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Molecular detection of some virulence factors of hypervirulent *Klebsiella pneumonia* that associated with pathogenicity

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Abstract : *The study aimed to isolation and identification of Hypervirulent K. Pneumonia (hvKp) from medical samples and virulence factors genes changed into detected. The gift has a look at included specimens gathered from (Urine, Diabetic Foot, sputum, Liver abscesses, Blood, CSF-, and Semen,) of patients infected with K. Pneumonia in hospitals in Najaf City for each sex. Males had been (34) and females (47) of age ranged between (10-50) years throughout the length September to December - 2022.*

Eighty (eighty/100) isolates of K. Pneumonia have been detected using Morphological, microscopy and biochemical and confirmed using the VITEK-2 machine as a (hvKp). The result of phenotypic detection of some virulence elements, which include biofilm, pill, siderophore, Outer membrane Protein(omp) and hemolysis), all isolates were biofilm manufacturers(100%), with 3 exclusive categories. According to the results of screening virulence genes, BssS (n= eighty, a hundred%) turned into time-honoured amongst all of the clinical isolates, observed with the aid of the rmpA (n=22, 27.7%), and magA (n=three .7%), this suggests that The hypervirulent lines display a placing potential to reason severe infections in young healthy people and immunocompetent hosts in addition to inflamed sufferers liking pyogenic liver abscess.

Keywords: *Virulence Factors, Klebsiella pneumonia, Phenotypic*

1.Introduction

A Gram-negative, encapsulated bacterium called hypervirulent *K. pneumoniae* (hvKp) terrible, encapsulated bacterium called hypervirulent *K. Pneumoniae* (hvKp) causes many illnesses, such as pneumonia, urinary tract infections, bacteremia, meningitis, and liver abscesses. It brought on some of lethal infections like a bacterium. Hypervirulent

(hvKp) and classical (cKp) pathotypes exist [1, 2][*K. Pneumoniae* has some pathogenicity traits that allow it to contaminate its host, along with the manufacturing of urea and enterotoxin, siderophores, fimbriae, serum resistance, and pill polysaccharide and lipopolysaccharide. A massive tablet of exacerbated acidic polysaccharide encases the *Klebsiella* cell. The primary process of the capsule is to shield the

bacterium from phagocytosis and prevent the bacteria from being killed [3].

Mostly *K. Pneumoniae* are hospitals associated with high fatality fees if incorrectly handled, and can be discovered inside healthy patients' breathing, urinary, and gastrointestinal tracts. Humans' mouths, pores and skin, and digestive tracts contain it as ordinary vegetation, wherein it does not immediately motivate ailment. However, it may become risky bacterial infection [4]. [It is the second one maximum not unusual motive of UTIs, pneumonia, infections of the bloodstream (BSI), meningitis, intraabdominal infections, and pyrogenic liver abscesses (PLAs).

Metastatic infections, such as meningitis, endophthalmitis, and pyogenic abscess, are the maximum tremendous hallmarks of *K. Pneumoniae* infections [5]. The majority of *K. Pneumoniae* clones diagnosed as hypervirulent convey virulence plasmids encoding the putative metabolite transporter, the regulator of mucoid phenotype A (*rmpA*), and aerobactin siderophore biosynthesis (*iucA*). To more correctly pick out hypervirulence, studies are increasingly using genotypic markers to display for widespread virulence genes [6].

Samples collection :

one hundred specimens of each sexes, 10 to 50 years of age, were collected from numerous hospitals in AL-Najaf City.- During September and November (2022), during a 3-month length. The clinical specimens, which consisted of urine, diabetic foot, liver abscess, sputum, burns, wound, blood, CSF, and semen swabs, have been selected based on the distinctive medical signs.

2. Methodology

Identification of *K. Pneumoniae*

An unmarried colony from every starting effective culture is installed on blood, MacConkey, and nutrient agar, after which categorised and checked out below a light

microscope after being coloured with the use of Gram's stain relying on its morphological capabilities (colony form, size, coloration, borders, and texture). Following inspection, biochemical checks were carried out on every isolate to complete the [6.7] and to be confirmed by the vitek2 system.

Phenotypic Detection of Hypermucoviscosity (HMV).

Single colonies have been examined for his or her ability to stretch a mucoviscous thread the use of an progressed string take a look at. The HMV phenotype changed into determined while the generated string stretched more than 10 mm in period [7]. Vitek System effects for spotting manose and ribenose in *K. Pneumoniae* isolates were also terrible [8].

Detection of Virulence Factors of *K. Pneumoniae* Isolates:

Blood Hemolysis. Blood Hemolysis. The bacterial isolates have been streaked on blood agar plates containing 5% (vol/vol) human blood for plate hemolysis. Take a look at. After twenty-4 hours of incubation at 37°C, complete (β) and partial (α) purple blood cellular rupture changed into carefully determined [9].

Capsule

The pill changed into dyed following the Cruickshank *et al.* Ten method: On one aspect of a glass slide, a drop of bacterial suspension changed into applied, and any other glass slide was used to unfold it out. It wasn't heated to fix, simply allowed to dry. Flood lightly for around four minutes after making use of 1% crystal violet. CuSO₄ (20%) turned into used to clean the smear. Allowed to dry within the air without the use of drying paper, then microscopically inspected.

Siderophore production

The test was carried the procedure [10] by transferring a tiny quantity of pure colony to M9 media using a sterilized wooden stick, then incubating the media at 37 C for 24 hours. The

findings were recorded based on the appearance of bacterial colonies on the media.

Serum Resistance.

The turbidimetric technique was utilized to examine serum resistance. After three hours of incubation at 37°C, the absorbance at the wavelength of 620 nm was carefully determined before and after. The final absorbance was measured using the average of two repetitions, and the mean of the residual absorbance about the unique absorbance before incubation was computed. The isolates became serum resistant if the ratio exceeded 100% [10].

Biofilm Detection: A single isolate colony was used to inoculate the plates in Congo crimson agar, and they underwent incubation under cardio conditions for (24– forty-eight h) at 37 °C. A favorable outcome was determined by using black colonies with a drying crystalline fine. The cores of the colonies on occasion darkened, however, the weak slime producers often stayed crimson. An uncertain final results become cautioned by the colonies turning darker and lacking a dry crystalline colonial morphology [11,12].

The mixture used for the reaction contained a total of 25 l of Dream Taq Green PCR MasterMix (2x), 12.5 l of bacterial lysate, 1 l of forward primer (10 M), 1 l of reverse primer (10 M), and 9.5 l of nuclease-free water. A PCR control that was negative was developed. the biking circumstances shown in picture 2.become evaluated [13]. Implied OD492 of the six wells became computed (OD) for every isolate. Three widespread deviations that were more than the suggest OD of the poor control wells were used to establish the reduce-off ODT (OD).

- The evolved biofilm's stage became said as
- (i) Weakly adherent: (<zero.A hundred and twenty) ODC < ODT ≤ 2ODC
 - (ii) Moderately adherent:(0.One hundred twenty-0.240) 2OC < ODT ≤ 4ODC
 - (iii) Strongly adherent: (>zero.240) 4ODC < ODT.

Polymerase Chain Analysis of Virulence Genes. .

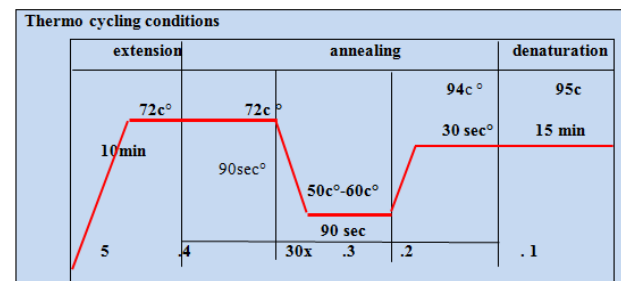
An unmarried colony of each isolated turned into located in a suspended country in 70 ul of DNase-loose water and heated at ninety-five °C for 10 mins. With the aid of the primers laid out in Table 1 and the Dream Taq PCR Master Mix from Fermentas (US), the genes chargeable for virulence BssS for biofilm formation, rmpA for the LPS gene, and megA for aerobactin gene have been amplified.

Table (1): Primers used all throughout the modern have a look at.

The mixture used for the reaction contained a

Primer Target	Oligo Sequence 5'→3'	Product Size (bp)	Ref.
<i>BssS</i>	F- 5- GATTCAATTTTGGCGATTCC TGC-3 R- 5TAATGAAGTCATTCAGACT CATCC-3	225	13
<i>rmpA</i>	F- 5- CATAAGAGTATTGGTTGAC AG-3 R-5- CTTGCATGAGCCATCTTTCA-3	461	13
<i>magA</i>	F- 5- GGTGCTCTTTACATCATTGC-3 R-5- GCAATGGCCATTTGCGTTAG -3	1283	13

total of 25 l of Dream Taq Green PCR MasterMix (2x), 12.5 l of bacterial lysate, 1 l of forward primer (10 M), 1 l of reverse primer (10 M), and 9.5 l of nuclease-free water. A negative PCR control was developed. The biking circumstances are shown in picture 2.



Statistical Analysis. Data representing the presence of different virulence elements associated with genes in *K. Pneumoniae*, were analyzed by acting the χ^2 test or Fisher exact test. The significance of differences turned into evaluated at $P \leq 0.05$.

3. Results and Discussion

Specimens distribution :

The general quantity of samples (100) showing tremendous cultures had been acquired from exceptional samples from patients with *K. Pneumoniae* infection of various while and sexes. *Klebsiella pneumoniae* is a recognised opportunistic pathogen which can motivate upper respiration tract infection, diarrhoea, pneumonia, urinary tract contamination (UTI), and sepsis [14].

K. Pneumonia characterization :

All isolates had been microscopically investigated, and a microscopic exam of *K. Pneumoniae* gram-negative bacteria disclosed Brevibacteria, which had been single-, quick-, or double-stranded [9]. Biochemical outcomes confirmed tremendous consequences for *Klebsiella pneumoniae* on Kriegler iron agar (KIA) for catalase, citrate usage, lactose and glucose, methyl pink and urease. At the same time, indole, H₂S, motility Sex and oxidase had been terrible [14].

In medical microbiology, the Vitek compact machine was used to verify eighty/a hundred specimens as *Klebsiella pneumoniae* isolates. The results showed that eighty isolates belonged to *Klebsiella pneumoniae*, and 20 isolates have been not related to other bacterial species.

The results advised that *K. Pneumoniae* changed into isolated extra normally coming from urine, diabetic feet (21.2%), as well as less tend to from burns (18.7%), wounds (12.5%), sputum, liver, and semen (, three.7%). However, two isolates of *K. Pneumoniae* have been discovered in blood samples, and one isolate (2.5%) became detected in cerebrospinal fluid (CSF).

Phenotypic detection of some virulence factors of hvK. *Pneumoniae*.

The effects of the studies counseled that eighty% (eighty/one hundred) of the isolates of *K. Pneumoniae* have tablet with (Hiss's Method) Capsule stains, even though 20% (20/a hundred) did now not have capsule. The study additionally found out that a hundred% (eighty/80) of *K. Pneumoniae* isolates can supply siderophores when the improvement of bacteria appears on the floor of the M9 medium. In this research, after incubating at 37 °C for 24 hours, all isolates 80 (100%) didn't broaden hemolysin on blood agar. The capability for producing biofilm changed into investigated in eighty isolates in all. From these isolates, fifty six.25 percent (45/eighty) produced strong biofilm, 31.25 percent (25/eighty) mild or weak biofilm, and 12.50 percentage (10/80) did not shape biofilm.

K. Pneumoniae has many virulence elements that improve its pathogenicity, mainly siderophores, adhesions and lipopolysaccharide (LPS) O-side chain (endotoxin), and capsular polysaccharide. The pathogenicity of *K. Pneumoniae* is drastically affected by the capsule polysaccharide (CPS), which has been separated into 77 serological differing types (K) [9].

Bacterial surfaces are engulfed using capsular layers, which inhibit phagocytosis. The most extensive capsular antigens are K1 and K2 [16]. The carbohydrate-rich pill of *K. Pneumonia* will increase pathogenicity and shields the micro organism inside from leukocyte absorption and the serum's bactericidal results [16]

These findings corroborated those of Aljanaby and Alhasany (17, 18, 19).

The outcomes of this study accept as true with AL-Salem and Bunyan's [19] finding that *K. Pneumoniae* can make siderophores in 92.86% of all isolates. Iron chelator structures are produced via sixty three% of *K. Pneumoniae*, consistent with Neslihan and Ufuk (2006). For the bulk of bacterial species, iron is a vital nutrient. It additionally performs an important

role inside the electron delivery chain and capabilities as a cofactor for several different enzymes. Pathogenic bacteria should have iron acquisition mechanisms with a view to proliferate. Since siderophores may additionally represent a vital front within the interplay among host and pathogen, the significance of iron absorption mechanisms is fundamentally giant [20].

The amount of available iron within the host tissue also limits the growth of bacteria there. Iron is essential in bacterial improvement, generally acting as a redox catalyst in proteins and taking element in sports regarding the delivery of oxygen and electrons [21].

Regarding the manufacturing of biofilm, the findings have been in comparison to those of According to data from [23], 69% (forty-five/65) of *K. Pneumoniae* isolates develop biofilms because of excessive attachment, 6% (four/65) are vulnerable or slight biofilm producers, and 25% (sixteen/sixty-five) of isolates do now not produce biofilms. Recorded the *K pneumoniae*(hvf) that changed into remoted from (Diabetic foot, Liver abscess, Blood, Sputum, CSF, Burns) are negative for manose and ribenose and this demonstrates they have been micro organism extra virulent contrast with bacteria that contain on mannose and ribose in assessment to (UTI, Ear swab, Vegetable swabs) that were greater motivate for macrophages that have been considered traditional *K pneumoniae*(ckp). Mannose and rhamnose, which may be diagnosed through macrophage lectin receptors to cause phagocytosis, had been determined in K1 hvKP, and its advanced phagocytosis was tested in a previous look at to be absent from K1 and K2 tablet sorts turned into identified in K1 hvKP. Its better expression should guard bacteria towards neutrophil killing under excessive glucose situation 24,[25].

Virulence genes identity :

The findings proven that the BssS gene, that's the maximum which might be typical of the genes beneath look at, turned into found in

all isolates with a percentage (100%) and that the bands seemed for all favorable isolates inside the anticipated length of the gene (225bp) in gel electrophoresis, as proven in figure 2. Niveditha [11], who determined that 37% of isolates produced red colonies on CRA, indicating non-biofilm growing, whereas 63% of isolates produced black colonies. Additionally, Sura [12] validated that 72% of isolates produced biofilms simultaneously as simply 20% of isolates did not. The formation and renovation of biofilms, which consist of adhesions and pollution, iron uptake, lipopolysaccharides, and the presence of capsules, are vital to the pathogenicity of micro organism. The connection between the staying power of bacteria within the urinary tract and the presence of virulence elements has been hypothesized (Ponnusamy and Nagappan, 2013). Production of biofilms lets in an unmarried cellular to briefly adopt a multicellular life, wherein collective behaviour allows survivorship in adversarial settings [22].

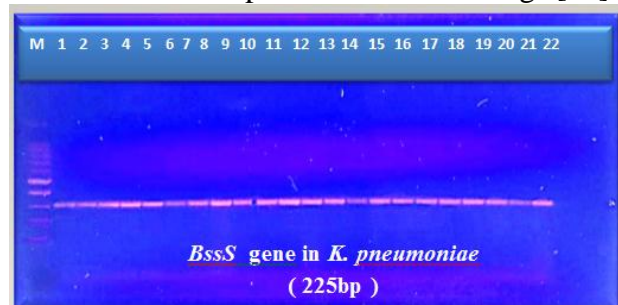


Figure (2) : Virulence genes amplification assay for *K. pneumoniae*, image of Agarose Gel electrophoresis for *BssS* gene in *K. pneumoniae* isolates with product size 225bp , M: Marker ladder (100-1500bp).

The findings of this study demonstrated the presence of the *rmpA* gene in *K. pneumoniae* with a percentage of (27%) as shown in Figure 3. This is because only six isolates and the associated sequence numbers have the *rmpA* gene, where

reas other isolates did not, as shown in Fig.3.

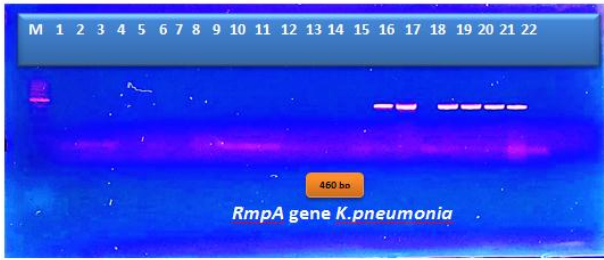


Figure (3): Virulence genes amplification assay for *K. pneumoniae*, image of Agarose Gel electrophoresis for *rmpA* gene in *K. pneumoniae* isolates-, size 461 bp , M: Marker ladder (100-1500bp):

The findings of molecularly detecting the mag A gene in numerous *K. pneumoniae* isolates are presented in figure (4).

along with the percentages of positive and negative isolates using Agarose Gel electrophoresis. For the magA gene, additional isolates produced negative results.

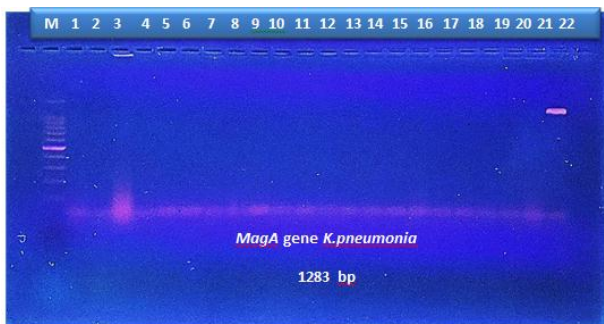
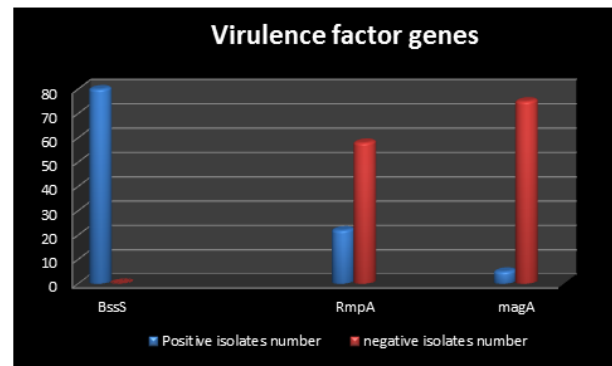


Figure (4): image of Agarose Gel electrophoresis for *magA* in *K. pneumoniae* isolates with product size 1283bp , M: Marker ladder (100-1500bp): Isolates numbers and that primer sequences for *magA* gene.

Prevalence of Virulence Factor Encoding Genes in Virulence *K. Pneumoniae* :

According to the results of screening virulence genes, *BssS* (n= 80, 100%) was prevalent among all the clinical isolates, followed by the *rmpA* (n=22, 27.7%), and *magA* (n=3.7%), as in figure (5).



Figure(5): Presence Virulence Factor Genes of *K. Pneumoniae* Isolates.

The presence of the *magA*, *kfu*, and *K2* genes encoding virulence factors from Kurdistan Province and Iran turned into decided via screening virulence genes for *kfu* (n=8, eleven.4%) and *magA* (n=1,1.Forty three%) (26,27). Data evaluation of these sequences the usage of the gene showed ninety eight% identification of those nucleotide sequences with *K. Pneumoniae* biofilm gene (*bssS*), that is found in other research and codes for virulence elements that growth gene expression in biofilm development [27].

The gene clusters *cps* (capsular polysaccharide synthesis), *mag A* (mucoviscosity associated gene A), *rmpA*, and *wb* (O-precise polysaccharide directed by using the *wb* gene cluster) make up the genome of the *K. Pneumoniae* capsule. As a *K1*-unique capsular polymerase gene that features as a transforming activator for the production of *cps*, *MagA* (35-Kbp) turned into determined. Additionally, *magA* shares homology with the genes chargeable for the manufacturing, transfer, and glycosylation of the LPS. *RmpA* has been tested to grow the colony mucoidy in exclusive *K. Pneumoniae* serotypes and function as a plasmid-mediated regulator for additional capsular polysaccharide synthesis [28,].

Conclusions

hyper-virulent *K. Pneumoniae* (hv KP) possess excessive percent of virulence factors phenotypically and genotypically— among *K.Pneumoniae* remoted from Diabetic foot

,liver and semen samples patients inflamed with- with Hypermucoviscous isolates-

Ethics

This study was carried out under approval through the scientific ethics committee on the University of Kufa (2017). Verbal and written consent was furnished by using parents and agreement for book turned into acquired from both individuals and researchers.

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