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ORIGINAL ARTICLE

K1 and K2 Capsule Identification of XDR-*Klebsiella pneumoniae* in UTI Patients

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ABSTRACT

Background: *Klebsiella pneumoniae is a significant human pathogen, causing a variety of infections. Its virulence is closely linked to its capsule, a protective outer layer. Over 79 different capsule types (K-types) have been identified in Klebsiella, contributing to its diverse disease-causing abilities.* ***Objective****: This study investigated Extensively drug-resistant (XDR) K. pneumoniae strains isolated from urinary tract infections (UTIs) with a specific focus on identifying prevalent capsular types.* ***Methodology****: The study was conducted in the period from Feb. 2024 till end of October 2024 in some private clinics in Iraq. One hundred urine samples were screened for the presence of the target isolates. Isolates were identified through culturing were then were subjected to XDR screening. Subsequently, genomic DNA was extracted from the isolates and screened by multiplex PCR to detect specific regions within the capsular type gene cluster of serotypes K1 and K2.* ***Results:*** *63 of UTI samples were identified as K. pneumoniae depending on pgi as housekeeping gene. 65% (41/63) of all isolates demonstrate a resistance to at least one agent of three or more antimicrobial categories and were considered as MDR isolates while 35% (22/63) were consided as XDR isolates, K2 capsule type were the common type in UTI Patient.* ***Conclusion:*** *Our findings highlight the critical need for increased monitoring and detailed genetic analysis of K. pneumoniae in healthcare environments, especially in areas grappling with widespread antibiotic resistance. The rise of K2 capsular types among extensively drug-resistant strains presents a serious public health threat, underscoring the importance of implementing focused infection control practices and exploring alternative treatment approaches.*

INTRODUCTION

Urinary tract infections (UTIs) are a significant global health concern, and *Klebsiella pneumoniae* is a major culprit. This Gram-negative bacterium has evolved into a formidable adversary, with the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains posing a serious challenge to treatment[1].

XDR-*K. pneumoniae* strains, resistant to nearly all available antibiotics, are particularly alarming[2]. These strains, often harboring resistance genes on plasmids, leave limited therapeutic options and increase the risk of treatment failure, prolonged hospital stays, and even death [3]. The situation is further complicated by the coexistence of drug resistance with hyper-virulence in certain *K. pneumoniae* strains, particularly those with K1 and K2 capsular serotypes. These hyper-virulent strains exhibit increased invasiveness, immune evasion, and virulence, making infections more severe[4].

Klebsiella pneumoniae possesses a significant virulence factor: a thick, capsule encasing the bacterial cell. This capsule, composed of complex polysaccharides, acts as a shield, protecting the bacterium from the host's immune defenses and other environmental threats [5]. Each *Klebsiella* strain produces a unique capsular structure, determined by its genetic makeup (cps locus). This variation in the capsule's composition leads to different capsular serotypes (K-types), with over 80 identified so far, including prominent types like K1 and K2 [6,7].

Klebsiella pneumoniae produces an acidic capsule polysaccharide that is important for survival in the host. The *K. pneumoniae* capsule Polysaccharides (CPS) biosynthesis is dependent on multiple genes that comprise a cps locus. Translocation and assembly of CPS onto the bacterial surface are regulated by proteins encoded by the conserved genes galf, orf2, wzi, wza, wzb and wzc at the 50 end of the cps locus (8).Variation in nucleotide sequence and number of genes underlies the differences in *K. pneumoniae* capsule types [9]. Studies have linked K1 serotypes of *Klebsiella pneumoniae* to severe, widespread infections. The mucoviscosity-associated gene (magA), crucial for the

production of the K1 capsule, is located within the cps gene cluster. While PCR targeting magA was initially used to detect K1 strains, the discovery of a similar allele in other K-serotypes necessitates a reassessment of this method [10]. The aim of this study investigated Extensively drug-resistant (XDR) *K. pneumoniae* strains isolated from urinary tract infections (UTIs) with a specific focus on identifying prevalent capsular types.

METHODOLOGY

Isolation and Identification of *K. pmneumonae* strains:

In this study, *K. pneumoniae* strains were isolated from urine samples of patients diagnosed with urinary tract infections (UTIs). Each urine sample was cultured on MacConkey agar and blood agar and incubated at 37°C for 24 hours. Presumptive K. pneumoniae colonies were identified based on morphological characteristics, Gram staining, and standard biochemical tests such as lactose fermentation, indole, methyl red, Voges-Proskauer, and citrate utilization tests. The study was conducted between February and October 2024 in (Ramadi city/Iraq) hospitals and some private clinics, to investigate urinary tract infections (UTIs) in 100 patients of both sexes and varying ages. Subsequently, the Vitek 2 Compact system with a GN (Gram-Negative) card was utilized for definitive species identification according to the manufacturer's instructions (bioMérieux, France).

Antibiotics susceptibility test:

For each pure isolate, the antimicrobial susceptibility test was done according to by Kirby-Bauer disc diffusion method as described in the guidelines of the CLSI Institute [11], and using AST card by automated Vitek-2 compact system. Ten antimicrobial agents were tested, including Amox-clav, Ceftriaxon, Cefotaxime, Ceftazidime, Meropenem, Amikacin, Levofoxaccin, Colistin ,Ciprofloxacin, and Piperacillin-Tazobactam, to screen for extensively drug-resistant (XDR) strains.

Molecular Screenings:

Bacterial genomic DNA was extracted using the Geneaid Genomic DNA Mini Kit. The quality and integrity of the extracted DNA were assessed by agarose gel electrophoresis. For genetic identification of *K. pneumoniae* strains, *pgi* gene was used as housekeeping target within conventional PCR with primer details listed in **Table 1**. Subsequently, multiplex PCR was performed targeting two specific genes (details provided in Table 1). The PCR reactions were conducted according to the methodology described in 2 reports[12,13].

Table 1: Sequence of primers and their size Gene

Gene		Sequence of forward and reverse Primer(5´- 3´)	Annealing Tm.	PCR Product Size bp	Ref.
Capsular type K1	**F**	**GGTGCTCTTTACATCATTGC**	**58**	**1283**	(13)
	R	**GCAATGGCCATTTGCGTTAG**			
Capsular type K2	**F**	**GACCCGATATTCATACTTGACAGAG**	**58**	**641**	
	R	**CCTGAAGTAAAATCGTAAATAGATGGC**			
Pgi	**F**	**GAG AAA AAC CTG CCT GTA CTG CTG GC**	**50**	**556**	
	R	**CGC GCC ACG CTT TAT AGC GGT TAA T**			

F: Forward sequences, R: Reverse sequences

RESULTS

Identification of *Klebsiella pneumoniae*

This study examined 140 urine samples obtained from patients with various ages and both sexes, and collected from Al-Ramadi Teaching General Hospital and Al-Ramadi Teaching for Children and Maternity Hospital. These patients were presented with urinary tract infections (UTIs). Of the collected specimens, 90 (64.2 %) yielded positive bacterial cultures, while 50 (35.0%) were negative. The identification of isolates according to colonial morphology (Fig.1), biochemical tests (Table 2), and confirmed by Vitek 2 compact device as a final identification of organisms by using a GN (Gram-Negative) card which showed that 70 isolates belong to *K. pneumonia*e.

Fig. 1: A: *K. pneumoniae* on Blood agar. B: *K. pneumoniae* mucoid, large, pink, lactose fermenter

Table 2: Biochemical tests for *K. pneumoniae* isolates

Test	*K.pneumoniae*
MacConkey agar medium	Lactose ferment (+)
Gram-stain	G- rod
"Catalase test"	+
"Oxidase test"	-
"Indole Test"	-
"Methyl Red Test"	-
VP Test	+
"Citrate Utilization Test"	+
Urease	+

More identification of *K. pneumoniae* was done by analyzing conserved housekeeping genes, which is consider a crucial for fundamental cell processes. These genes were remarkably similar across species, effectively differentiate closely related organisms. Among these, the *pgi* gene stands out as a dependable genetic marker. PCR result indicated that all isolates were detected as target bacteria as shown in agarose electrophoresis (Fig. 2).

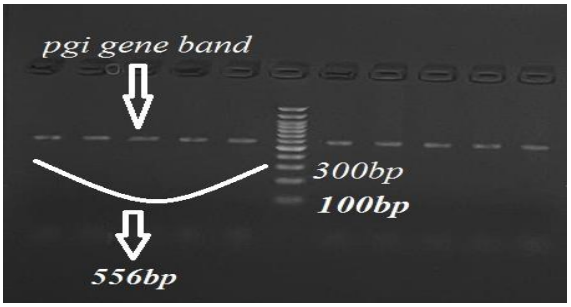

Fig. 2: Electrophoresis of PCR product of pgi gene as genetic detection. (1.5%agarose, 7 V/cm^{2}for 90min)

Antimicrobial Susceptibility

Our study investigating the antibiotic resistance profiles of 70 *K. pneumoniae* isolates employed Kirby-Bauer disk diffusion and the Vitek-2 compact system [11]. The results revealed a high degree of antibiotic resistance diversity among the *K. pneumoniae* isolates. Notably, over 65% of the isolates exhibited resistance to at least one agent from three or more antimicrobial categories, classifying them as multidrug-resistant (MDR). Furthermore, 35% of the isolates were categorized as XDR.

Table 3: Antimicrobial Susceptibility Results.

		Klebsiella pneumoniae					
		Resistant		**Intermediate**		**Sensitive**	
		No.	**%**	**No.**	**%**	**No.**	**%**
1	**Amox-clav**	68	97%	1	1.5%	1	1.5%
2	**Ceftriaxon**	63	90%	4	6%	3	4%
3	**Cefotaxime**	58	82%	10	14%	2	4%
4	**Ceftazidime**	55	78%	10	14%	5	7%
5	**Meropenem**	18	26%	12	17%	40	57%
6	**Amikacin**	35	50%	25	35%	10	15%
7	**Levofoxaccin**	30	42%	23	33%	17	25%
8	**Colistin**	25	35%	15	22%	30	42%
9	**Ciprofloxacin**	23	32%	35	50%	12	18%
10	**Piperacillin-Tazobactam**	64	91%	4	6%	2	3%

Molecular Screening of Capsules:

The results of gel electrophoresis of PCR amplification (Fig. 3) revealed that the K2 capsular type was the most prevalent among XDR *K. pneumoniae* isolates. However, PCR amplification failed to detect other capsular types, suggesting a limited distribution of these capsular types in Iraqi patients with XDR *K. pneumoniae* infections.

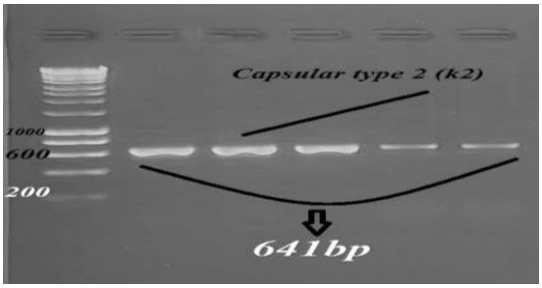

Fig.3: Agarose Gel Electrophoresis of multiplex-PCR product for the detection of capsular type in XDR *K. pneumoniae*

DISCUSSION

Identification of K1 and K2 capsular types aids in tracking outbreaks and understanding the spread of hyper-virulent and XDR *K. pneumoniae* strains in healthcare settings. The negative culture cases were attributed to the reasons that some patients were under antimicrobials chemotherapy during culture time.

The high mortality rates associated with infections caused by carbapenemase-producing *K. pneumoniae* are due to the fact that these infections are difficult to treat. Plasmids within *K. pneumoniae* play a crucial role in spreading and acquiring genes that confer antibiotic resistance and virulence. These extrachromosomal DNA molecules are responsible for many key bacterial traits, leading some researchers to consider them as independent organisms [14]. Scientists discovered some bacteria that are almost impossible to treat and others that are completely untouchable by current antibiotics, this is a worrying development as it could make even common bacterial infections extremely difficult to control in the future [15,16].

Carbapenem-resistant bacteria are a growing public health concern, and *Klebsiella pneumoniae* is a prime example. While carbapenems are typically the last line of defense against multi-drug resistant infections, carbapenem-resistant *K. pneumoniae* (CRKP) strains are emerging, leading to high mortality rates in infected patients [17]. Resistance to carbapenems in *K. pneumoniae* is due to either production of carbapenemase enzymes, alteration of porin or upregulation of efflux pump [18]. When bacteria are resistant to more drugs than multidrug-resistant microbes (MDR) and often require use of relatively toxic drugs, high doses of drugs or drug combinations are considered as Extensively Drug Resistant (XDR) isolates

Our study revealed that the K2 capsular type was most prevalent among XDR *K. pneumoniae* isolates, partially aligning with the findings of other reports [19,20]. Our study findings suggest that *K. pneumoniae* (K2) strain is most frequently linked to invasive infections and may exhibit increased pathogenicity among Iraqi patients, particularly nosocomial infections. The presence of this association suggests that these bacterial strains may be resistant to antibiotics. Globally, determining the capsular type of *Klebsiella pneumoniae* is a crucial future goal in treating infections caused by *K. pneumoniae*. This is because *K. pneumoniae* infections are often difficult to treat due to the bacteria's frequent resistance to multiple antibiotics.

The strains of *K. pneumoniae* possessing a K2 capsule, often exhibit high levels of antibiotic resistance. This resistance may be attributed to the capsule's ability to hinder the entry of antibiotics into the bacterial cell, thereby preventing the drugs from reaching their intended targets. Additionally, the presence of a capsule, particularly the K antigen, can mask the O-antigen, a component of the bacterial lipopolysaccharide (LPS). This masking of the LPS can impede the recognition and detection of the bacteria by the immune system, potentially contributing to the bacteria's ability to evade phagocytosis [21].

K. pneumoniae strains possess a Capsule Polysaccharide (CPS), a key virulence factor that significantly impacts the severity of infections. This capsule acts as a shield, hindering the ability of immune cells (phagocytes) to engulf and destroy the bacteria [22]. The specific type of CPS, known as the K antigen, plays a crucial role in determining the severity and invasiveness of the infection. For instance, strains with the K2 capsule are notorious for causing severe infections like pyogenic liver abscesses in the community. In contrast to encapsulated strains, those lacking a capsule (unencapsulated) are more vulnerable to the body's defenses. They are more easily killed by serum complement, a part of the immune system, and are readily consumed and eliminated by phagocytic cells [23].

CONCLUSION

Our findings highlight the critical need for increased monitoring and detailed genetic analysis of *K. pneumoniae* in healthcare environments, especially in areas grappling with widespread antibiotic resistance. The rise of K2 capsular types among extensively drug-resistant strains presents a serious public health threat, underscoring the importance of implementing focused infection control practices and exploring alternative treatment approaches.

Ethical approval

The study was approved by Ethics Committee of the Al Anbar Medical Research University (approval number 122, Feb. 22, 2023). All individuals have given consent to participate in the current study.

Declarations

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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