant to trimethoprim.[26] Likewise, the result of a community-based research project carried out by Al shomrani et al. in a healthcare centre in Riyadh, showed that 91% for trimethoprim and 97% for gentamicin.[21]

Antimicrobial Susceptibility of Klebsiella pneumoniae

In the present study, the researchers observed differences in the resistance rates of *K. pneumoniae* to 8 antibiotics, indicating that different antibiotic treatment schemes should be adopted for patients with catheters. Resistance to amoxicillin and trimethoprim was observed in 66.6% of isolates, as compared to 11.1% of isolates who showed resistance to ciprofloxacin. One of the mechanisms of resistance in K. pneumoniae consists in the alteration of penicillin- binding proteins (PBPs), enzymes that catalyse peptidoglycan synthesis and specific target of the β-lactam antibiotics. In addition, a mechanism of fluoroquinolone resistance is due to point mutations in specific areas of DNA gyrase. A dissimilar finding was observed by Aminul et al. in their study, where 7.4% of isolates were resistant to gentamicin and ciprofloxacin, and 0.0% to amoxicillin and ceftriaxone.[27] In accordance with Ullah et al. in Pakistan, dissimilar results displayed varied resistance to gentamicin, ceftriaxone, amikacin and ciprofloxacin (52.17%, 54.35%, 32.61% and 52.1%, respectively).[28]

Detection of Virulence Genes

Detection of Virulence Genes of Staphylococcus aureus

In this study, molecular analysis revealed that both S. aureus isolates (100%) carried the clfA gene. Clumping factor A is a crucial virulence factor for S. aureus, playing a key role in its ability to invade and colonise infection sites. Momtaz et al. suggested that the presence of clumping factors varies among S. aureus strains and is essential for colonisation.[29] Our findings align with those of Ghasemian et al and Omara et al., who reported that all the S. aureus strains they studied carried the clfA gene.[30,31] However, our results differ from those of Gowrishankar et al., who found that only 58.7% of isolates harboured this gene,[32] and from those of Degaim et al., who reported that 87.5% of isolates were positive for clfA.[14] Our study found the presence of the capsular polysaccharides (Cap5) gene in 50% of isolates. This is similar to the findings of Sutter et al., who reported that 56% of isolates contained the genes for CP type 5 (Cap5).[33]

Detection of Virulence Genes of Klebsiella pneumoniae

In this study, all *K. pneumoniae* isolates were positive for the virulence genes fimH-1 and Ycfm. Molecular analysis confirmed the presence of the fimH-1 gene in both isolates (100%). This finding is consistent with the study conducted by Jasim et al., who reported that 100% of isolates produced the fimH gene.[34] However, it differs from the results of Al-Kraety et al., who found the fimH gene in only 14 out of 40 *K. pneumoniae* isolates.[9] Additionally, Ferreira et al. reported that the fimH gene was present in 88% of *K. pneumoniae* isolates obtained from a Brazilian Intensive Care Unit.[35] Fimbriae are believed to play a crucial role in the attachment of bacteria to epithelial cell surfaces. Type-

ISSN: 0019-5138
DOI: https://doi.org/

1 fimbriae, in particular, are important for determining the virulence of an organism. Experiments mentioned by Al-Kraety et al demonstrated that mannose-resistant hemagglutinin is required for Klebsiella to attach to target cells.[9] this study found the Ycfm gene in all *K. pneumoniae* isolates. This finding aligns with the results reported by Aljanaby, who found the Ycfm gene in 100% of isolates at 160 bp.[11] Similarly, Tomaz et al. reported the presence of the Ycfm gene in 91 of their isolates, [36] and Kuş et al. found it in 86.8% of isolates.[37] Based on these findings, it appears that the fimH-1 and Ycfm genes are commonly present in *K. pneumoniae* isolates and may play a key role in the bacterium's pathogenicity.

When looking for virulence genes, the PCR approach is usually the one to go with. Many branches of microbiology make heavy use of this molecular strategy. Pathogenic bacteria including Proteus mirabilis, [38, 39] Staphylococcus aureus[40] and Pseudomonas aeruginosa[41] have been identified using it, and it has also been used to assess the severity of Coronavirus disease[42]. A number of studies have made use of PCR to investigate mutations in gastric cancer, [43] roles of bacterial neuraminidase and hyaluronidase in in vivo cancer cell contacts, [44, 45] and to quantify gene expression levels of biomarkers in a variety of diseases[46, 47]. In addition, PCR has been used to study the shRNA host gene 3 as a potential metabolic reprogramming treatment target in breast cancer,[48] determine the significance of mitochondrial DNA quantification for blastocyst transfer potential,[49] and find anti-testicular antibodies in cases of male infertility[50].

Limitations

This study has some limitations that need to be considered. Firstly, the sample size was relatively small, which may not accurately represent the entire population of urinary catheterised patients. A larger sample size would provide more reliable results. Secondly, the collection and processing of urine samples could potentially introduce contamination with other microorganisms, leading to false-positive results, and thirdly, the study was conducted in a limited geographical area. Additionally, the cross-sectional approach of the study only produces a brief overview of the incidence of virulence genes at a specific time point. A longitudinal study would be necessary to assess the dynamics of virulence gene carriage over time and identify risk factors for acquiring or losing these genes.

Conclusions

Staphylococcus aureus and *Klebsiella pneumoniae* are common causes of UTIs in patients with urinary catheters. These bacteria possess various virulence factors associated with urinary catheters. In this study, the fimH1 and Ycfm genes, which encode for adhesion and biofilm formation, were found in almost all *K. pneumoniae* isolates. These genes play a significant role in UTIs and appear to be fundamental to the classic pathogenicity of *K. pneumoniae*. Furthermore, S. aureus and *K. pneumoniae* have become increasingly resistant to antibiotics. Our findings suggest a positive correlation between antibiotic resistance and the prevalence of virulence factors in these bacteria. This highlights the need for further research to understand the complex interplay between antibiotic resistance and virulence in UTIs caused by S. aureus and *K. pneumoniae*.

Conflict of Interest: None

Source of Funding: None

Authors' Contribution: Both HSK and SSK equally contributed to all stages of the research, including sample collection and analysis, data acquisition, statistical evaluation, research conceptualization and design, manuscript preparation, and critical revision of the final draft.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

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ISSN: 0019-5138
DOI: https://doi.org/