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Original Research Article

Molecular Study of K1, K2, MagA genes in High Virulent *Kebsiella pneumonia* in Kirkuk City, Iraq

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Abstract: This study aimed to detect K1, K2, and MagA genes in *Kebsiella pneumonia* patients and IL-10 levels. The study included 30 samples from variant patients' swabs (urine, wound). Then, cultured in McConkey media for 24 hours, purified the isolated samples and extracted bacteria DNA to work on conventional PCR. The study shows the advanced assay acceptably well-known ten isolates (33.3%) as K1 and 11 isolates (36.6%) as K2 serotypes, magA 14 isolates (46,6%) among 30 isolates of *K. pneumonia* serotypes. The immunological detection of IL-10 from 30 patients in the current study showed a high increase (83.3%) in *K. pneumonia*. There was high virulence from k1, k2 and magA in K. pneumonia patients, and the levels of interleukin-10 increased to inhibit the infection.

Keywords: *Kebsiella pneumonia*, Conventional PCR, MagA genes,Interleukin -10.

1. INTRODUCTION

Klebsiella pneumonia is a prevalent Gram-negative bacterium in the environment, including soil and water. Additionally, it may be a typical component of the human respiratory, gastrointestinal, and urinary tract flora [1]. While it usually has no negative effects on healthy people, it can result in infections, especially in those hospitalized for specific medical illnesses or compromised immune systems. Infections caused by *Klebsiella pneumonia* can range in severity from minor urinary tract infections to more serious infections for example pneumonia, bloodstream, and wound infections. Although siderophores, fimbriae and lipopolysaccharide have all been identified as virulence factors, the capsule is the record well-researched virulence component of *Klebsiella pneumonia*. The *Kebsiella pneumonia* capsule is categorised into 78K antigen types using a serological antigenic classification method. [2;3]. The use of antibiotics in treatment is common. However, several *K. pneumonia* strains have developed resistance to various medications, making treatment efforts more challenging. The frequency and, molecular epidemiology of K1 and K2 isolates in clinical cases of K. pneumonia in Japan are unclear. Out of 257 clinical isolates of *K. pneumoniae*, surveillance studies carried out 1980s found 13 (5.1%) K1 isolates and 9 (3.5%) K2 isolates. A regional surveillance study was carried out over 30 years ago [4]. Determining if this figure accurately depicts the state of affairs in Japan as a whole is crucial. Out of the 120 clinical isolates of K. mostly pneumonia that were collected in 2010, individual isolates after the blood of pneumonia patients were recovered; however, later regional study found 13 (10.8%) K2 isolates and 9 (7.5%) K1 isolates, correspondingly [5]. Both Taiwan and South Korea, which are close to Japan, have endemic isolates of *K. pneumoniae* K1-ST23 and occasional cases of clone-induced community-acquired liver abscesses. [5]. It is crucial to clarify the epidemiology of virulent *K. pneumonia* clones, namely K1-ST23. The most virulent strains of *K. pneumoniae* are thought to be the capsular serotypes K1 and K2 [6]. Numerous studies on bacterial pathogenesis have found serotype K1, magA, to be a conceivable virulence factor for *K. pneumonia* liver abscess (LA) [7]. Therefore, magA PCR analysis provides a rapid and exact technique of identifying capsular K1 bacteria. Additionally, the occurrence of K2 isolates proposes that rapid identification of K2 serotypes is necessary in adding to K1 [8].

Almost all leukocytes generate IL-10, a significant anti-inflammatory cytokine that broadly suppresses various immune cell types. Because IL-10 regulates the proinflammatory immune response during bacterial infections, the pathogen can be eliminated with less tissue damage. Overproduction of IL-10 results in persistent infections or increased host mortality because the immune system is not strong enough to eradicate the infectious pathogen [9].

2. MATERIALS AND METHODS

2.1. Assortment of Examples:

2.2 Cultivation of the Samples for Isolation of Klebsiella:

Swabs were cultured in nutrient broth and incubated aerobically for 18 to 24 hours at 37°C. A loopful of contaminated nutrient broth was inoculated onto MacConkey's agar medium. After 24-48 hours of aerobic incubation at 37°C, the injected medium was examined for bacterial proliferation.

2.3 Purification of the Isolates: On MacConkey's agar plates, suspected colonies were subcultured and incubated for a further 24 hours at 37°C.

2.4 Identification of the Isolates:

A. Morphological characterization: Gram stain was applied to the films, and the isolates' morphological traits were visually assessed under a microscope. B. Cultural traits: An examination of the colonial morphology of MacConkey's agar was conducted. C- Biochemical characterization: tests for urease, citrate utilization, oxidase, indole, methyl red, Vogasproskauer, and catalase were carried out.

2.5 Using the (PCR)

The genus Klebsiella specific genes (K1, K2 gene) and the virulence genes of Klebsiella isolates (magA gene) were found PCR:

2.6 Extraction of Bacterial DNA:

One milliliter is extracted from the expanding Klebsiella colony in Macconkey media and put into an Eppendorf tube. To extract the DNA genome, which is in a suspended form, special solutions for dissolving the cell wall and proteins are added to it. The DNA genome is then stored to be used with the PCR.

2.7. Oligonucle Utide prime Ss:

1. Oligonucleotide primers set MagA: According to Aher *et al*., 2012[10].
2. Oligonucleotid primer set K1: According to Aher *et al*., 2012[10].

Table1: *K. pneumoniae* primer sizes and sequences utilised in this investigation

Primer	Forword	Reverse	Bp
K1	5-GTAGGTATTGCAAGCAAGCCATGC	5-GCCCAGCTTAATGAATCCGT-3	531
K2	5-GGAGCCATTTGAATTCGGTG	5-TCCCTAGCACTGGCTTAAGT	531
Mag A	TAGGACCGTTAATTTGCTTTCT	GAATATTCCCACTCCCTCTCC	1283

Table 2: PCR settings for capsular typing and a primer unique to *K. pneumoniae* species

Gene name	Temperture ˚C / Time					Cycles
	Intial denaturation	Cycle conditions			Final extention	
		denaturation	annealing	Extension		
Mag A &K1	95°C/5 min	94 °C/ 45 s	55°C/ 60s	72 °C/ 1min	72°C/ 10	35
K2	94°C/5 min	94°C/60s	58 °C/1min	72 °C/1min	72°C/10 min	35

2.8. Immunological Detection

Interleukin Leveles (IL-10)

Each subject included in the research had a vein punctured using a syringe to provide three millilitres of blood. After being put into sterile test tubes, blood samples were centrifuged for 15 minutes at 3000 rpm. Using an automated micropipette, the collected sera were aspirated, transferred to Eppendorf tubes, and frozen until the IL-10 (Sunlong kit) test was performed using the ELISA method.

3. RESULT

The investigation comprised thirty clinical isolates of *K. pneumonia* obtained from patients at Lab Gynaecological and Pediatric Hospital (15 isolates from urine and 15 from wounds). Five isolates from urine samples and five from wounds comprised the ten isolates with a positive K1 serotype. The 11 K2 serotype isolates included nine samples from the urine and three samples from wounds. We devised a PCR to identify isolates containing K1 and K2 capsular polysaccharides by using K1 and K2 unique MagA genes essential for the production of these capsular polysaccharide types. The negative control, which had no DNA, showed no band. Of the 30 *K. pneumoniae* serotypes, the proposed test correctly identified ten isolates (33.3%) and 11 more isolates as K1.

4. DISCUSSION

Enterobacterium *K. pneumonia* is an opportunistic UTI; pneumonia and wound infections are caused by a pathogen, according to Feizabadi et al [8]. The predominant infections among patients in this investigation were urinary K1 and K2 serotypes, contrasting with other studies that indicated a higher prevalence of liver abscesses and a lower incidence of urinary tract or biliary tract infections. [11,12]. The current study's findings are at odds with those reported by Turton et al., (2007), Aher et al., 2012 Fang et al., 2004 and Ma et., 2005 MagA and Kfu genes were determined to be prevalent at rates of 12.5% and 25%, respectively [13], according to the person who discovered the virulence-associated gene prevalence [14]. Although K1 and K2 loci are more common in hypervirulent *Klebsiella pneumoniae* isolates, the capsule type alone cannot adequately explain hypervirulence since K1 and K2 capsule kinds coexist with additional virulence genes. The genetic replacement of the capsule from a reduced amount of virulent strain with that of a hypervirulent *Klebsiella pneumoniae* strain cannot be the only explanation for the virulent phenotype in mice, suggesting that the capsule does non primarily control virulence. To determine the capsule's contribution, inconsistent efforts have been made to swap capsule sites among isolates genetically. [15]. Nonetheless, capsule is believed to increase (hvKp) survival and spread while blocking phagocytosis. Repetitive mannose or rhamnose carbohydrates are recognized by macrophage lectin receptors, which trigger phagocytosis. Nevertheless, the K1, al K2, K5, and K57 capsule types do not include these repetitive sugars, which makes them more resistant to lection phagocytosis and macrophage death. Neutrophils have been shown to kill K1 isolates more resistantly than cKp isolates, both extracellularly and intracellularly. This finding may facilitate systemic spread in addition to minimizing the use of extracellular antibiotics [16]. The advantages these unique structures provide are probably what led to the emergence of (hvKp) with specific capsule types [17]. When infections occur, there is a high expression of IL-6 and IL-10, which has been observed in individuals with hemophagocytic lymphocytosis and neutropenic pediatric malignancy. To coordinate the body's immune response, pathogen-stimulated CD4+ T helper (Th) lymphocyte subsets would activate and develop into several subgroups, such as Th1, Th2, Th17, and Treg cells, among others. Through cell-mediated immunity, Th1 cells primarily release interleukin (IL)-2, interferon-gamma (IFN-γ), and TNF-α to initiate pro-inflammatory responses. The Th2 cell subset is thought to be primarily responsible for humoral-mediated immunity and to have a greater anti-inflammatory response [18]. While there are several processes at play in this process, research indicates that a disproportionately elevated release of interleukin (IL)-10 is linked to host tolerance against infection. The anti-inflammatory cytokine IL-10 prevents macrophages from engaging in several pro- inflammatory and anti-microbial immune effector pathways. We postulated that IL-10 production by KP modifies macrophage iron metabolism and enhances bacterial access to this vital resource since this anti-inflammatory cytokine directly impacts macrophage iron metabolism [18].

5. CONCLUSION

There was high virulence from k1, k2 and magA in *k.penumonia* patient and the levels of interleukin-10 increasesto to inhibit the infection.

Competing Interests: The author declares no conflict of interest.

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