Al-Kufa University Journal For Biology: 2023. Vol. 15 No. 3 P: 122 -128
ISSN (Print)2073-8854 ISSN (Online): 2311-6544

Original Research Paper: *Journal homepage : https://journal.uokufa.edu.iq/index.php/ajb/index*

Genotypic And Phenotypic Surveillance Of Multidrug Resistance Of Hypermucoid *K. Pneumoniae* Among Clinical Isolates In AL Najaf City /Iraq

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Article history
Received: 4 / 6 /2023
Revised: 10 / 8 /2023
Accepted: 12 / 8 /2023
DOI:
10.36320/ajb/v15.i3.14615

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Abstract: *Hypervirulent Klebsiella pneumoniae strains are the major cause of liver abscesses ,diabetes foot ulcers and other infections throughout Iraq, and these strains are usually antibiotics susceptible. Recently, hypervirulent K. pneumoniae isolates have emerged due to acquiring antimicrobial resistance determinants or the transfer of a virulence plasmid into a classic isolates ,.Klebsiella pneumoniae is responsible for hospital- and community-acquired infections. This study aimed to determine the prevalence of hyper irulence K. pneumoniae and investigate the antibiotics resistance profile among clinical specimens at Al Najaf Hospitals in/ Iraq, and detect the genes for molecular identification of K. pneumoniae in comparison with phenotypic and biochemical methods. In total, 100 clinical specimens were collected from patients infected with K. pneumoniae, between January 2023 and May 2023 from both sexes males (38) and Females (62) with age ranged between (20-70) years. The Hypervirulent Klebsiella pneumoniae isolates were cultured on general and selective media as (Blood agar and MacConkey agar and CHROM agar), and confirmed by using Vitek-2 system. The isolates were reported with high resistance towards various types of antibiotics, especially penicillins and cephalosporins. In contrast, K. pneumoniae showed very low resistance to imipenem and amikacin. According to the results the range of multidrug-resistant K. pneumoniae isolates in this study was estimated at 100%.*

Keywords*: *K. pneumoniae, hypervirulent . Antibiotics resistance, gnr Gene, Vitek-2 system

1. Introduction

K.Pneumonia may cause various types of health-related infections, including meningitis, pneumonia, bacteremia, urinary tract infections, as well as wound or surgical site infections. *K. pneumoniae* have frequently developed antimicrobial resistance with a high fatality rate if wrongly treated [1]

K. pneumoniae is known as one of the major causes of hospital- and community-acquired infections and plays a significant role in the propagation of antibacterial resistance genes from environmental bacteria to pathogenic bacteria [2]. This bacterium has developed resistance to antibacterial agents more readily than most bacteria by the production of Carbapenemase and Extended- Spectrum β-Lactamase enzymes[1, 3]. The most significant risk factor of AMR is exposure to antibiotics, and the main cause which contributes to expanding the spreading of resistant bacteria strains is the prolonged and intensive use of antimicrobial agents in health care settings [4].

Extended spectrum beta-lactamases are a group of enzymes produced by certain bacteria that are able to hydrolyze extended- spectrum antibiotics belonging to the penicillin and cephalosporin groups and monobactam. ESBLs are found in Gram-negative bacteria, especially in Enterobacteriaceae that are often located on plasmids that are transferable between bacterial species [5].This study aimed to determine the prevalence of *K. pneumoniae* and investigate the antibiotics resistance profile genes among clinical specimens at Al Najaf Hospitals.

2.Methodology

K. pneumoniae isolation and identification

A total of 100 clinical specimens were collected from patients infected with *K. pneumoniae* (38 males and 62 females, with age ranging between: 10-70 years, including urine semen swabs, wound diabetes foot ulcer, and liver, between January 2022 and May 2023. The samples were streaked on blood and MacConkey agar and CHROM agar Orientation medium, then incubated at 37°C for 24 h. and confirmed by the Viteck system. hypermucoviscous *K. pneumoniae* were detected by string test and Viteck to distinguish from classical *K. pneumoniae* [6] .

Antibiotic Sensitivity Test

Kirby-Bauer disc diffusion test was done to detect the susceptibility of *K. pneumoniae* to Gentamicin (10 µg), Cefotaxime (30 µg), Meropenem (10 µg), Ciprofloxacin(5µg), Tobramycin (10 µg), Ampicillin (10 µg), Ceftazidime (30 µg), Nitrofurination (30 µg), Imipenem (10 µg), Aztreonam (30 µg), and aztreonam. Resistance to three or more various classes of antibiotics was considered MDR *K. pneumoniae* (7).

Polymerase Chain Reaction :

DNA of bacterial isolates was extracted by using the Bioneer kit. Polymerase chain reaction (PCR) test was performed with species-specific primer of ***blaKPC***: **Fw-** 5-ATGTCACTGTATCGCCGTC-3 **and Rev-**5-TTACTGCCCGTTGACGCC-3', ***bla NDM*** ' was **Fw-** 5-CCAGCTCGCACCGAATG-3 **Rev**-5 -AACGCCGCACCAAACG-3 ***qnrA*** was **Fw-** 5-CAAGAGGATTTCTACGCCAT-3 **Rev**-5- TCGGCAAAGGTC AGGTCACAGC-3 and ***acc*** was **Fw**-5-AGTACAGCATCGTGACCAACA-3 **Rev**-5-CTCGAATGCCTGGCGTGTTT-3 that used for the amplification of the *K. pneumoniae* target genes ... The reaction was performed in a 20 µl volume, 3 µl of a ready Master Mix, 2 µL of each primer, and 5 µL of DNA, while nuclease-free water was used to complete the volume. The PCR program included initial denaturation in one cycle for 5 min at 95°C, amplification in 35 cycles each of 30 sec. at 94°C, 30 sec. at 55°C, and 30 sec. at 72°C, followed by a final extension cycle for 7 min. at 72°C [8].

Data Analysis

The data were analyzed in SPSS software (version 16.0), and a p-value less than 0.05 was considered statistically significant.

3.Results and Discussion

The prevalence of hypermucoviscous among clinical specimens was diabetes foot ulcer and liver abscesses while classical *K. pneumoniae* present in urine ,wound and other samples .The diagnosis that based on microscopic examination and characteristic of the bacterial colonies, a total of 100 clinical specimens of clinical specimen's were cultured on selective medium CHROMagar Orientation the isolates of *K.pneumoniae* appeared as

mucoid metallic blue colonies as in figure (1) [2.3,9.10].

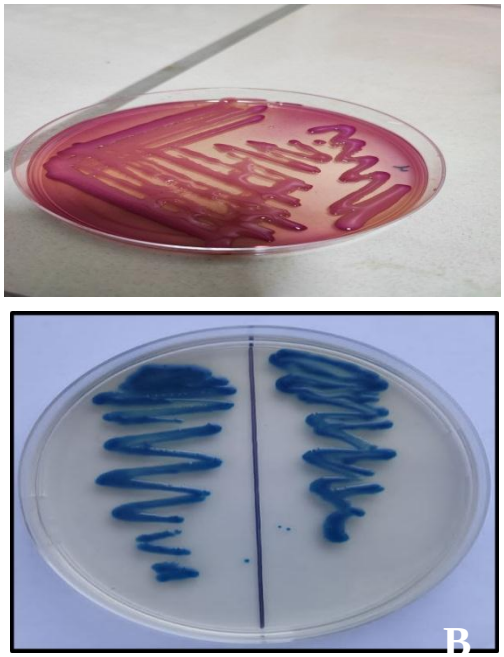

Figure (1): Mucoid colonies of *K. pneumoniae* on CHROMagar **(A)and MacConkey agar (B).**

The string test, figure (2) which uses an inoculation loop to generate a viscous string from a bacterial colony, has been used to evaluate for the hypermucoviscous phenotype**,** Many hypervirulent *K. pneumoniae* strains are hypermucoviscous due to increased or altered capsule production, which helps protect against phagocytosis[10,11.12].

Figure (2): String test for Hyperverlant *K. pneumoniae.*

Phenotypic detection Antibiogram of Hyperverlant *K. pneumoniae*

Regarded to *K. pneumoniae* showed a varied levels of resistance to; Ampicillin with percentage 100%, Piperacillin 40% and Nitrofurantion 55%; Norfloxacin 10%, Cefotaxime 10µg 50%, Cefotaxime 30µg 40%,Aztreonam 40% and Ticarcllin with percentage of 10 % figure (3)..

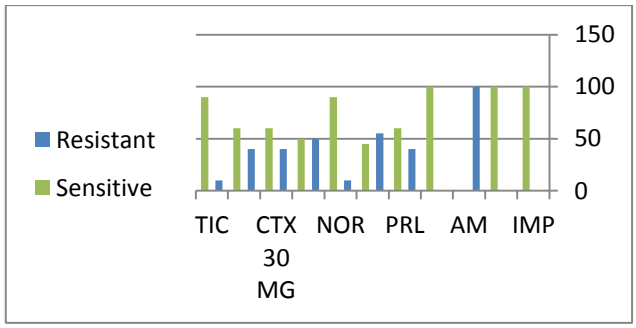

Figure (3): Susceptibility of isolates *K. pneumoniae* to antibiotics

This corresponds with the findings [13]. in which imepenam, cefaperazone + sulbactam, cefepime + tazobactam, and piperacilllin + tazobactam are reported as the most effective drugs against ESBL-producing gram-negative bacilli. A 53.7% of the bacteria were ESBL producers, with the highest production by *K. pneumoniae*. This is similar to other studies in which 44.7–57.4% are ESBL producers [14], but significantly lower than the 80 % ESBL formers found by [15,16.]. Resistance to β-lactam agents is most commonly mediated by constitutively expressed or inducible chromosomal β -lactamases or efflux pumps .[17,18.19]

Genotypic detection *Aac* , *NDM* , *QnrA* and *bla KPC* genes among *K. pneumoniae*

The results that obtained in this study showed that the presence of *aac* gene in *K. pneumoniae* isolates was (54.5%) as in Figure (4) , the *aac* gene were distrusted into one of the urine isolates , (4) isolates from burn samples , (7) from blood , (3) from diabetes (2) isolates from liver, CSF and semen , While other isolates do not contain this gene.

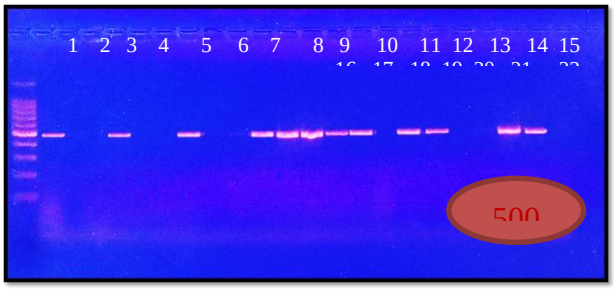

Figure (4-): PCR amplification products of *K. pneumoniae* isolates that amplified with *aac* gene primers with product 545 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1, 4,7, 10, 11, 12, 14, 16, 17, 20, 21) show positive results with the *aac* gene.

blaNDM in *K. pneumoniae* showed that (40.9%) , as in Figure (5) that presence *blaNDM* gene among isolates. The (3) isolate from urine samples, (5) isolate from burn sample , (8,9) isolates from blood samples , (10,13) isolates from Diabetes patients, (14) isolate from Liver samples and (17,18) isolates from CSF samples , While other isolates *blaNDM* gene was absence .

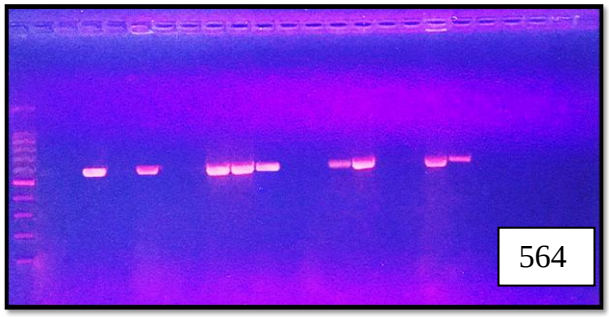

Figure (5): PCR amplification products of *K. pneumoniae* isolates that amplified with *NDM* gene primers with product 564 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (3,5 , 8, 9, 10, 13, 14, 17, 18) show positive results with the *NDM* gene.

The results that obtained in this study showed that the presence of *qnrA* in *K. pneumoniae* with (13.6%) as in Figure (6) ,the *qnrA* gene was presence in isolates (1,2) from urine samples. The isolate number (21) from diabetes patients. While other isolates do not contain on *qnrA* gene.

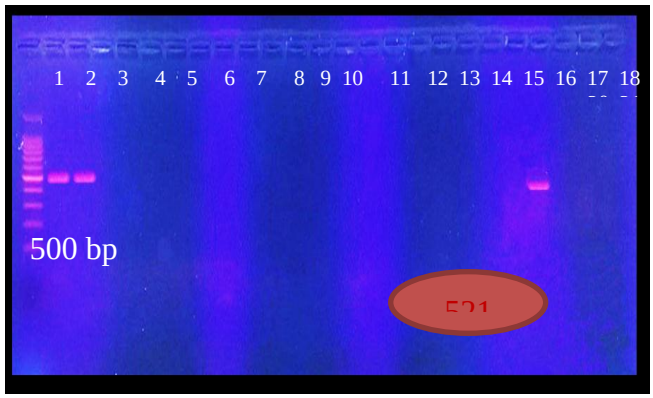

Figure (6): PCR amplification products of *K. pneumoniae* isolates that amplified with *qnrA* gene primers with product 521 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1, 2, 21) show positive results with the *qnrA* gene.

The results that obtained in this study showed that the *blaKPC* in *K. pneumoniae* (0%) , the number of isolated from (1-11) that is classical *K. Pneumoniae* and isolates from (12-22) that is hyperverlant *K. Pneumoniae*. Table (1) showed the distribution of genes among classical *K. Pneumoniae* and hyperverlant *K. Pneumoniae*.

In region of Iraq, there were clear differences regarding the presence of carbapenemases, as [20,21] studied the distribution of *bla*IMP, *bla*VIM and *bla*KPC among *K.pneumoniae* and the results were negative, while [22,23] were showed that the prevalence of VIM metallo ß-lactamase in 82.3% of clinical *K. pneumoniae* isolates in Hilla hospitals also this study were in compatible with different studies conducted by [24,25] in Iraq recorded the rates of the prevalence of those genes have been *bla*VIM 33.3and *bla*IMP 1.1[26.27] recorded the rates of prevalence of those genes have been *bla*VIM (5%) while whereas no amplicons were observed for *bla*IMP and *bla*KPC [28,29,30,31].

Table (1) percentage of *K. pneumoniae* genes in theses study

Table (4-7) percentage of *K. pneumoniae genes in theses study*

Gene	*K.pneumoniae hyprvelant*	*K.pneumoniae classical*
***Aac* (54.5%)**	31.82%	22.72%
***NDM* (40.9%)**	18.18%	22.72%
***Qnr A* (13.6%)**	4.54%	9.09%
***KPC* (0%)**	0%	0%

4.Conclusion

The antibiotics that are suggested in this study for proper and accurate management because a high prevalence of MDR *K. pneumoniae* infections, this will lead to decreasing the rate of mortality and morbidity. The development of antibiotics policies and regular surveillance of antimicrobial sensitivity patterns may lead to overcome the overuse of among pathogens.

Ethical approval

All subjects involved in this study were informed and the agreement will obtained verbally from each one before the collection of samples. All procedures performed in this study were in accordance with the ethical standards of the Kufa University/Iraq.

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