Virulence Factors of Klebsiella pneumoniae Isolates from Iraqi Patients

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ABSTRACT
The aim objective of this research is a review study of the virulence factors of Klebsiella pneumoniae isolated from Iraqi patients, where the world is currently facing a major problem where say a war between bacteria and antibiotics. The most important of these bacteria is K. pneumoniae and it is one of the opportunistic bacteria and is considered one of the causes of nosocomial infection, contamination of wounds, Urinary tract infection and contamination of operating theaters. Where these bacteria are distinguished by their resistance to β-lactam antibiotics to produce β-lactamase as well as having a lipopolysaccharide and an important virulence factor is the capsule that resists phagocytosis. An important factor for these bacteria is their formation of biofilm, which is primarily responsible for chronic infections because of their resistance to phagocytosis and killing due to humoral and cellular immunity. The excessive use of antibiotics, which led to the resistance of bacteria to these antibiotics and is the main cause of the current problems of the world, as microorganisms have the ability to develop mechanisms that enable them to overcome these antibiotics more quickly and difficult to find alternatives to these antibiotics. Most of the changes that make bacteria resistant to many antibiotics are acquired changes that are caused by genetic changes or acquisition of resistant genes that make them resistant to many antibiotics.

Keywords: K. pneumoniae, virulence factors, β-lactamase, Lipopolysaccharide, Capsule.

INTRODUCTION
We will focus on the virulence factors of isolated samples from Iraqi patients to see how the K. Pneumoniae have developed resistance to antibiotics. The excessive use of antibiotics and the resistance of bacteria in general, especially the K. pneumoniae, to many of these antibiotics, despite the development taking place in the antibiotics, led to great risks in obtaining alternatives to these antibiotics due to the development of resistance in the bacteria to inhibit the action of these antibiotics and increase their virulence [1]. K. pneumoniae is considered one of the important cause pathogen from nosocomial infection especially for a patient who suffers from Immunocompromised or who are taking drugs immunosuppressed and who suffer from increase iron concentration in blood [2].

Virulence factors
K. pneumoniae possess a number of virulence factors which share with pathogen and include capsule antigens, adhesion factors, enterotoxin produce like lipopolysaccharide as well as resistance killer effect for serum and system the gain on iron (Siderophage) and multi resistance for antibiotics which considered the main reason in spread acquired infections in hospitals, as the percentage infections 80% which led to find alternative treatments and we will mention some Iraqi research on these factors:

The capsule
The capsule is considered fundamental to the virulence of Klebsiella, as it protects the bacterium from phagocytosis and prevents the bacteria by bactericidal serum factors [3]. Some serotypes or capsular types of K. pneumoniae, e.g. (K1, K2, K5, K54 and K57), have been correlating with invasive human infection illness. K1 was found among isolates causing Friedländer’s pneumonia, and has more recently been associated with pyogenic liver abscesses [4].

[5] studied the association between K-type, sequence type (ST) and virulence gene content. The authors concluded that K-types are not associated with specific K. pneumoniae clones and that K-types are published among unrelated clones by horizontal transmit of the cps operon, which encodes the synthesis of capsular polysaccharides. During recent years, several genes encoding virulence factors K. pneumoniae have been described: the plasmid-borne rmpA regulates the mucoid phenotype [6], wcaG is associated with reinforced bacterial get-away from phagocytosis [7], Kfu is involved in iron acquirement, fimH encodes type 1 fimbriae, mrkD encodes type 3fimbriae and c29A encodes the non-fimbrial adhesion factor CF29K [5].

In a study conducted by [8], the genotype K1 and K2 were used. Of the 46 isolate from these bacteria, eight were positive for K1, fourteen positive for K2 and 3 positives for both, while eighteen isolate did not contain either K1 or K2. In another study conducted by [9], forty isolates showed twenty three isolate carrying the K1 genotype, while eleven positive isolates of the K2 genotype and six isolates did not carry either type.

Other study by [10], one hundred sample of urine and sputum were collected from patients at Al-Yarmouk Teaching Hospital in Baghdad. 38 sample were obtained from Klebsiella pneumoniae and were only from UTI. These isolates showed resistance to most of the antibiotics such as Cefazidime Augmentine, Ceftriaxone, and Cefotaxime, while lower resistance was shown to both Imipenem and Meropenem. The results also showed only four samples that were positive for the wzy gene, represented by K19 and K20, K21, K22.

Thirty nine isolate of K. pneumoniae were diagnosed in Al-Diwaniya Teaching Hospital for various cases (sputum,
urine, burns and wounds). The presence of capsule antigen (K) was detected by Multiplex PCR technique. The results showed that there were three serotypes K1, K57 and K2 that were more present than other types [11].

In another study by [12] of seven isolates of K. pneumoniae out of 34 sample. Thirty four samples were collected from Ramadi Teaching Hospital and Burns Specialized Hospital in Baghdad, where the results showed that the isolates were resistant to most antibiotics as these seven isolates were diagnosed through a PCR to detect the mag A gene for serotype K1 and also for rcsA gene for genotype K2, the results showed that 5 isolates were related to type K2 and the remaining two isolates did not have any of the two types.

**Lipopolysaccharide**

Lipopolysaccharide represents an important and essential factor in bacterial pathogenicity, especially K. pneumoniae, as it is one of the superficial compositions of bacteria that help it to resist phagocytosis, and it is characterized by its ability to activate the complement factor [3]. It participates in protecting bacteria against the host’s Complement System. LPS consists of three parts: Lipid A, Core polysaccharide, and O antigen, which consists of a side chain of the polysaccharide, and the antigen O is responsible for the bacteria’s resistance to killing [13]. K. pneumoniae have eight serotypes, and serotype O1 and O2 are the most common. K. pneumoniae O-antigen-deficient strains, community acquired pneumonia (CAPS) protects the micro-organism against complement killing [14]. K. pneumoniae serotype O1K1 plays a study role in virulence by transfer resistance to serum killing and by promoting bacterial dissemination to and colonization of internal organs after the start of bacteraemia [15].

In a study conducted by [16] where the CPS genotype was used, among 46 isolate of these bacteria, 43 were positive for this gene while only 3 isolates were missing for this gene. Two types of core polysaccharide (Type I and Type II) have been diagnosed produced by these bacteria, which are synthesized by two different groups of wa gene cluster. [17].

**Outer membrane proteins**

Is one of the important proteins of the gram negative bacteria are present in the outer membrane OmpA, which is characterized by most of the Enterobacteriae. OmpA is independent of the core polysaccharide in K. pneumoniae, which has an important role in preventing the activation of epithelial cells in the airway as it acts on NF-kB-p38- and p44 / 42- dependent pathways and thus participate to the attenuation these cells through the inflammatory response [18].

An important factor that is produced by the Klebsiella pneumoniae is the expression of efflux pump AcrAB, whose action is not limited to exporting antibiotics only such as (quinolones and β-lactams), but also works to export antimicrobial agents derived from the host. The loss of AcrAB leads to a loss of the ability to cause pneumonia [19].

In a study by [20], on the resistance of multiple drugs by K. pneumoniae, which has the wide resistance to many antibiotics in Iraqi hospitals, Efflux pumps AcrAB was studied where 100 samples were taken from various sources from the medicine city and diagnosed by 16S-23S rDNA gene. It is present that 60 isolate were for K. pneumoniae, after which efflux pump AcrA gene was detected using a specific primer and it appeared that there are 26 isolates producing this gene. The results showed by studying the gene expression AcrAB-TolC efflux pump using q(RT-PCR) that there is a relationship between the gene expression and chloramphenicol concentration, as well as the gene expression of the same gene increases from exposure to the Imipenem with some differences, while there was a decrease in the expression of Amikacin and Ciprofloxacin.

In a study in which the researcher collected 195 different clinical samples from three hospitals in the city of Al-Najaf and 50 samples from the hospital environment, where the K. pneumoniae was diagnosed by biochemical and cultural tests and the results showed that 89 isolates were for the K. pneumoniae where the resistance to quinolone antibiotics was examined by their growing on the MacConkey agar medium supported with the ciprofloxacin, it showed that 34 isolates were resistant to the ciprofloxacin antibiotic. As for the sensitivity test for 18 antibiotics, the results showed that 34 isolates had the characteristic of Multidrug resistant (MDR). Also in this study, the presence of resistance genes (aac (6)’-Ib-cr, qepA, qnrS and qnrB) was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected, where the plasmid resistance gene showed aac (6)’-Ib-cr, qepA, qnrS and qnrB was detected.

**Hypermucoviscous phenotype**

A distinguishing factor of hvKP strains is its hypermucoviscous phenotype. The great majority, but not all of the strains cause community-acquired pyogenic liver abscesses (CA-PLA) possess this phenotype [6].

In a study conducted by [16] where the caps viscosity gene was investigated for 46 isolates of these bacteria, it gave 21 positive isolates, and the genes responsible for regulating viscosity and mucous matter were studied using three types of genetic indicators (rmpA1, rmpA rmpA2). Results showed that 21 isolates contained the rmpA gene, while only 19 isolates gave a positive result for these indicators rmpA1 and rmpA2.

In another study, forty isolates of K. pneumoniae were used, where the results showed that eleven strains were carriers of the rmpA gene, where five strains were carrying type K1 and also five strains of type K2 and only one strain of the two serotype together. The results also showed that the positive strains of the serotype K1 serotype gave amplification of both magA, rmpA and 16S rRNA genes while the K. pneumoniae strains of type K2 and non-carrier of the rmpA gene gave only amplification of serotype K2 and 16S rRNA and also the strains carrying type K1 and non-carrier of the gene were magA and 16S rRNA and finally the non-carrier
strains of the two types K1 as well as the K2 and non-carrier of the rmpA gene gave a positive result for the 16S rRNA gene. Through the results above, the results show that the genotype magA and k2A as well as any possible that is useful in detecting serotype K1 and K2 for K. pneumoniae.[9].

Siderophores and Fimbrial adhesins
Sixty-one isolates of K. pneumoniae were isolated and diagnosed from 433 samples of children suffering from diarrhea in Kirkuk Hospitals, where virulence factors were determined for these bacteria and the results showed that all K. pneumoniae isolates were produced for the capsule, urosepsis and siderophore. The results also showed that all isolates have the ability to adhere to human buccal cavity epithelial cells.[22].

In a study in which K. pneumoniae were found to be able to remain in the urinary tract and their relationship to the genes responsible for forming the biological membranes fimA, fimH, mrkA and mrkD, the study included 50 isolated K. pneumoniae isolates from patients with UTI and these isolates were diagnosed by the VITEK system apparatus or their ability to produce biofilms by using tissue culture plate method. Female infections were more than males 50/44 and 6/44 respectively, and results also showed that about 72% of isolates were biofilm formation. Using the PCR technique, 12 isolates were detected, all carrying the fimA, fimH, mrkA and mrkD genes.[23].

Virulence factors were detected for 32 isolates of K. pneumoniae isolated from various cases (stomach, urine, burns and blood) in Al-Najaf Governorate. As the capsule, hypermucoviscosity, and ability to form biofilms, produce siderophores and as well as the production of β-lactamase were revealed, PCR was used to detect genes that encode these factors (fimH, ycfM), (Kfu: iron uptake system, entB: enterobactin, irp-2: yersiniabactin), capsule synthesis or invasions (rmpA, uge, wabG) and β-lactamase (SHV, TEM). The results showed that 100% of the isolates produced the capsule, biofilms and the siderophores, while for the hypermucoviscosity only 62.5% of the isolates had the ability to produce this factor. Also, most virulence genes appeared as fimH-1, ycfM and entB (100%), uge and TEM (93.75%), wabG and SHV (87.5%). While the Kfu and rmpA genes appeared 65.62 and 62.5%, respectively. The lowest percentage was for the irp-2 gene (37.5%). For as its ability to produce β-lactamase, 62% of the K. pneumoniae isolates showed their ability to produce this enzyme.[24].

β-Lactam antibiotics
Due to their diversity, broad spectrum of activity and low toxicity, β-lactams are the most prescribed antibiotics worldwide. All β-lactam ring in common. Due to differences in their side chains, β-lactams may be classified into the following main groups: penicillins, cephalosporins, monobactams and carbapenems.[25]. β-lactams target the bacterial cell wall synthesis and act by binding covalently to penicillin binding proteins (PBPs).

Carbapenem-hydrolyzing β-lactamases (Carbapenemases)
Carbapenemases are clinically important because they destroy and so may confer resistance to carbapenems (and usually most other β-lactams). K. pneumoniae that produce class A Carbapenemases (KPCs) are frequently identified worldwide.[26]. K. pneumoniae Carbapenemases (KPCs) It is considered one of the important enzymes that work to destroy β-lactam antibiotics, as it works to break down the β-lactam ring and inhibit the action of these antibiotics, especially class A.

The researcher [27] collected 42 isolates of K. pneumoniae from burn infections in Baghdad hospitals, where sensitivity test was tested for all isolates for a number of antibiotics and the results showed that the ratios appeared as doxycycline (100%), tetracycline (95.23%), cefotaxime and piperacillin (85.71%), ceftriaxone (88.09%), trimethoprim-sulfamethoxazol (83.71), ticarcillin (78.57), aztreonam (71.2%) ceftazidime (69.4%) ciprofloxacin (59.52%), gentamycin (26.16%) , imipenem (21.42%) and finally amikacin and meropenem (19.04%).

In a study conducted by the researcher [28] to detect the presence of blaOXA-23 gene between 117 isolate of K. pneumoniae obtained from the hospitals in Al-Hilla, where the results showed that the highest percentage was found in stool followed by sputum samples. The initial sensitivity test of β-lactam antibiotics showed that 91 isolates were resistant to ampicillin and amoxicillin. About 17 isolates showed their resistance to carbapenem antibiotics, and the presence of blaOXA-23 gene was detected in these isolates using PCR technique, the results showed that 15 isolates of K. pneumoniae were carrier of this gene.

In another study, 135 urine samples were collected from patients with cystitis and confirmed by clinical diagnosis at Al-Hilla Teaching Hospital. The samples were diagnosed by biochemical and VITEK2 system tests. The results showed that 23 isolates were K. pneumoniae (31.9%) of the total samples. All of these isolates were tested for sensitivity to antibiotics, and the isolates showed high sensitivity to the amikacin (87.7%), norfloxacin (73.40%) and tobramycin (93.91%), while it showed resistance to the antibiotics ampicillin 23(100%), ceftazidim 20(91.3%), cefotaxime 19(82.6%) and cefepime 17(73.9%) and ertapenem 10(43.5%). The researcher used DDST as well as chromatic ESBL medium, MIC test strip and chromatic CRE medium for KPC for investigation of ESBL and KPC.[29].

The (blaKPC1) and (blaKPC2) genes were detected for a number of Iraqi samples isolated from different hospitals for patients with wounds, burns, sputum and urine in 2015 using specific primers of the first gene blaKPC1 and the second gene blaKPC2 where most of the samples showed their resistance to the carbapenem antibiotics and production of Carbapenemases, there are a number of variations showed compared to NCBI [30,31].

In another study conducted by [32], there was a variation in the resistance of carbapenem antibiotic. Among the 53 isolates of Klebsiella pneumoniae most isolates were sensitive to Meropenem and Imipenem antibiotic 90.5% and 77% respectively. The isolates showed higher resistance to third generation cephalosporins. Carbapenemases production was detected by the modified Hodge test, five carbapenem resistant K. pneumoniae isolates (K2, K3, K4,
In this study, detection of \( \text{Lactamase} \) \( \text{Antimicrob. Agents} \) was carried out on all fifty-three K. pneumoniae isolates. Even though five isolates gave positive modified Hodge test, only one isolate \( \text{K2} \) gave specific identification for \( \text{blaKPC} \) gene.

Another study, the results showed that 27 isolates of K. pneumoniae bacteria in Al-Hilla Teaching Hospital for isolated samples of urine and wound infection, that there is resistance to tetracycline and ceftiraxone antibiotics, and showed sensitivity to amikacin as well as imipenem [33].

In a study that collected 61 urine and stool samples in Al-Diwaniyah governorate to detect encoded genes for \( \beta \)-lactamase from patients with bladder and colon cancer. Where the results showed that E. coli and K. pneumoniae in urine samples 17 out of 23 samples, while the stool samples were 19 out of 26 samples and the samples were resistant to three classes of antibiotics. The PCR technique was used to detect the \( \text{blaTEM} \) and \( \text{blaSHV} \) genes, where it was found that most isolates carried at least one of the genes, and the highest was the \( \text{blaSHV} \) gene, then \( \text{blaTEM} \) at a rate of \( (66.7\%) \) and \( (55.6\%) \), respectively [34].

In a study conducted by [35] eighty isolates of K. pneumoniae among elderly patients (smokers and non-smokers) with chronic pneumonia in Al-Najaf Hospital. The results showed the sensitivity of a number of antibiotics that there was a variation in the levels of resistance between the antibiotics (amoxicillin, nitrofurantoin, Amoxiclav, Cefotaxime, Ceftriaxone, Gentamicin, Amikacin, Tobramycin and Tetracycline), and it was also found through the results that all the isolates of K. pneumoniae from smoking patients were resistant to all antibiotics compared to non-smokers. The results revealed that through detection of genotypic, 45 were carriers of the \( \text{blaTEM} \) gene, 31 were carriers of the \( \text{blaSHV} \) gene, while 18 isolates were carriers of both genes.

Due of the necessity of detection resistance genes, the researchers [36] collected eighty seven isolated urine samples from Al-Hilla Teaching Hospital for patients suffering from UTI. 34 isolate were gram negative bacilli, including 14 isolates that were for K. pneumoniae was produced for carbapenemase. The genotype was detected by the PCR of the \( \text{blaIMP} \) and \( \text{blaOXA-48} \) genes, where results showed that 5 \( (35.7\%) \) and 3 \( (21.4\%) \) of K. pneumoniae were positive for \( \text{blaIMP} \) genes and \( \text{blaOXA-48} \) genes respectively.

**Histamine-producing bacteria (HPB)**

In a study by [37], fifty one specimen were collected from patients with respiratory infections in the Basra city. K. pneumonia was diagnosed by morphology and biochemical characteristics, as well as colonies culture on the Niven’s agar media as the colonies of purple color are an indicator of bacteria producing histamine. All samples of K. pneumonia are diagnosed by the HDC gene and its expression is detected. The results showed that only 11 strains of Klebsiella pneumonia were positive for the production of histamine and most of them were sensitive to Trimethoprim but are resistant to Ampicillin, Sulbactam / Ambicillin.

In a diagnostic study by the researcher [38] for a number of isolated samples from different clinical sources (Sputum, wounds smear and Urine) through the 16Sr RNA gene, the results showed that among the 25 isolates 10 samples from the K. pneumoniae showed this evidence of the pathogenicity of these bacteria and their resistance to antibiotics.

**CONCLUSION**

Through a review of many research studies of the Iraqi isolates of K. pneumoniae withing 5 years a go, it became apparent that over time, the ability of these bacteria to resist antibiotics increases through the development of the virulence factors they possess or possess by acquiring new characteristic. This indicator shows the difficulty of discovering new antibiotics that work to kill these bacteria therefore, plant extracts have been used recently to inhibit them.

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**REFERENCES**


