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ORIGINAL ARTICLE

Molecular Detection of *Temoniera* gene (*TEM*) and Aminoglycosides (*Aac)* gene among *K.Pneumoniae* isolates

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ABSTRACT
A total of 100 isolates of K.Pneumonia were collected from various clinical specimen's such as UTI were (35%) ,(29%) of diabetic foot isolates,(16%) of burnsisolates, (10%) of Liver isolates, , (5%) of Blood isolates, (3%) of Semen isolates and (2%) CSF isolates, which identified by Vitec-2 system. The first amplification of genes by PCR technique was done for the isolates of K. pneumoniae to detect antibiotic resistance included: the (TEM, andaac) genes, The genomic DNA of Pneumonia isolates was extracted by the boiling method and PCR by using specific primers to detect the antibiotic genes and visualized by gel documentation, The present study showed the TEM gene were (45.5%) and the aac gene showed(54.5%), of isolates of K. pneumoniae, among all isolates .
Keywords: Temoniera gene (TEM), Aminoglycosides (Aac), K.Pneumoniae, PCR

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INTRODUCTION

TEM-1 is the most important β-lactamase in Gram negative bacteria, Both *TEM-1* and *TEM-2* is capable of hydrolyzing penicillin and first-generation cephalosporin but are not able to invade oxyimino–cephalosporin. Amino acid replacement can be created in many places of *TEM-1*. β-lactamase in vitro without losing the most activity. These changes are responsible for phenotypic extended spectrum β-lactamases (ESBL) change and enzyme activity site that gives access to oxy Mino β-lactamase. More than 130 common *TEM* enzymes have been identified, which provide a useful method for the distribution of resistant genes [1]. *Escherichia coli* and *Klebsiella pneumoniae* are the main gram-negative opportunistic pathogens in nosocomial infections [2]. *K. pneumoniae* can cause clinical infections including respiratory tract infection, pneumonia, urinary tract infection, wound infection, and bacteremia (Killingbeck *et al*., 2020). Mutations in topoisomerase IV and DNA gyrase and overexpression of AcrAB efflux system are the main mechanisms of quinolone resistance in *E. coli and K. pneumoniae* isolates. Plasmid-mediated quinolone resistance (PMQR) mechanisms have also been reported [3, 4, 8].

MATERIAL AND METHODS

K. pneumoniae isolation and identification

A total number of 100 samples *K. pneumoniae* were collected from various clinical samples during the period from November,2023 to March, 2023. All isolates cultured on MacConkey Agar medium were identified according to some biochemical test [5]. Then all suspected isolate was streaked on CHROM agar Orientation medium (CHROMagar™, France) and finally, the diagnosis was confirmed using Vitek2 compact system (Biomeriux, France) using ID-GN cards [10].

Extraction of Genomic DNA

DNA of *K. pneumoniae* was separated by boiling method. Briefly, colonies were suspended in 100 microliters of sterile distilled water and boiled at 100^0C in the water bath for 15 minutes than rapidly cooled at -20^0C for one hour, then centrifugation and the supernatant were preserved for the used in the

amplification-processes [6, 7]. The concentration and purity of DNA can be determined by reading the optical density of a sample at 260 and 280 nm in spectrophotometry, 5 µl of each DNA sample were added to 995 µl. Of distilled water and mixed well. The reading at 260 nm. Allows calculation of the concentration of nucleic acid in the sample, For pure double-stranded DNA, 1 OD260 = 50 ug/ mL [8], The ratio between the readings at 260 and 280 nm (OD 260/OD 280) provides an estimate of the purity of the nucleic acid. Pure preparation of DNA has an OD 260/OD 280 value of 1.8, the concentration of DNA was calculated by the formula:

DNA concentration (µg/ml) = O.D 260nm × 50 × dilution factor

Detection of Antibiotic resistance genesby PCR

The DNA primers (from Alpha DNA company, Canada) were resuspended by dissolving the lyophilized product after spinning down briefly with TE buffer molecular grad depending on manufacturer instruction as stock suspension. Working primer tube was prepared by diluted with TE buffer molecular grad. The final picomoles depended on the procedure of each primer [11, 12].

Polymerase Chain Reaction Protocol

The primers of antibiotic resistance genes were TEM gene Fw-5-GATCTCAACAGCGGTAAG-3 Rev-5-CAGTGAGGCACCTATCTC-3 and Aac gene were Fw-5-AGTACAGCATCGTGACCAACA-3, Rev-5-CTCGAATGCCTGGCGTGTTT-3 by using PCR. The protocol was used depending on Promega Biosystem manufacturer's instruction. Single reaction (final reaction volume 20 µl) . All PCR components were assembled in PCR tube and mixed by refrigerated microcentrifuge at 50 rcf for 10 second [13, 14].

RESULTS AND DISCUSSION

Identification of *K. pneumoniae* isolates

The present study revealed that features of *K.pneumoniae* isolates were gram negative bacterium, encapsulated, Rod-shape, mucoid colonies and pink color on MacConkey agar while metallic blue color on CHROM agar Orientation medium at 370C for 24 h According to the results of characteristics of microscopic, colony morphological, biochemical tests and Vitek-2 system were revealed that 100 isolates were identified as *K. pneumoniae* [15, 16, 21]. .

Detection of *Temoniera* gene in *K. Pneumoniae (TEM)*

The result showed that the *TEM* resistance gene was detected in (45.5 %) of *K. Pneumoniae* isolates that positive and 54.5 % it is negative for *TEM gene* as in figure (1) , The positive result of *TEM* gene in 100 isolate that belong to different isolates , (4.5 %) Isolates of Urine , (13.64%) Isolate of Diabetes isolate, Isolate (9.09%) of Liver isolate ,(4.5 %) of CSF isolate and (13.64%) of Semen , While other isolated does not contain on *TEM* gene as shown Figure (1), ESBLs are one of the main leading causes of resistance to ß-lactam antibiotics among Gram-negative bacteria. These enzymes are plasmid-encoded β-lactamases that mediate resistance to penicillin's, first-, second- and third- generation cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime. TEM, SHV, and CTX-M are the major genetic groups of ESBLs amongst clinically important Gram-negative bacteria [17, 3, 1].

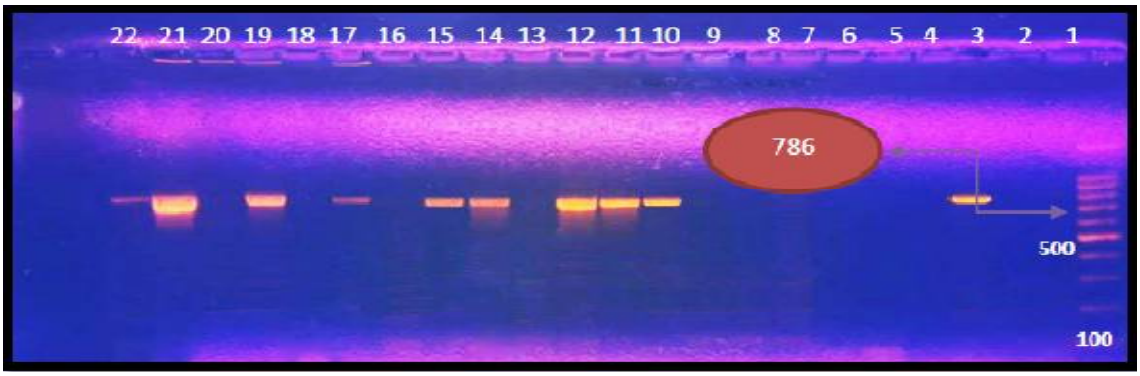

Figure (1): PCR amplification products of *K. pneumoniae* isolates that amplified with *TEM* gene primers with product 786 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1-22) show positive results with the *TEM* gene.

Aminoglycosides (*Aac)* gene

The result finding showed that the positive of Aac in *K. pneumoniae* with percentage (54.5%) and (45.5%) that negative result according sequences isolated that use in PCR , as show Figure (2), The positive result of *Aac* gene in 100 isolate that belong to different isolates , (4.54 %) Isolates duo to Urine

, (4.54 %) Isolate due to Burn isolate , (4.54 %) Isolate due to Blood isolate , (13.64%) Isolate duo to Diabetes isolate, Isolate (9.09%) duo to Liver isolate ,(9.09%) duo to CSF isolate and (9.09%) duo to Semen ,In this study, among 100 strains the resistant to aminoglycoside, genes encoding aac gene 54.5 % , The hypervirulent *K. Pneumoniae* that is encoding *Aac* gene more than classical *K. Pneumoniae* [5, 18].

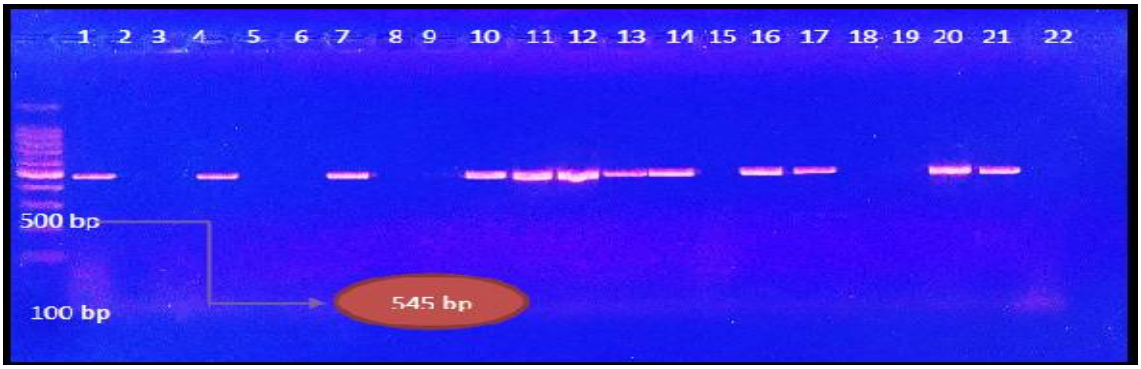

Figure (2): PCR amplification products of *K. pneumoniae* isolates that amplified with *aac* gene primers with product 545 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1, 4,7, 10, 11, 12, 14, 16, 17, 20, 21) show positive results with the *aac* gene.

CONCLUSION

The antimicrobial agents are choosing in this study for proper and accurate management. the high prevalence of MDR *K. pneumoniae* infections, will aid in decreasing the rate of mortality and morbidity. The development of antibiotic policies and regular surveillance of antimicrobial sensitivity patterns may aid to overcome the overuse of antibiotics that is the main cause of drug resistance development among pathogens.

Ethics

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Kufa University/ Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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