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Detection of Some Capsule Genes (*ugE, wabG and ycfM*) in Carbapenem Resistance *Klebsiella pneumoniae* Clinical Isolates from Baghdad Hospitals

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Abstract: The rising prevalence of carbapenem-resistant *K. pneumoniae* has emerged as a significant concern in the field of public health. Consequently, it is imperative to conduct an in-depth study of this issue, particularly in relation to the potential association between capsule genes and the development of carbapenem resistance. The aim of the study identifying effective strategies to reduce the dissemination of carbapenem antibiotic resistance. The 120 samples were collected from different clinical specimens (burns, sputum, urine, and wound) between October 2022 and January 2023.), according to culturing , microscopic examination, biochemical tests and vitek 2 system , only 50 isolate (41.6%) isolates were found to be *Klebsiella pneumoniae*. The percentages of isolation were found to be (46%) by sputum (22%), (20%), and (12%) in urine, sputum, wounds and burns respectively. Antibiotic sensitivity testing on three types of carbapenem discs, namely imipenem, meropenem, and doripenem, revealed that meropenem was the most effective antibiotic, followed by imipenem, and finally doripenem, with resistance percentages of (30%, 40%, and 56%, respectively). By using PCR specific primers designed particularly for this study, capsule associated genes were detected in twenty-five resistant and sensitive isolates. *cfm* was found to be the most predominant gene, followed by *wabG* and *uge*, with percentages of (88%, 84%, and 72%, respectively). It was concluded there is an association between capsule associated genes and carbapenem resistance in *K. pneumoniae*.

Keywords: *Klebsiella pneumoniae, uge, ycfm, wabg*, Carbapenem-resistant

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Introduction

Carl Friedlander first described *Klebsiella pneumoniae* in the late 19th century as a microorganism that was isolated from the respiratory tract of those who had passed away from pneumonia, at the time, it was known as Friedlander's bacteria. Trevisan (1885) discovered the first strains of this genus, which he renamed to honor the scientist Edwin Klebs (1) The genus is known to be Gram-negative, nonmotile, has a capsule, and may be found in the environment, on medical equipment, in soil and on blue water (water on the earth's surface) (2). According to Gorrie *et al*. (3), *Klebsiella* species tend to be an opportunistic bacteria that colonize the mucous membranes without producing disease. on the other hand, it can migrate from mucosae to other tissues and produce infections that could be potentially lethal such as infections of the urinary tract, sepsis, infections of the bloodstream, and respiratory tract infections. In the early 1990s, a novel beta-lactamase was found in Klebsiella, labeled "*Klebsiella pneumoniae* carbapenemase" (KPC) (4). The extremely resilient bacteria *K.*

pneumoniae appears to work better as a pathogen by following the adage that "the best defense for a pathogen is a good defense" as opposed to "the best defense for a pathogen is a good offense." And one of the first defense mechanism is the capsule that surround the bacterial cell (5). This research aims to detect some capsule genes specifically (*ycfm, wabG and uge)* in carbapenems resistant *Klebsiella pneumonia* in clinical isolates from Baghdad hospitals and detect capsule genes in resistant isolates.

Material and methods

Collection of samples

This research was carried out in Baghdad, Iraq, for a year period, from October 2022 to January 2023. 120 clinical specimens obtained from patients (both males and females) with infections of the urinary system, burns, wounds, and also respiratory systems. who were admitted to a number of different hospitals in Baghdad, including Ghazi Hariri, Baghdad Teaching Hospital, Central Educational Laboratories, Burn Hospital, and Al-Imamain Al-Kadhimin Medical City. The specimens were immediately transferred to the Microbiology Laboratory in aseptic conditions and cultured using selective and differential methodes.

Klebsiella pneumoniae isolation and identification

Klebsilla pneumoniae got isolated from different human samples using the streaking technique. The samples were streaked onto MacConkey agar (Himedia, India) plates (6) and then incubated at 37°C for twenty four hours. The bacterial colonies were identified through their mucoid appearance, which showed up in a shiny pink color. Subculturing on MacConkey agar plates were used to purify isolated colonies.

Morphological and biochemical tests

Every bacterial culture underwent gram staining to determine if they were Gram positive or if they were Gram negative (7). Microscopic observations were used to define morphological traits such as color and shape. The margin together with the elevation of the colony was observed to determine its shape. under a microscope, the stain of the bacterial wall will be observed (8) The following biochemical tests were performed for further characterization: IMViC (9), and catalase (10). Testing via vitek2 (Bio-Meriaux, France) was used for further confirmation and to ensure that all isolates were *Klebsiella pneumonia* Species The manufacturer's report presented the findings as follows: excellent identification ranged from 96% to 100%, very good identification ranged from 93% to 95%, good identification ranged from 89% to 92%, and acceptable identification ranged from 85% to 88%.

Antibiotic susceptibility testing

Antibiotic susceptibility test was carried out using the Kirby-Bauer technique (disc diffusion method) where 50 *K. pneumonia* isolates were tested using three types of carbapnemes discs: meropenem (10µg), imipenem (10µg), and doripenem (10 µg), as proposed by the Clinical Laboratory Standards Institute (CLSI, 2023)(11). All isolates were cultured overnight at 37°C in Mueller Hinton broth (Oxoid,UK). pure colonies of bacteria were quantified to 0.5 on the MacFarland nephelometer scale (1.5 10^8CFU/ml) then by using a sterile swab the bacterial suspension was streaked onto Mueller Hinton agar (Oxoid, UK). According to CLSI guidelines (2023), then the dimeter of the isolate inhibition zone was measured to determine antimicrobial sensitivity and resistance.

Detection of metallo-β-lactamase activity using phenotypic method

All isolates that were found to be resistant to carbapenem were tested ,imipenem (the zone dimeter ≤19 mm according to CLSI, 2023 recommendations) was used by employing disc diffusion method on Mueller Hinton agar were tested for metallo-β-lactamase activity using the imipenem+EDTA combination disc test as described in previous research (12) In short, a colony from an overnight growth of the test organism was placed on a Muller Hinton agar plate the suspension was ensured to be 0.5 McFarland, which is the equivalent of 1.5×10^8 CFU/ml of bacterial suspension density. A disc of imipenem (10 µg) was embedded into an inoculated plate, and a similar antibiotic disc but with EDTA was placed on the opposite side of the plate 5 mm apart. at 37°C the plates were incubated for 16-18 hours. the inhibition zones of both discs were compared.

DNA extraction

DNA was extracted from 50 confirmed isolates from an overnight culture that was adjusted to extract the whole DNA from the bacterial isolates, the procedure of Easypure genomic DNA extraction kit was utilized.

Detection of capsule-associated genes via Polymerase chain reaction assay(PCR)

Conventional PCR testing for 25 isolates to detect the outer membrane lipoprotein (*ycfM*) and invasions (*uge, wabG*), It was done following the instructions provided by the manufacturer. Primers specially designed for this study were listed in (Table 1). The OneTaq2X Master Mix (NEB) was used for amplification. (Table 2) showed the thermal cycling conditions for (PCR).

Table (1): A list of the primers utilized in this research

Target genes	Sequences of the primers	Size of the product (bp)	Refrence
wabG	F (GTTGGGGAACGGATCGTAGAG) R (GGTGGTCGGCAAAGATAAGGA)	163	This study
ugE	F (ATGGACTACATCACTGCCCTG) R (AAAGCCCACCAGGTCATACAG)	192	This study
ycfM	F (CGATATTCCTCGCAGGCTGTG) R (TGATCCTGATGCTCAATCGGC)	163	This study

Table (2): List of the thermo cycling conditions

Amplification steps	Temperature °C	Time	Number of cycles
Initial denaturation	95	5 min	1
Denaturation	95	30 sec	
Annealing	55 ^{ycfm},54 ^{uge},55^{wabG}	30 sec	30
Extension	72	1 min	
Final extension	72	7 min	1
Hold	10	10 min	

Results and discussion

Isolation and identification of *klebsiella pneumonia*

Based on the results of the morphological and biochemical tests that was later on confirmed by VITEK2 analysis, 50 *K. pneumoniae* isolates were identified from 120 distinct clinical specimens with a probability of (99%). The isolates were given the designations (KP1, KP2, KP3,... KP50). (Table 3) presents the percentage of isolates.

Table (3): Number and percentage of isolation for each specimen

Specimen type	Number of Specimen	Number of isolates	Percentage of isolation from 50 isolates	Percentage of isolation from 120 specimen
Sputum	35	11	22%	29.2%
Wound	27	10	20%	22.5 %
Burn	19	6	12%	15.8%
Urine	39	23	46%	32.5%
Total	120	50	100	100

The data indicated that urine had the largest percentage of the clinical specimens isolated then followed by sputum, wound, and burn. The high percentage of *K. pneumonia* isolated from urine may be attributed to long-term hospitalization of patients (chronic dieses patients), and the infection is often associated with urethral catheterization associated with inpatient (13) The percentage of other specimens isolated varied according to the overall number of specimens as well as the isolation condition. (Table 4) shows the findings from biochemical tests that are used to determine a clinical diagnosis:

Table (4): Biochemical tests results

Biochemical test	Result
Gram stain	G-ve bacilli
Growth on MacConkey agar	Pink (lactose fermenter), large, mucoid colony
Indol	–
Methyl red	–
Vogousproskur	+
Simmone citrate	+
Catalase	+

Antibiotic susceptibility results of *klebsiella pneumonia*

Result of the antibiotic resistance of all fifty local *K. pneumoniae* isolates from different infection sites against three antibiotics were briefed in (Figure 1), it showed that (56%) of the isolates were resistant to doripenem and (40%) were resistant to imipenem and (30%) were resistant to meropenem. Considering the bacteria's resistance mechanisms towards carbapenems includes the synthesis of lactamases and mutations that impair the expression as well as activity of porins and PBPs, One indicator of resistance development is the increase in the prevalence of resistant *K. pneumoniae* bacteria. (14) Numerous local studies found that the *K. pneumoniae* isolates showed relatively small degree of resistance to imipenem and meropenem (15). previous studies demonstrated the significant resistance to aminoglycoides and cephalosporins seen in isolates of *K. pneumoniae*. This bacterium was shown to be highly resistant to third and fourth generation cephalosporines, according to many local researchs (16,17). Lack of standardized criteria for identifying drug-resistant isolates, insufficient laboratory infrastructure, a lack of communication between physicians and microbiologists, and poor sanitation may all be contributing factors to Iraq's rising numbers of drug-resistant isolates (18). In three prominent tertiary care institutions in Baghdad, Iraq, it was discovered that *K. pneumoniae* was resistant to carbapenems, one of the of final resort drugs (19).

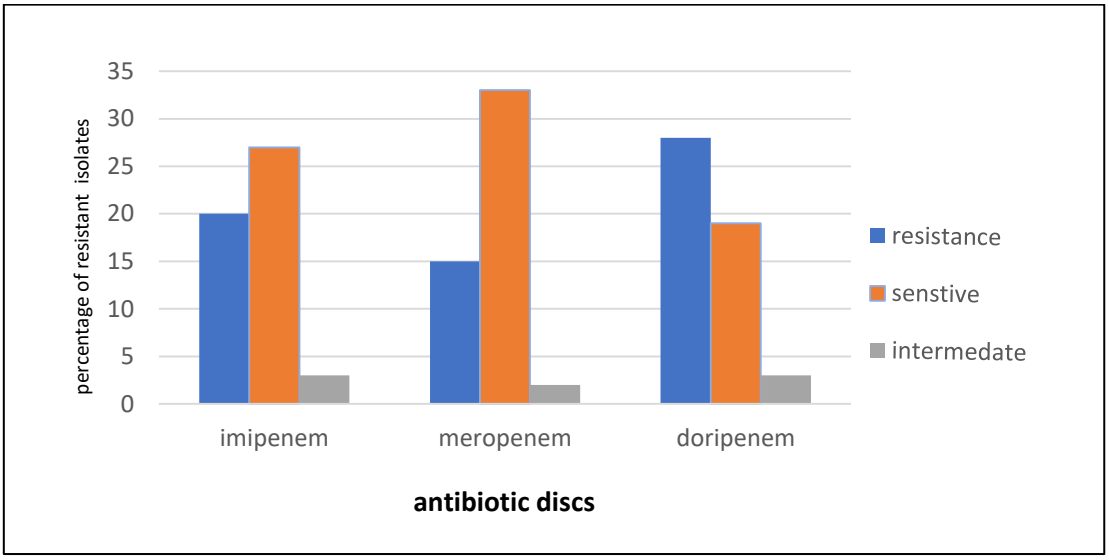

Figure (1): Results of *K. pneumoniae* susceptibility to carbapenems antibiotics. (Imipenem, Meropenem and doripenem)

Phenotypic detection using imipenem disc with EDTA

The 20 imipenem-resistant isolates were examined using the imipenem/ EDTA combined disc test to ascertain if metallo-β-lactamase production is the root cause of imipenem resistance or whether there are additional mechanisms involved (Figure 2). The addition of EDTA to Imipenem-resistant bacteria resulted in increased susceptibility due to its inhibitory effect on the formation of MBL (20). From these 20 isolates, 18 (90%) were positive for metallo-β-lactamase, according to this phenotyping. Gram-negative bacilli that produce metallo-beta-lactamase (MBL) have the potential to transfer resistance genes to other microbes, hence giving rise to significant medical complications in hospitals worldwide Therefore, it is crucial to identify the presence of MBL-producing gram-negative bacilli during the early stages in order to prevent their dissemination (21).

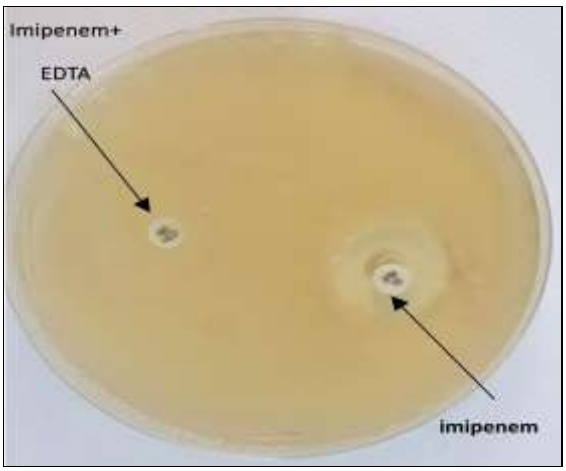

Figure (2): Inhibition zone of imipenem resistance isolate compared to the inhibtion zone using imipenem disc containing EDTA to determine metallo-β-lactamase production.

Detection of capsule associated genes in *k. pneumoniae* by (PCR)

The current study assessed the detection of capsule-associated genes in 25 isolates, including carbapenem-sensitive (n=12) and carbapenem-resistant (n=13). The results of this analysis are presented in (Figures 3,4,5). the most prominent gene was ycfm with a percentage of (88% of isolates), *wabG* (84%), *uge* (72%), and all were capsule assoiated genes which encodes the capsule, capsule lipoprotein and outer membrane protein synthesis (22). It is compelling to mention that the carbapenem sensitive strains lacked at least one of the three capsule associated genes meanwhile the isolates that got all the three genes were in fact carbapenem resistant, and this is in an agreement with previous research that virulence factors in this case it's the capsule in pathogenic bacteria have been associated with antibiotic resistance (23). Although these genes are not directly responsible for carbapenems resistant in *K. pneumoniae* but it's well known that virulence factors and antibiotic resistance are conncted and are usually important in the pathogenesis of bacteria (24). All three genes (*ycfm, uge* and *wabg*) were deemed to be of importance to the bacterial pathogenicity, the prevalence of the special structure in the capsule of *K. pneumoniae* (25), is in line with the large percentage of *ycfm* gene seen in the results followed by The *WabG* gene, and despite the fact that it is prevalent in patients with severe and invasive infections and has a significant role in pathogenicity, its mechanism of action is still unclear (26) The *uge* gene was the least found in the 25 isolate however, according to previous research which indicates that *K. pneumoniae* mutant strains (by eliminating its *uge* gene) were found to have lost their virulence in experimental animals, which indicates the importance of *uge* gene for the pathogenicity *K. pneumoniae* (27).

Figure (3): Gel electrophoresis for capsule gene *ycfm* of PCR product size (163 bp) run using 2% agarose and 80 volt for 80 minutes . Lane L: 100bp DNA marker ; lane (1-25) : *k.pnumoniae* isolates detected for the gene presence. Isolates (1,9,16) tested negative for this gene.

Figure (4): gel electrophoresis for capsule gene *wabG* of PCR product size (163 bp) run using 2% agarose and 80 volt for 80 minutes . Lane L: 100bp DNA marker ; lane (1-25) : *k.pnumoniae* isolates detected for the gene presence. Isolates (7,12,20,22) tested negative for this gene.

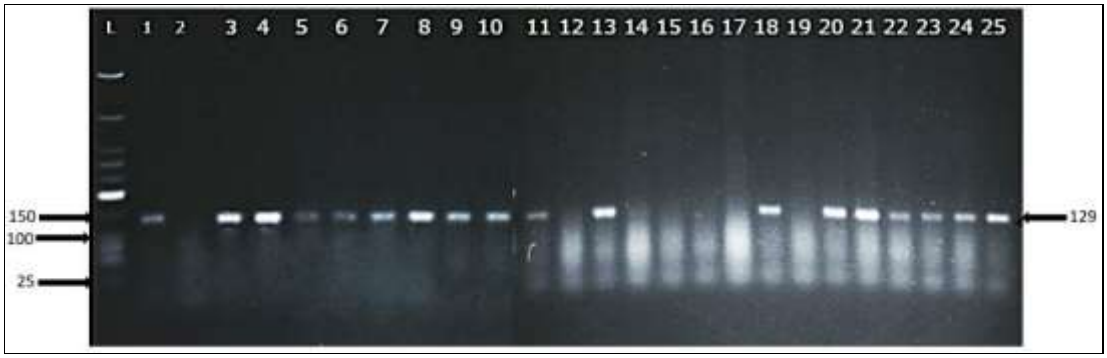

Figure (5): gel electrophoresis for capsule gene *uge* of PCR product size (163 bp) run using 2% agarose 80 volt for 80 minutes . Lane L: 100bp DNA marker ; lane (1-25) : *k.pnumoniae* isolates detected for the gene presence. Isolates (2,11,13,14,15,16,18) tested negative for this gene.

Conclusion

Carbapenem-resistant *K. pneumoniae* plays a role in infection at several body sites, and antibiotic resistance may be attributable to virulence genes, particularly capsule-associated genes .In the current investigation, the dominant virulence factor gene was ycfm which is responsible for adhesion as well as outer membrane protein synthesis. Clinical sources of urine and tracheal infections were shown to include the most prevalent virulence genes specifically capsule associated ones. The frequency of virulent factors in *K. pneumoniae* was positively correlated with antibiotic resistance. Carbapenem-resistant *Klebsiella pneumoniae* poses a serious danger to our local hospitals' healthcare systems.

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