Detection of Some Virulence Genes In Multidrug-Resistant Klebsiella Pneumoniae Isolated From Urethritis Patients In Wasit Province, Iraq

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SUMMARY

Urinary tract infections (UTIs) are the most prevalent type of nosocomial infection, and *K. pneumoniae* is the second most frequent Gram-negative bacterium cause of UTIs. A major worldwide health worry is the emergence of bacterial pathogens that are immune to numerous medications. Antibiotic overuse has limited our options for treating *K. pneumoniae* and made effective control of this bacterial disease more difficult..

For this study, we tested all *Klebsiella pneumonia* isolates for resistance to 17 antibiotics from various classes. We found that 83% of the isolates were resistant to the class polymyxin antibiotic (colistin), followed by 81% to ampicillin, and 80% to cephalosporin class antibiotics (cefotaxime, ceftriaxone) (57.5 percent). About half of the bacteria tested were resistant to ceftazidime, 40% were resistant to tetracyclines, and 37% were resistant to macrolides like azithromycin (37,5) Comparatively few strains are resistant to the quinolones levofloxacin and ciprofloxacin (7.5%) or the pipracilline family (3%), an aminoglycoside (5%), or a tobramycin (5%). Antibiotics like gentamicin (an aminoglycoside), carbapenem (imipenem), and quinolone (naldixic acid) (10 percent)

16S rRNA confirms the molecular identification of k pneumonia isolates. Utilizing the NanoDrop instrument, the concentration and purity were determined. Purity was between 1.6 and 2.0, while concentration ranged from 50 to 360 ng/l. The AcrAB gene, with a molecular weight of 312bp, was detected using multiplex-PCR, and the findings indicated that all of the isolates tested positive for its presence. Molecular weight 1250 bp *rmpA* gene was found in 25% of the isolates, 226 bp *mrkD* gene gave 70% positive results, and *rcsA* and *rcsB* genes were found in 35%, 35%, and 95% of the isolates, respectively.

1 INTRODUCTION

In contemporary medicine, urinary tract infections (UTIs) are among the most common diseases. (Rashed *et al.*, 2008). Every year, 150 million people around the globe get UTIs. (Stamm and Norrby, 2001). Among the most common bacterial infections, both in nosocomial infections and outpatient facilities are the infections (UTI). Enterobacteriaceae are commonly responsible for urinary infections (Akram *et al.*, 2007). Because of the length of the urethra and its proximity to the anus, which enable fecal bacteria entering the urinary tract, females are more susceptible to urinary tract infections (Holt *et al.*, 2002). Urinary tract infections commonly show symptoms like greater frequency, urgency, pain when urinating, and a pungent smell. These diseases frequently have urethritis, or urethral inflammation, as a precursor. 75% to 90% of all instances of

urinary tract infections are caused by the Enterobacteriaceae bacterium *E. coli*. (Stamm and Norrby, 2001). Uropathogens come in a wide variety of forms, but E. coli is still the most prevalent. *Klebsiella pneumoniae*, *Enterobacter*, *Proteus* spp., and *Enterococcus* spp. are some other typical uropathogens. (Hender *et al.*, 2007).

The increased utilization of the cephalosporins was combined with the appearance of the Enterobacteriaceae that possess the ESBLs. Even though many genera were discovered to be carrying the ESBL, K. pneumoniae are responsible for most isolates (Aljanaby and Alhasnawi, 2017). Genes encoding the ESBL enzymes often reside in the plasmids, however, they may be discovered as well in chromosomal DNA (Guiral *et al.*, 2018).

The hydrolysis of numerous distinct beta-lactam medications, including carbapenems, is facilitated by a diverse group of enzymes known as metallocarbapases. (Palzkill, 2013). A family of anti-microbial agents known as carbapenems has been set aside for infections brought on by multi-drug resistant microorganisms. One of the major threats to the public's health was the development of carbapenem resistance. An worrisome rate of antimicrobial resistance has led to significant outbreaks and treatment failures of nosocomial infections and community-acquired infections caused by clinically significant carbapenem resistance. (Elshamy and Aboshanab, 2020).

2 MATERIALS AND METHODS

2.1 Specimens Collection and Culture

Between January 2022 and April 15 of the same year, 336 samples were taken from patients with urinary tract infections at Al-Karama and Al Zahraa teaching hospitals, Al Kut hospital, and private clinics in Wasit province. The patients' ages ranged from 18 to 98 years old.

2.2 Isolation and identification of bacteria

The samples (urine and urethral swab) were culture on MacConkey agar, Blood agar, and incubated aerobically at 37°C for 24 hours (Rajash and Rattan, 2008). The isolated bacteria were identified according to morphological, biochemical tests and Vitek2

2.3 Antibiotic Susceptibility testing

Antibiotic Susceptibility testing has been conducted by the Kirby-Bauer process on the Muller Hinton agar (Bauer, 1966). Different antibiotics were used, including (Amikacin, Amoxillin-clavulanic acid, Ampicillin, Aztreonam, Azithromycin, Ceftazidime Cefixime, Ciprofloxacin, Ceftriaxone, Colistine, Cefotaxime, Gentamycin, Imipenem, Levofloxacin, Meropenem, Nalidixic Acid, Nitrofurantoin, Piperacillen, Tetracycline, and Tobramycin).

2.4 Phenotypic detection of ESBL producers

Phenotypical detection of ESBL production was accomplished using the disk diffusion method (screening test), and this was then validated using the double disc synergy test (DDST). Screening test was done according to CLSI (2021). Confirmatory test was carried out according to (Ugwu *et al.*, 2020). It was performed to seek cephalosporin/clavulanic acid synergy in cephalosporin resistant isolates by double disk synergy test (DDST).

2.5 Phenotypic detection of Carbapanamase

Isolates were tested for their ability to produce metallo-Blactamases (MBL) by looking at how susceptible they were to impenime or

Meropenem, and resistant isolates were flagged as being able to do so by showing resistance to either drug in an impenime or meropenem (IMP or MEM)-EDTA, combined disc test (Mathur *et al.*, 2008)**.**

2.6 Extraction of DNA from *Klebsiella* isolates:

Using a genomic DNA micro kit, genomic DNA was extracted from all *K. pneumoniae* isolates (40). Extraction was done according to the manufacturer's instructions of Geneaid Company after cultured on the Luria - Bertani broth. DNA was extracted to provide a PCR template for amplification.

Table 1 Primers' sequence of *16S rRNA, AcrAB* F, *mdtK* , *rmpA, rcsA*, and *rcsB*

Primer names	Sequences (5'-3')	Tm (°C)	Amplicon size (bp)	Reference
16S rRNA	F: GTATCTAAACCAGTTCGCACC R: TGCATATCTGCTGTTGCATC	58	145	(Makhrmash *et al.*, 2022)
AcrAB	F: ATCAGCGGCCGGATTGGTAAA R: CGGGTTCGGGAAAATAGCGCG	58	312	(Wasfi *et al.*, 2016)
mdtK	F: GCGCTTAACTTCAGCTCA R: GATGATAAATCCACACCAGAA	52	453	(Wasfi *et al.*, 2016)
rmpA	F: ACTGGGCTACCTCTGCTTCA CTTGCATGAGCCATCTTTCA	54	535	(Liu *et al.*, 2018)
rcsA	F: GGTCAGCCGAACGATATGAT R: ACGGGATATCTGACCAGTCG	57	537	This study
*rcs*B	F: TTTTTGGCGATCTCGGTTAC R: CACGGCCCTTATCAACAATC	56	408	This study

2.7 Statistical Analysis

The results were statistically analysed using the (SAS) Statistical Analysis System, version 9.1. The numbers were compared using a Chi-square analysis. A disparity between two groups is considered significant if it is less than 5% (P 0.05).

3 RESULT

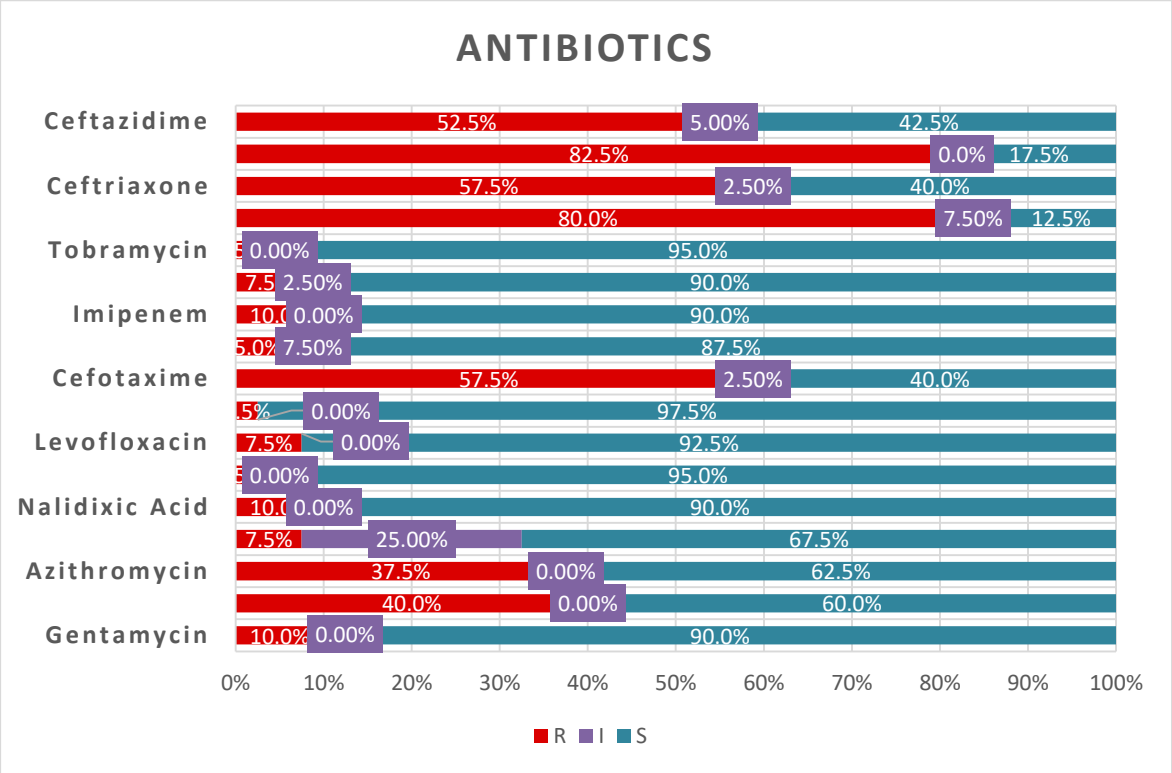
3.1 Isolation and Identification of *Klebsiella pneumoniae*

A total of 336 clinical samples (urine and urethral swabs) were obtained from patients with suspected UTIs from different age groups (18-98) years old and gender admitted to: AL-Zahraa teaching hospital, AL-Karama teaching hospital, AL-Kut hospital for gynaecology obstertrics and pediatrics, and from private clinics in Wasit province, during the period from December 2021 to December 2022. About 105 of these samples Traditional societal, microscopic, and molecular traits were used to identify K. pneumonia. Additionally, the identity is verified by the API 20 System, Vitek 2 System, and 16s rRNA.

3.2 Antibiotic resistance of *K. pneumoniae* isolates:

The findings of using the disc diffusion technique to screen 40 K. pneumoniae isolates for sensitivity to 17 various antibiotics are shown in the NCCL's handbook. According to Figure 1, of the 40 K. pneumoniae samples

examined, 82.5% were resistant to colistin, 80% to ampicillin, and then 57.5% to cefepime and ceftriaxone. The results also show that 39 out of 40 K. pneumoniae isolates examined reacted favourably to nitrofurantoin treatment, or 97.5%. Following penicillin in terms of sensitivity were amikacin (95.0%) and levofloxacin. (92.5%).



- P-Value < 0.05 (significant)
- P-Value > 0.05 (no significant)

Figure 1 Antibiotic resistant of *K. pneumoniae* isolates

3.3 Phenotypic detection of extended spectrum beta-lactamases (ESBL)

Penicillins, cephalosporins, monobactams, and carbapenems are all examples of antibiotics in the β-lactam class that are widely used across the world. (Joey, 2011; ur Rahman *et al.*, 2018).

A positive result from initial screening was followed up with phenotypic confirmatory test by disk approximation method.

Table 2 Phenotype of Percent of Extended spectrum beta-lactamases

Parameters	Sensitive		Resistant	
	N.	%	N.	%
CRO	13	32.5%	27	67.5%
ATM	17	42.5%	23	57.5%

CTX	12	30.0%	28	70.0%
CAZ	15	37.5%	25	62.5%
Screening test	(31) 77.5%			
Confirmatory test (DDST)	(13) 32.5%			
ESBLs: extended spectrum ß-lactamase; CTX: cefotaxime ; CAZ: ceftazidime; CRO: ceftriaxone; Positive: Resistance to CTX (≤ 27mm), CAZ (≤ 22mm) and CRO (≤ 25mm) .				

3.4 Phenotypic detection of carbapenem producers

The results of the investigation showed that sixteen of the forty *K. pneumoniae* isolates were resistant to carbapenemsm, and this resistance could be identified by the fact that imipenem EDTA was able to stop it. This percentage represents forty percent. Imipenem or meropenem plus EDTA double-disk synergy testing method has been employed.

3.5 Molecular Detectoin

All of the isolates 40 were submitted to molecular identification utilizing PCR amplification of the *16S rRNA* using K *16S-F* and K *16S-R* primers, which are specific primers for the PCR amplification of the *16S rRNA* of *K. pneumonia*. The amplified fragments were around 130 bp in size.

All 40 isolates underwent molecular detection by PCR amplification of *AcrAB* gene using specialized primers that are specific primers for PCR amplification of *K. pneumonia AcrAB* gene. As can be seen in Figure, the outcomes indicated that the amplified fragments were 312 bp in size. All 40 (100%) of the isolates produced good findings (312 bp).

Using customized primers designed specifically for the PCR amplification of the K. pneumonia mdtK gene, all 40 isolates underwent molecular detection. Figure from the data demonstrates that the amplified fragments were 453 bp in size. 19 out of 40 isolates, or 47.5%, produced successful findings (453 bp).

In the current investigation, the *rmpA* gene was amplified using PCR using a primer designed specifically for the *rmpA* gene. The PCR product produced by the amplified DNA using the *rmpA* primers has a band with an approximate molecular size of 1250 bp. The rmpA gene was amplified using customized primers designed specifically for the PCR amplification of the *K. pneumonia rmpA* gene to perform molecular detection on all 40 isolates. As can be seen in Figure, the outcomes indicated that the amplified fragments were 1250 bp in size. About 10 out of 40 isolates (about 25%) produced favorable findings (1250 bp).

All 40 isolates underwent molecular detection by PCR amplification of *rcsA* gene using specialized primers that are specific primers for PCR amplification of *K. pneumonia rcsA* gene. the size of the amplified fragments was determined to be 537 bp. All (14 of 40) isolates (35.0%) gave positive results (537 bp).

The prevalence of the *rcsB* gene in *Klebsiella pneumoniae* isolates from patients with urethritis was investigated using a specialized primer. The results of amplification using the polymerase chain reaction showed that 38/40 have the *rcsB* gene, which represents 95%.

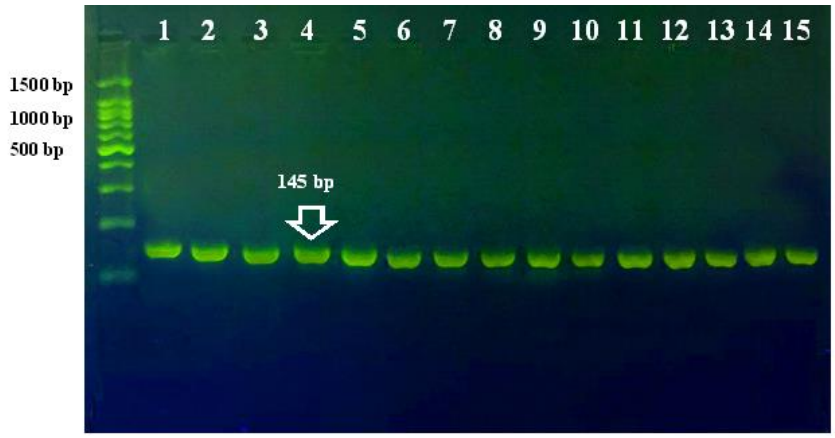


Figure 2 PCR product of 16s rRNA with band size 145 bp. The product was electrophoresis on 1.5% agarose at 70 volt/cm2. 1x TBE buffer for 0.5 hours

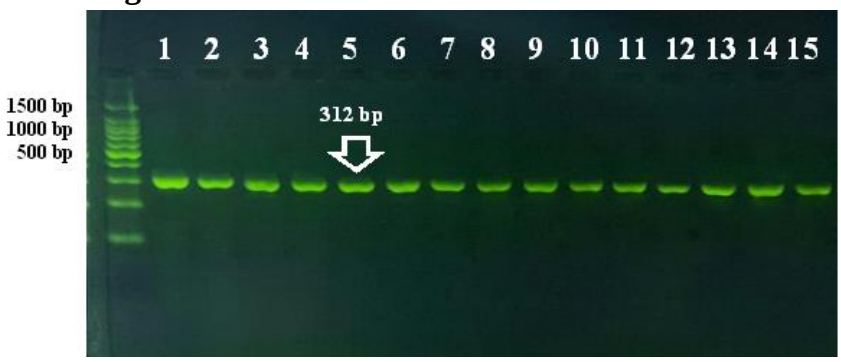


Figure 3 PCR product of *AcrAB* with band size 312 bp. The product was electrophoresis on 1.5% agarose at 70 volt/cm2. 1x TBE buffer for 0.5 hours.

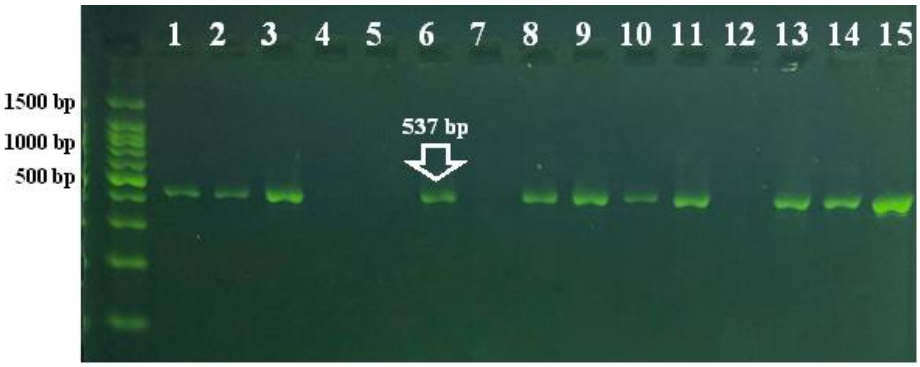


Figure 4 PCR product of *mdrk* with band size 453 bp. The product was electrophoresis on 1.5% agarose at 70 volt/cm2. 1x TBE buffer for 0.5 hours.

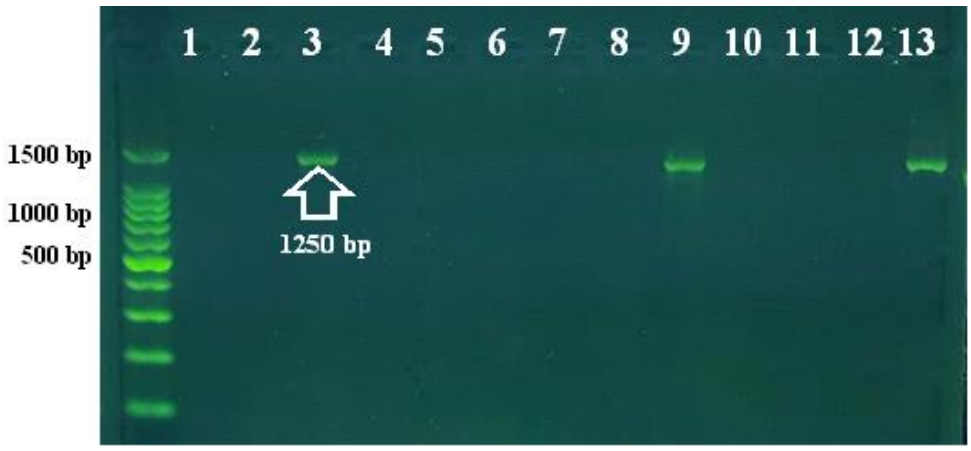


Figure 5 PCR product of *rmpA* with band size 1250 bp. The product was electrophoresis on 1.5% agarose at 70 volt/cm2. 1x TBE buffer for 0.5 hours.

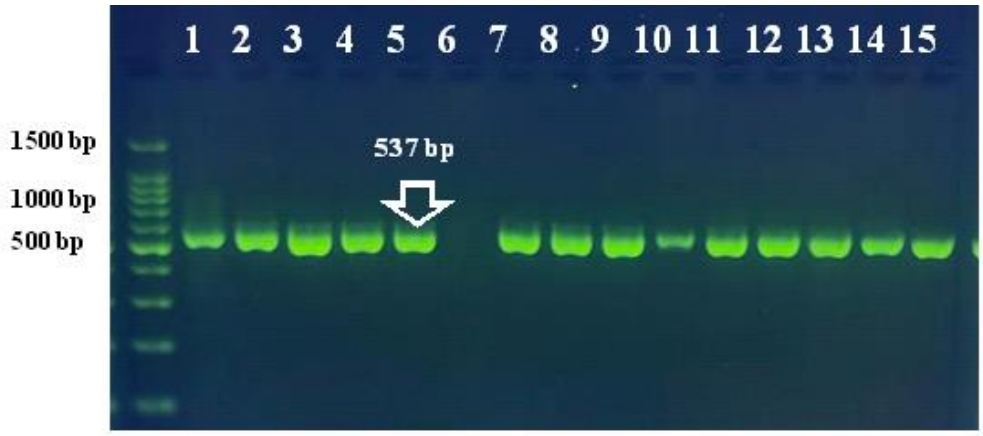


Figure 6 PCR product of *rcsA* with band size 537 bp. The product was electrophoresis on 1.5% agarose at 70 volt/cm2. 1x TBE buffer for 0.5 hours.

3.5.1 Detection of *rcsB* gene

Figure 7 PCR product of *rcsB* with band size 408 bp. The product was electrophoresis on 1.5% agarose at 70 volt/cm2. 1x TBE buffer for 0.5 hours.

4 DISCUSSION

These numbers are lower than those found by Amin et al. (2009) in Pakistan, who found that 82.5% and 85% of cefotaxime and ceftriaxone resistant strains, respectively, were prevalent there (Amin *et al.*, 2009). Our result also was higher than, Nasehi et al. (2010) result who mention that *K. pneumoniae* isolates were 27 % resistant to ceftriaxone (Nasehi *et al.*, 2010). This may be because these bacteria produce an enzyme called -lactamase (cephalosporinase) that cleaves the -lactam ring of cephalosprins, rendering the medicine ineffective.

There was an 80% resistance rate to ampicillin and a 7.5% resistance rate to piperacillin, both members of the penicillin family. In contrast to the findings of Al-Mulla (2003), who reported that one hundred percent of isolates were ampicillin-resistant, the current data show that only a small percentage of isolates (Al-Mulla, 2003). One of the most used medicines for treating urinary tract infections is ampicillin (Rice *et al.*, 1996), Therefore, the widespread resistance in Iraqi isolates poses a serious challenge to antimicrobial therapy. In Scotland, 88.2% of *Klebsiella* isolates from infections were ampicillin-resistant, according to research by (Thomson and Amyes, 1993). While Ahmad (2000) found that most *K. pneumoniae* isolates were resistant to piperacillin, our findings showed the opposite (Ahmad, 2000).

A possible cause of K. *pneumoniae* resistance to cephalosporins and penicillins is the presence of β-lactamase enzymes (cephalosporinase and penicillinase) that cleave the β-lactam ring of the medication, rendering the antibiotics ineffective (Stock and Wiedemann, 2001). β- lactamase are chromosomally or plasmid encoded and most of these plasmids are self-transmissible plasmids (Prescott *et al.*, 1999).

Twelve percent of *K. pneumoniae* tested positive for ciprofloxacin resistance, according to a study by (Amin *et al.*, 2009).

Study also demonstrated that 5.0% and 10% of *K. pneumonia* isolates were resistant to the aminoglycosids category, which contains drugs like amikacin and gentamicin. Similar results about amikacin resistance were observed by Nasehi et al. (2010), who showed that 17.5% of bacteria were resistant to the antibiotic (Akindele and Rotilu, 1997). In contrast, Akindele and Rotilu's (2000) findings diverged significantly from the available evidence. In their study, they found that 79% of *Klebsiella* isolates were resistant to the antibiotic gentamicin (Akindele and Rotilu, 1997). All Klebsiella bacteria tested in the research by Reish et al. (1993) and Roilides et al. (2000) were also resistant to the antibiotic gentamicin (Reish *et al.*, 1993; Roilides *et al.*, 2000). As such, *Klebsiella* isolates that produced extended spectrum β-lactamase enzymes were reported to be resistant to aminoglycosids by (Feizabadi *et al.*, 2007).

K. pneumoniae resistance to amikacin is due to three different mechanisms: First, the antibiotic is altered by enzymes, next chromosomal mutations occur in the genes that code for the target proteins, and finally, bacterial membrane permeability is reduced (Marranzano *et al.*, 1996).

There was a 40% resistance rate to tetracycline in the group that had previously been treated. Ten percent of *K. pneumoniae* isolates were reported to be resistant to the tetracycline by (Marranzano *et al.*, 1996).

The percentage of *Klebsiella* isolates resistant to the quinolone antibiotic ciprofloxacin was 7.5%. Antibiotic-enzyme (GyrA) binding site mutations have been linked to the development of quinolone resistance (Livermore and Brown, 2001).

And lastly, it was shown that 10% of *K. pneumoniae* isolates were resistant to the Carbapenem drug imipenem. Strong action against extended-spectrum beta-lactamases (ESBLs) from *Klebsiella* spp. was found for carbapenem (imipenem) antibiotics by (Livermore and Brown, 2001). The strain of *Klebsiella pneumoniae* isolated from a human respiratory tract and tested responsive to imipenem was discovered by (Feizabadi *et al.*, 2007). Another investigation found that *K. pneumoniae* isolated from a variety of clinical samples was imipenem-sensitive (Feizabadi *et al.*, 2008). The Carbapenemase enzymes produced by *K. pneumoniae* are to blame for the 156 strain's resistance to the antibiotic class known as Carbapenems. The imipenem susceptibility finding for *K. pneumoniae* was consistent with those found by Lim et al. (2009) and Nasehi et al. (2010), who found that just one isolate was resistant to imipenem (Lim *et al.*, 2009; Nasehi *et al.*, 2010). This finding suggests that imipenem is an effective therapy for *K. pneumoniae*

One third-generation cephalosporin (potential ESBL producers). Cefotaxime had a success rate of 70.5%, ceftriaxone of 67.5%, and ceftazidime of 62.5%.

Isolates of *Klebsiella pneumoniae* that showed resistance to three or more classes of antibiotics were considered MDR (Basak *et al.*, 2016).

The confirmatory approach by disk approximation was used on all isolates that showed antimicrobial resistance to any of the third generation cephalosporins examined. One of the major challenges with administering antibiotics is the emergence and rapid development of drug resistance among *K. pneumoniae* isolates, which is causing widespread concern (Zhang *et al.*, 2011; Sánchez-Romero *et al.*, 2012).

Extended-spectrum beta-lactamase (ESBLs), which is a part of group A-beta-lactamases and can lead to the hydrolysis of broad-spectrum cephalosporin and lead to resistance to penicillin and cephalosporins, has been found to be responsible for beta-lactam resistance. This resistance has been detected through chromosomal or plasmid genes. On the other hand, beta-lactamase inhibitors like clavulanic acid are able to stop them in their tracks (Mesa *et al.*, 2006; Mocktar *et al.*, 2007). Previous research has demonstrated that *K. pneumoniae* strains that are resistant to a wide variety of antibiotics are fast spreading, and this is especially true in instances where the bacteria are capable of building biofilm (Vuotto *et al.*, 2014).

Study demonstrated that phenotypic detection of ESBL production *K. pneumoniae* were (96%) (Ugwu *et al.*, 2020).

Carbapenems regarded as the last line and the most efficient antibiotic prescribed for treating serious infections that result from the multiple antibiotic resistance Gram negative bacteria since the 1980s (El-Gamal *et al.*, 2017). Over the course of the past several years,

carbapenem-resistant *K. pneumoniae* has emerged as one of the most critical public health challenges affecting countries all over the world (Braun *et al.*, 2014). The study was previously recorded in Iraq by (AlThahab *et al.*, 2013) who found that 33 % of *K. pneumoniae* isolates were resistant to this antibiotic

Efflux pumps are a mechanism exploited by *K. pneumoniae* strains that are multidrug resistant (Maurya *et al.*, 2019). The efflux pumps may lessen the amount of antibiotics present inside of cells, which is crucial for bacterial survival (Xu *et al.*, 2019). Compared to *mdtK*, the *AcrAB* efflux pump was more prevalent in K. pneumoniae strains in our investigation. It had a strong correlation to the MDR phenotype. Our findings are in accordance with recent studies that found that antibiotics, particularly fluoroquinolones like ciprofloxacin, tetracycline, and beta-lactam antibiotics in MDR isolates, are reabsorbed by *K. pneumoniae* strains via the multidrug efflux pump system (*AcrAB -TolC*) (Mirzaie and Ranjbar, 2021).

The *K. pneumoniae AcrAB* system causes resistance against antimicrobial peptides that are reportedly prevalent in the lung, according to research by Padilla et al. As a result, airway epithelial cells produce HBD-1 and HBD-2, while neutrophils drawn to the infection site release HNP-1 (Padilla *et al.*, 2010).

Ferreira *et al.* investigated the prevalence of *AcrAB* gene in *Klebsiella pneumoniae* isolated in a Brazilian Intensive Care Unit. The study showed that *AcrAB* was 100% of isolate (Ferreira *et al.*, 2019).

According to Mirzaie and Ranjbar, 41 (41%) of the strains had efflux pump genes, including *AcrAB*. They also point out that compared to other efflux pump genes, *AcrAB* was more common in *K. pneumoniae* strains. Additionally, compared to other clinical specimens, urine samples had a higher prevalence of the *AcrAB* efflux pump gene (Mirzaie and Ranjbar, 2021).

Efflux pump systems have been reported as essential mechanisms of resistance and cause of MDR in *K. pneumoniae* (Mahamoud *et al.*, 2007; Meletis *et al.*, 2012). In *K. pneumoniae*, the *AcrAB* and *mdtK* complexes are the best-characterized efflux pumps (Wasfi *et al.*, 2016).

Ferreira *et al.* investigated the prevalence of *mdtK* gene in *Klebsiella pneumoniae* isolated in a Brazilian Intensive Care Unit. The study showed that *mdtK* was 88% of isolate (Ferreira *et al.*, 2019).

Mirzaie and Ranjbar revealed that the efflux pump genes including *mdtK* were observed in 26 (26%) of the strains (Mirzaie and Ranjbar, 2021).

The 180-Kb virulence plasmid on which the rmpA gene was found was described by Suescun et al. (2006). (Suescún *et al.*, 2006). This plasmid is a multi-copy plasmid and responsible for expressing the mucoid phenotype of *K. pneumoniae*. It was found that *rmpA* carrying plasmid of the *K. pneumoniae* isolates, the plasmid contained also many virulence-associated genes (Yeh *et al.*, 2006).

Mirzaie and Ranjbar revealed that the virulence-related genes including mucoid phenotype A (*rmpA*) were found in 48 (48%) of isolates (Mirzaie and Ranjbar, 2021).

72 out of 151 (48 %) isolates have the rmpA gene, according to Yu *et al*. (2006) (Yu *et al.*, 2006). rmpA gene was found in 96 percent of the isolates when Yu *et al.* (2008) evaluated the prevalence of several virulence factors in the causative isolates. Additionally, they discovered that the frequency of the rmpA gene was 97.8% among 45 liver abscess isolates with a positive

hypermucoviscosity phenotype (Yu *et al.*, 2008).

The most common ones are rmpA, and the latter is connected to *K. pneumoniae* hypermucoviscosity and high pathogenicity (Kawai, 2006). The early identification of the infection in vulnerable hosts is made possible by the molecular detection of these genes. Further research is required to clarify the function of other host and pathogen variables, which may contribute to the physiological and molecular development of illness in light of all the findings. (Aher *et al.*, 2012).

Capsular polysaccharide is an important virulence factor of *K. pneumoniae*, which helps bacteria escape immunity by resisting macrophage phagocytosis, inhibiting the early inflammatory response, resisting the action of anti-microbial peptides, and inhibiting dendritic cell maturation. Increased capsular production is associated with the hypervirulence phenotype of *K. pneumoniae*, and the *rcsA/B* genes are involved in regulating and affecting the synthesis of capsular polysaccharides(Brisse *et al.*, 2009; Peng *et al.*, 2018). In our current study, the prevalence of *rcsA* and *rcsB* genes was (35 and 95%), respectively, and this is close to what Jin *et al.* found, as they indicated in their study that the prevalence of both genes is 100% (Jin *et al.*, 2022).

Reference

1. Aher, T., Roy, A. and Kumar, P. (2012) 'Molecular detection of virulence genes associated with pathogenicity of Klebsiella spp. isolated from the respiratory tract of apparently healthy as well as sick goats', Israel Journal of Veterinary Medicine, 67(4), pp. 249–252.
2. Ahmad, A. (2000) Urinary tract caliculi and its bacterial correlation. Salahaddin University.
3. Akindele, J.A. and Rotilu, I.O. (1997) 'Outbreak of neonatal Klebsiella septicaemia: a review of antimicrobial sensitivities.', African journal of medicine and medical sciences, 26(1–2), pp. 51–53.
4. Akram, M., Shahid, M. and Khan, A.U. (2007) 'Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India', Annals of clinical microbiology and antimicrobials, 6(1), pp. 1–7.
5. Al-Mulla, H. (2003) The effect of salicylate compounds on Klebsiella isolated from different clinical infections. Al-Nahrain University.
6. AlThahab, A.A., Almohana, A.M. and Al Sehlawi, Z.S. (2013) 'Occurrence and detection of carbapenemase-producing Klebsiella pneumoniae clinical isolates in Najaf hospitals', Magazine of Al-Kufa University for Biology, 5(2), pp. 29–37.
7. Amin, A., Ghumro, P.B., Hussain, S. and Hameed, A. (2009) 'Prevalence of antibiotic resistance among clinical isolates of Klebsiella pneumoniae isolated from a Tertiary Care Hospital in Pakistan', Malaysian Journal of Microbiology, 5(2), pp. 81–86.
8. Basak, S., Singh, P. and Rajurkar, M. (2016) 'Multidrug Resistant and Extensively Drug Resistant Bacteria: A Study', Journal of Pathogens, 2016, pp. 1–5.
9. Braun, S.D., Monecke, S., Thürmer, A., Ruppelt, A., et al. (2014) 'Rapid Identification of Carbapenemase Genes in Gram-Negative Bacteria with an Oligonucleotide Microarray-Based Assay', PLoS ONE. Edited by J. Kluytmans, 9(7), p. e102232.

10. Brisse, S., Fevre, C., Passet, V., Issenhuth-Jeanjean, S., et al. (2009) 'Virulent clones of Klebsiella pneumoniae: identification and evolutionary scenario based on genomic and phenotypic characterization', PloS one, 4(3), p. e4982.
11. El-Gamal, M.I., Brahim, I., Hisham, N., Aladdin, R., et al. (2017) 'Recent updates of carbapenem antibiotics', European Journal of Medicinal Chemistry, 131, pp. 185–195.
12. Elshamy, A.A. and Aboshanab, K.M. (2020) 'A review on bacterial resistance to carbapenems: epidemiology, detection and treatment options', Future Science OA, 6(3).
13. Feizabadi, M.M., Etemadi, G., Rahmati, M., Mohammadi-Yeganeh, S., et al. (2007) 'Antibiotic resistance patterns and genetic analysis of Klebsiella pneumoniae isolates from the respiratory tract', TANAFFOS (Respiration), 6(3 (summer)), pp. 20–25.
14. Feizabadi, M.M., Farahani, A.S., Rahmati, M. and Asadi, S. (2008) 'Phenotypic characterization and plasmid analysis of Klebsiella pneumoniae strains from Iranian patients'.
15. Ferreira, R.L., da Silva, B.C.M., Rezende, G.S., Nakamura-Silva, R., et al. (2019) 'High Prevalence of Multidrug-Resistant Klebsiella pneumoniae Harboring Several Virulence and β-Lactamase Encoding Genes in a Brazilian Intensive Care Unit', Frontiers in Microbiology, 9.
16. Guiral, E., Pons, M.J., Vubil, D., Marí-Almirall, M., et al. (2018) 'Epidemiology and molecular characterization of multidrug-resistant Escherichia coli isolates harboring blaCTX-M group 1 extended-spectrum β-lactamases causing bacteremia and urinary tract infection in Manhiça, Mozambique', Infection and drug resistance, 11, p. 927.
17. Hender, T.C., Wesley, J.C., Bialek, J., Bondeson, A., et al. (2007) 'MHD stability, operational limits and disruptions', Nuclear fusion, 47(6), p. S128.
18. Holt, R.A., Mani Subramanian, G., Halpern, A., Sutton, G.G., et al. (2002) 'The genome sequence of the malaria mosquito Anopheles gambiae', Science, 298(5591), pp. 129–149.
19. Jin, Z., Wang, Z., Gong, L., Yi, L., et al. (2022) 'Molecular epidemiological characteristics of carbapenem-resistant Klebsiella pneumoniae among children in China', AMB Express, 12(1), p. 89.
20. Joey, F.L. (2011) 'On the homotopy perturbation method and its applications'. Ministry of Higher Education.
21. Kawai, T. (2006) 'Hypermucoviscosity: an extremely sticky phenotype of Klebsiella pneumoniae associated with emerging destructive tissue abscess syndrome', Clinical Infectious Diseases. The University of Chicago Press, pp. 1359–1361.
22. Lim, K.T., Yeo, C.C., Md Yasin, R., Balan, G., et al. (2009) 'Characterization of multidrug-resistant and extended-spectrum β-lactamase-producing Klebsiella pneumoniae strains from Malaysian hospitals', Journal of medical microbiology, 58(11), pp. 1463–1469.
23. Liu, C., Shi, J. and Guo, J. (2018) 'High prevalence of hypervirulent Klebsiella pneumoniae infection in the genetic background of elderly patients in two teaching hospitals in China', Infection and Drug Resistance, Volume 11, pp. 1031–1041.
24. Livermore, D.M. and Brown, D.F.J. (2001) 'Detection of β-lactamase-mediated resistance', Journal of

antimicrobial chemotherapy, 48(suppl_1), pp. 59–64.
25. Mahamoud, A., Chevalier, J., Alibert-Franco, S., Kern, W. V, et al. (2007) 'Antibiotic efflux pumps in Gram-negative bacteria: the inhibitor response strategy', Journal of Antimicrobial Chemotherapy, 59(6), pp. 1223–1229.
26. Makhrmash, J.H., Al-Aidy, S.R. and Qaddoori, B.H. (2022) 'Investigation of Biofilm Virulence Genes Prevalence in Klebsiella pneumoniae Isolated from the Urinary Tract Infections', Archives of Razi Institute, 77(4), pp. 1421–1427.
27. Marranzano, M., Agodi, A., Romeo, M., Saporito, A., et al. (1996) 'Molecular typing of Klebsiella pneumoniae isolates from a neonatal intensive care unit', Microbiologica-Quarterly Journal of Microbiological Sciences, 19(4), pp. 293–300.
28. Mathur, P., Das, A., Kapil, A., Sharma, V., et al. (2008) 'An evaluation of four different phenotypic techniques for detection of metallo-β-lactamase producing Pseudomonas aeruginosa', Indian Journal of Medical Microbiology, 26(3), p. 233.
29. Maurya, N., Jangra, M., Tambat, R. and Nandanwar, H. (2019) 'Alliance of efflux pumps with β-lactamases in multidrug-resistant Klebsiella pneumoniae isolates', Microbial Drug Resistance, 25(8), pp. 1155–1163.
30. Meletis, G., Exindari, M., Vavatsi, N., Sofianou, D., et al. (2012) 'Mechanisms responsible for the emergence of carbapenem resistance in Pseudomonas aeruginosa', Hippokratia, 16(4), p. 303
31. .Mesa, R.J., Blanc, V., Blanch, A.R., Cortés, P., et al. (2006) 'Extended-spectrum β-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage)', Journal of Antimicrobial chemotherapy, 58(1), pp. 211–215.
32. Mirzaie, A. and Ranjbar, R. (2021) 'Antibiotic resistance, virulence-associated genes analysis and molecular typing of Klebsiella pneumoniae strains recovered from clinical samples', AMB Express, 11(1), p. 122.
33. Mocktar, C., Govinden, U. and Essack, S. (2007) 'TEM-145 and TEM-146 ß-lactamases produced by Escherichia coli isolates from state hospitals in KwaZulu-Natal, South Africa', African journal of Biotechnology, 6(5).
34. Nasehi, L., SHAH, C.F., Nikbin, V.A.S. and Nematzadeh, S.H. (2010) 'PER, CTX-M, TEM and SHV Beta-lactamases in clinical isolates of Klebsiella pneumoniae isolated from Tehran, Iran'.
35. Padilla, E., Llobet, E., Doménech-Sánchez, A., Martínez-Martínez, L., et al. (2010) 'Klebsiella pneumoniae AcrAB Efflux Pump Contributes to Antimicrobial Resistance and Virulence', Antimicrobial Agents and Chemotherapy, 54(1), pp. 177–183.
36. Palzkill, T. (2013) 'Metallo-β-lactamase structure and function', Annals of the New York Academy of Sciences, 1277(1), pp. 91–104.
37. Peng, D., Li, X., Liu, P., Zhou, X., et al. (2018) 'Transcriptional regulation of galF by RcsAB affects capsular polysaccharide formation in Klebsiella pneumoniae NTUH-K2044', Microbiological research, 216, pp. 70–78.
38. Prescott, L.M., Harley, J.P. and Klein, D.A. (1999) 'Microbiology 4 th Edition WCB McGraw-Hill'. Boston.
39. Rajash, B. and Rattan, L.I. (2008) 'Essential of medical microbiology'. New Delhi: Jayppe Brothers Medical Publishers.
40. Rashed, M.F., RAHNAMAEI, F.M., Saremi, M. and Sabouri, R. (2008) 'A

survey on urinary pathogens and their antimicrobial susceptibility among patients with significant bacteriuria'.
41. Reish, O., Ashkenazi, S., Naor, N., Samra, Z., et al. (1993) 'An outbreak of multiresistant Klebsiella in a neonatal intensive care unit', Journal of Hospital Infection, 25(4), pp. 287–291.
42. Rice, L.B., Carias, L.L., Bonomo, R.A. and Shlaes, D.M. (1996) 'Molecular Genetics of Resistance to Both Ceftazidime and β-Lactam-β-Lactamase Inhibitor Combinations in Klebsiella pneumoniae and In Vivo Response to β-Lactam Therapy', The Journal of Infectious Diseases, 173(1), pp. 151–158.
43. Roilides, E., Kyriakides, G., Kadiltsoglou, I., Farmaki, E., et al. (2000) 'Septicemia due to multiresistant Klebsiella pneumoniae in a neonatal unit: a case-control study', American journal of perinatology, 17(01), pp. 35–40.
44. Sánchez-Romero, I., Asensio, Á., Oteo, J., Muñoz-Algarra, M., et al. (2012) 'Nosocomial Outbreak of VIM-1-Producing Klebsiella pneumoniae Isolates of Multilocus Sequence Type 15: Molecular Basis, Clinical Risk Factors, and Outcome', Antimicrobial Agents and Chemotherapy, 56(1), pp. 420–427.
45. Stamm, W.E. and Norrby, S.R. (2001) 'Urinary Tract Infections: Disease Panorama and Challenges', The Journal of Infectious Diseases, 183(s1), pp. S1–S4.
46. Stock, I. and Wiedemann, B. (2001) 'Natural antibiotic susceptibility of Klebsiella pneumoniae, K. oxytoca, K. planticola, K. ornithinolytica and K. terrigena strains', Journal of Medical Microbiology, 50(5), pp. 396–406.
47. Suescún, A.V., Cubillos, J.R. and Zambrano, M.M. (2006) 'Genes involucrados en la biogénesis de fimbrias afectan la formación de biopelículas por parte de Klebsiella pneumoniae', Biomédica, 26(4), pp. 528–537.
48. Thomson, C.J. and Amyes, S.G.B. (1993) 'Molecular epidemiology of the plasmid-encoded TEM-1 β-lactamase in Scotland', Epidemiology and Infection, 110(1), pp. 117–125.
49. Ugwu, M.C., Shariff, M., Nnajide, C.M., Beri, K., et al. (2020) 'Phenotypic and molecular characterization of β-lactamases among enterobacterial uropathogens in Southeastern Nigeria', Canadian Journal of Infectious Diseases and Medical Microbiology, 2020.
50. ur Rahman, S., Ali, T., Ali, I., Khan, N.A., et al. (2018) 'The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases', BioMed Research International, 2018, pp. 1–14.
51. Vuotto, C., Longo, F., Balice, M., Donelli, G., et al. (2014) 'Antibiotic Resistance Related to Biofilm Formation in Klebsiella pneumoniae', Pathogens, 3(3), pp. 743–758.
52. Wasfi, R., Elkhatib, W.F. and Ashour, H.M. (2016) 'Molecular typing and virulence analysis of multidrug resistant Klebsiella pneumoniae clinical isolates recovered from Egyptian hospitals', Scientific reports, 6(1), pp. 1–11.
53. Xu, Q., Jiang, J., Zhu, Z., Xu, T., et al. (2019) 'Efflux pumps AcrAB and OqxAB contribute to nitrofurantoin resistance in an uropathogenic Klebsiella pneumoniae isolate', International journal of antimicrobial agents, 54(2), pp. 223–227.
54. Yeh, K.-M., Chang, F.-Y., Fung, C.-P., Lin, J.-C., et al. (2006) 'magA is not a specific virulence gene for Klebsiella pneumoniae strains causing liver abscess but is part of the capsular polysaccharide

gene cluster of K. pneumoniae serotype K1', Journal of medical microbiology, 55(6), pp. 803–804.
55. Yu, W.-L., Ko, W.-C., Cheng, K.-C., Lee, C.-C., et al. (2008) 'Comparison of prevalence of virulence factors for Klebsiella pneumoniae liver abscesses between isolates with capsular K1/K2 and non-K1/K2 serotypes', Diagnostic Microbiology and Infectious Disease, 62(1), pp. 1–6.
56. Yu, W.-L., Ko, W.-C., Cheng, K.-C., Lee, H.-C., et al. (2006) 'Association between rmpA and magA Genes and Clinical Syndromes Caused by Klebsiella pneumoniae in Taiwan', Clinical Infectious Diseases, 42(10), pp. 1351–1358.
57. Zhang, R., Wang, X.D., Cai, J.C., Zhou, H.W., et al. (2011) 'Outbreak of Klebsiella pneumoniae carbapenemase 2-producing K. pneumoniae with high qnr prevalence in a Chinese hospital', Journal of Medical Microbiology, 60(7), pp. 977–982.