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Molecular Characterization of Efflux Pump Genes among Carbapenems Resistant *Klebsiella pneumoniae* Isolated from Burns Infections

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Abstract : The emergence of multi-drug resistance in carbapenem-resistant *Klebsiella pneumoniae* (CRKP) poses an increasing risk, particularly for individuals suffering from burn injuries. The efflux pump system in drug-resistant *K. pneumoniae* is dangerous. Consequently, the purpose of this study was to determine whether antibiotic resistance in *K. pneumoniae* isolated from wound patients is related to the *smvA*, *smvR*, and *cepA* efflux pump genes. Ninety clinical isolates of *K. pneumonia* bacteria were obtained from 250 burn patients (36%) who were consulted in the hospitals in Baghdad, Iraq, between August 2023 and March 2024. After identifying the isolates, the disc diffusion method was used for antibiotic susceptibility. The polymerase chain reaction (PCR) method was employed to investigate the frequency of efflux genes (*smvA*, *smvR*, and *cepA*). Additionally, online software (NCBI) was used to analyze the *cepA* gene's sequence. The results showed that Tigecyclin (87.77%) and Colistin (72.22%) had the highest percentage of antibiotic sensitivity against *K. pneumoniae*, while Meropenem (56.66%) and Imipenem (53.33%) had moderate sensitivity. On the other hand, Amoxicillin-clavulanic acid (94.44%) had the highest percentage of antibiotic resistance by *K. pneumoniae*, and 47 (52.2%) of the isolates were Multi-Drug Resistant (MDR) *K. pneumoniae*. The results of PCR revealed that the efflux pumps genes *smvA*, and *smvR* were present in all isolates, while *cepA* gene was identified in 72 isolates (80%). The carbapenem-resistant local isolate K85's *cepA* sequence was aligned with a gene from reference strains found in GenBank, yielding results that showed 99% identity and some variations in the query's nucleotides in the positions 97, 101, 307, 394, 580, 583,656,798 (delesion), and 922 of the subject. In conclusion, there was a high prevalence of *smv* efflux pump genes and present a variations among *cepA* gene among the carbapenem resistant local isolates from burn patients.

Key words: *Klebsiella pneumoniae*, Carbapenem-resistant, Efflux pump genes.

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Introduction

An important pathogen that causes pneumonia, burn infections, bacteremia, and urinary tract infections is *Klebsiella pneumoniae*. Globally, there has been an increase in the prevalence of carbapenem-resistant *K. pneumoniae* (CRKP), which makes antimicrobial treatment challenging and raises disease-related mortality rates. The only two options for treating bacterial infections that are resistant to multiple drugs are imipenem and meropenem. Treatment options are significantly more restricted if and when CRKP becomes resistant to other antibiotics (1, 2).

The indiscriminate use of antibiotics, particularly carbapenems (such as imipenem and meropenem), has led to

an increase in *Klebsiella pneumonia* resistance to these drugs. To counteract this, various strategies have been developed, including the efflux pump mechanism. Drugs can be selectively transported out of bacteria by membrane pumps, which are controlled by internal regulators. Antimicrobial medication has been extensively rendered inactive due to pumps overexpression, either for a single class of these drugs or for many more than two, particularly for clinical multidrug resistance (MDR) isolates (3). Many local studies indicated to the high emergence of multidrug resistant *Kleibsiella pneumoniae* strains in Baghdad Hospitals (4, 5).

Several genes related to disinfectant resistance, including SMR family members *smvA* and *smvR*, have been confirmed to be present in multidrug-resistant *K. pneumoniae*. *K. pneumoniae's* chromosomally encoded *cepA* gene, which provides protection against chlorhexidine, is linked to another mechanism of biocide tolerance. Disinfectant resistance, however, now poses a serious risk to public health and safety as well as the wise use of available resources. These genes are closely linked to *K. pneumoniae* strains that are less susceptible to antibiotics (6, 7).

In this study, carbapenem-resistant *Klebsiella pneumoniae* isolates were examined for the prevalence and molecular traits of efflux pump genes (*cepA*, *smvA*, and *smvR*). The *cepA* gene's sequence analysis was also presented.

Materials and Methods

Isolation and identification of *Klebsiella pneumoniae*

A total of 250 clinical samples as burn swabs were collected from August 2023 to March 2024 from patients who admitted in five of Baghdad hospitals, *Klebsiella pneumoniae* were identified using MacConkey agar, Blood agar, and CHROMagar. As directed by the manufacturer, these isolates were identified by biochemical tests using the VITEK 2 system (bioMerieux, France).

Antibiotic Susceptibility Test

The disc diffusion technique was used to assess antimicrobial susceptibility. After growing overnight on CHROMagar, *K. pneumoniae* was resuspended in regular saline. Mueller-Hinton agar (Oxoid) plates were inoculated with the suspension after its turbidity was adjusted to 0.5 McFarland. Amkacin (AK), Gentamicin (GEN), Imipenem (IPM), Meropenem (MEM), Levofloxacin (LEV), Ciprofloxacin (CIP), Tigecycline (TCG), Ceftriaxone (CTR), Amoxicillin-clavulanic acid (AMC), Colistin (CL), Piperacillin (PI), Cefipime (FEP), Trimethoprim/Sulfamethoxazole (SXT), and Cefoxitin (CX) were the antibiotic discs utilized in this investigation. After the agar plates were incubated at 37 °C for 24 hours, the inhibition zone was evaluated and interpreted using the proportion of susceptible, intermediate, or resistant isolates in accordance with CLSI breakpoint interpretative criteria (8).

Molecular study

All *K. pneumoniae* isolates which resistant to carbapenems had their bacterial DNA extracted using ready kits (Promega, USA). The purity of the isolated DNA was assessed using Thermo Scientific's NanoDropper 2000.

Primers and conditions of PCR

The specific primers for the *16S rRNA* gene and the *K. pneumoniae* efflux pump genes (*smvA*, *smvR*, and

cepA) are listed in Table (1) for the PCR assay.

Table (1): Oligonucleotide primers sequences of type 1 fimbriae genes used in this study.

Gene name	Primer name	Primer sequence 3'-5'	Product size(bp)	Reference
smvA	*smvA* (F)	CGCTGATGGCGATTTTGCTG	81	(9)
	smvA (R)	CGCTGAGGGTCGGCG		
smvR	*smvR* (F)	ATGATCGGCAGCGAGGATG	101	This study
	smvR (R)	AGGTAGGCCGCTTTGATGTC		
cepA	*cepA*(F)	CAACTCCTTCGCCTATCCCG	1051	(10)
	cepA(R)	TCAGGTCAGACCAAACGGCG		
16S rRNA	*16S* (F)	GACGATCCCTAGCTGGTCTG	95	(11)
	16S (R)	GTGCAATATTCCCCACTGCT		

For each gene, 10 µl of Go Taq® green master mix 2X (Promega, USA) and 1 µl of each primer (10 pmol/µL) are added to 20 µL of PCR reaction to amp up 2 µl of DNA template. The final volume is 20 µl with nuclease-free water. To aid in the contents sinking to the bottom of the tubes, the PCR premix, extracted DNA, and primers are rapidly centrifuged after being defrosted at 4°C and vortexed. The polymerase chain reaction was optimized following several trials. All the elements of the negative control were there, but D.W. was used in place of the DNA, and the PCR programs were run on a thermal cycler.

Table (2): Conditions of the uniplex PCR reaction for the identification of *16S rRNA* and efflux pump genes.

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	58, 60, and 62	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

58(*16S rRNA*), 60 (*smvA*, *smvR*), and 62(cepA).

Agarose gel electrophoresis was used to verify the existence of amplification following PCR amplification. When it came to the extracted DNA requirements, PCR was totally reliable. After taping the two edges of the gel tray shut with cellophane, the agarose solution was added, and the mixture was allowed to solidify for half an hour at room temperature. The gel was put in the gel tray after the comb was carefully taken out. 1X TAE-electrophoresis buffer was added to the tray until it covered the gel PCR products by 3 to 5 mm. The gel products were then loaded directly onto the tray. 5µl of the PCR product was put straight into the well. Power was turned on for 60 minutes at 100 volts per milliampere. DNA travels from the plus anode to the cathode

poles. The Gel imaging system was used to visualize the gel's Ethidium bromide-stained bands.

Sequence analysis of *cepA* gene

Four *K. pneumoniae* local isolates with minimal inhibitory concentrations (MICs) (≥ 16µg/ml) resistant to imipenem and meropenem antibiotics were used to sequence the nucleotide sequence of the *cepA* genes (≥ 16µg/ml) and chlorohexidine resistance (also one isolate that was sensitive to carbapenems and biocides was selected for comparison). Agarose gel electrophoresis was used to identify the PCR products for the *cepA* genes of the five isolates, and the Applied Biosystem (AB) capillary system (Macrogen Research, Seoul, Korea) was utilized for sequencing. Direct sequencing was applied to the PCR products, and an automatic sequencer was used to sequence both strands of the product. DNA sequences were analyzed and similarity searches were conducted using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov). We obtained the nucleotide sequences of the *cepA* genes of the reference strains of *K. pneumoniae* from the public database GenBank. These strains have been reported from different parts of the world. The phylogenetic analysis and alignment were conducted using the NCBI tools.

Results and Discussion

Isolation and Identification of *K. pneumoniae*

Following the morphology identification of the isolates using Gram's staining, cultural characteristics, and biochemical traits, the isolates were identified (12).

All specimens were cultured for 24 hours at 37° C on HiCromeTM Agar, MacConkey agar, and Blood agar plates. The isolates that were obtained from these media were identified based on the characters that were observed.

Urinary tract infections were isolated specifically using HICrome agar Orientation medium. Figure (4-1, A) illustrates how *Klebsiella* isolates looked as metallic blue colonies on HICrome agar after 24 hours at 37°C. These bacteria have mucoid structures and bright pink colonies when they grow on MacConkey agar, two traits that set them apart from other bacteria (Figure 4-1, B).

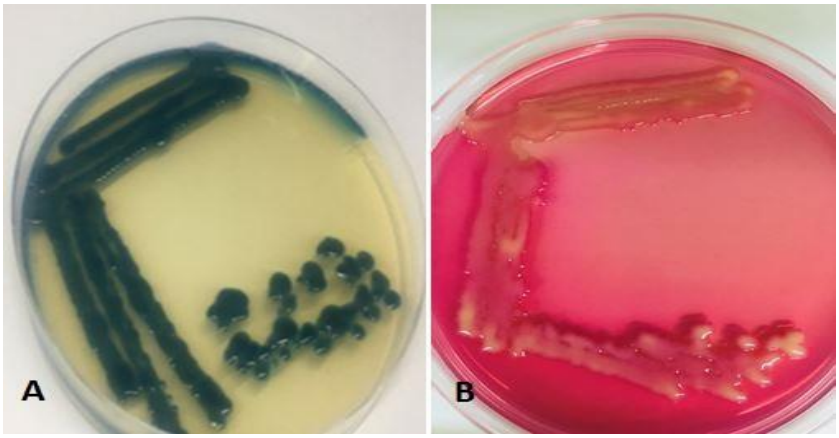

Figure (4-1): (A) *Klebsiella* mucoid colonies on CHROM agar Orientation medium agar (B) Colonies of *K. pneumoniae* on MacConkey agar medium at 37˚C for 24 h.

Gram negative pathogens were specifically isolated using the HICrome agar Orientation medium. *Klebsiella* isolates appeared as metallic blue

colonies on CHROMagar after being cultured at 37°C for 24 hours. This medium is also selective for other Gram-negative pathogens, with distinct colors for each bacterial genus; *Escherichia coli* colonies were pink red on CHROMagar.

Because it is simpler to distinguish between distinct colonies on chromogenic agar, these media are dependable for the detection of aerobic Gram-negative bacteria. Because of its great accuracy, quick identification, and extremely low false positive rates, CHROM agar Orientation medium is the recommended option (13). When compared to Blood agar and MacConkey agar, CHROM agar Orientation medium significantly reduces the workload in the microbiology laboratory and can be used as a cost-effective substitute for conventional urine culture methods. It should be taken into consideration as an alternative to conventional culture methods for uropathogen detection and reporting (14). By cultivating on blood agar medium, all suspected *Klebsiella* colonies were found to be large, shiny, mucoid, round, whitish-grey, and no hemolysis.

Identification of *Klebsiella pneumoniae* bacteria by VITEK 2

Klebsiella species were identified using the VITEK 2 system which has a 99% accuracy rate in diagnosing bacteria. Ninety (36%) *K. pnemoniae* isolates were found after identification, whereas 93 isolates were found after biochemical testing. Many factors affect the results achieved by automated biochemical identification system: the age of the culture, saline diluents concentration, medium, cell suspension density, the database, pH and algorithm of the machine. So, VITEK 2 was used to identify the isolates with high-resolution.

Distribution of samples among Patients

This study was conducted among patients with burns infections attending 5 of Baghdad hospitals. A total of 250 burn swabs samples were collected from patients. Only 171 samples showed significant growth. Ninety of positive cultures were identified as *K. pneumoniae* (36%). Most of the patients were of the males 54/90 (60%), while the percentage of the females was 36/76 (40 %).

Global sepsis and mortality rates from burn wound infections have unexpectedly increased due to the emergence of multidrug-resistant (MDR) bacteria. *Acinetobacter baumannii* and *Klebsiella pneumoniae* are known to be among the most common bacterial pathogens linked to multi-drug resistant wound infections (15). The prior local study, which comprised 250 clinical specimens (sputum, urine, and swabs from burns and wounds), was sourced from private laboratories in Baqubah and Diyala, Iraq, as well as the Al-Batool and Baqubah Teaching Hospitals. *E. coli* (19.2% and 8.8%) and *K. pneumoniae* (23.2 and 12%) are the two types of pathogenic bacteria that are more common in nosocomial and community-acquired infections than other pathogenic bacteria (16).

Antibiotic Susceptibility of *Klebsiella Pneumoniae* isolates

A test for antimicrobial susceptibility was conducted on all 90 *K. pneumoniae* isolates to 14 antibiotics, as indicated by the disc diffusion method (Table (4-1)).

Table (4-1): The percentages of 90 *K. pneumoniae* isolates' susceptibility to 14 antimicrobial agents.

Antibiotics	Resistance (No.(%))	Intermediate (No.(%))	Sensitive (No.(%))	P-value
MEM	38 (42.22)	1 (1.11)	51 (56.66)	0.0001**
IPM	40 (44.44)	2 (2.22)	48 (53.33)	0.0001**
PI	75 (83.33)	5 (5.55)	10 (11.11)	0.0001**
LEV	33 (36.66)	4 (4.44)	53 (58.88)	0.0001**
CIP	52 (57.77)	3 (3.33)	35 (38.88)	0.0001**
AK	36 (40.00)	2 (2.22)	52 (57.77)	0.0001**
GEN	54 (60.00)	3 (3.33)	33 (36.66)	0.0001**
CL	20 (22.22)	5 (5.55)	65 (72.22)	0.0001**
SXT	41 (45.55)	0 (0.00)	49 (54.44)	0.0001**
CTR	81 (90.00)	2 (2.22)	7 (7.77)	0.0001**
FEP	82 (91.11)	2 (2.22)	6 (6.66)	0.0001**
CX	80 (88.88)	0 (0.0)	10 (11.11)	0.0001**
AMC	85 (94.44)	3 (3.33)	2 (2.22)	0.0001**
TCG	10 (11.11)	1 (1.11)	79 (87.77)	0.0001**
P-value	0.0001**	**0.0927**NS	0.0001**	**----**
**(P≤0.01)				

Amkacin (AK), Gentamicin (GEN), Imipenem (IPM), Meropenem (MEM), Levofloxacin (LEV), Ciprofloxacin (CIP), Tigecycline (TCG), Ceftriaxone (CTR), Amoxicillin-clavulanic acid (AMC), Colistin (CL), Piperacillin (PI), Cefipime (FEP), Trimethoprim / Sulfamethoxazole (SXT) and Cefoxitin (CX).

According to the present findings, the most antibiotics that were found to be effective against *K. pneumoniae* were Tigecyclin (87.77%) and Colistin (72.22%). Levofloxacin (58.88%), Meropenem (56.66%), Imipenem (53.33%), and Amikacin (57.77%), as well as Trimethoprim/Sulfamethoxazole (54.44%), were found to be moderately effective against the bacteria. The most isolates that were found to be resistant to Amoxicillin-clavulanic acid (94.44%), Cefipime, Cefoxitin, Ceftriaxone, and Piperacillin. Of the 90 isolates of *K. pneumoniae*, 47 (52.2%) were resistant to more than three classes of selected antibiotics; these isolates were known as multi-drug resistant (MDR) *K. pneumoniae*. The antibiogram results demonstrated significant resistance to the majority of the antibiotics used in this study. Forty isolates (16%) were identified as *K. pneumoniae* by the Vitek2 system, according to a local study that included 250 clinical specimens in the form of burn swabs from inpatients with burn infections admitted to four hospitals in Baghdad. The majority of *K. pneumoniae* isolates showed high resistance to Erythromycin (100%) and Ceftazidime (85%), while the clinical isolates of the bacteria showed moderate resistance to most of the antibiotics tested, with the lowest percentages of resistance being observed for Imipenem (25%) and Meropenem (38%). Furthermore, it was evident that there was resistance to ceftriaxone, cefepime, and cefotaxime (17).

A prior study comprising 100 isolates collected from various wards of Azerbaijan state hospitals in Iran between 2019 and 2020 showed that ampicillin exhibited the highest antibiotic resistance (96%), while tigecycline showed the highest susceptibility (9%). Additionally, 85% of the isolates were multidrug resistant, and the most frequently detected ESBL gene in the tested isolates was *blaSHV-*

1, which accounted for 58% of the isolates, followed by *blaCTXM-15* (55%) and *blaSHV-11* (42%) (18). The US Centers for Disease Control and Prevention, the UK Department of Health, and the World Health Organization have all classified *K. pneumoniae* as a serious health risk among these multidrug resistant (MDR) bacteria. Infections with *K. pneumoniae* are especially problematic for newborns, burn patients, the elderly, and people with compromised immune systems in the hospital setting, but they are also a major cause of community-acquired infections, such as sepsis and pneumonia (19).

Between September and November of 2016, a study was carried out at the Burn Unit of the Cipto Mangunkusumo Hospital (RSCM). The results demonstrated that *Acinetobacter baumannii* (11%) was the most common isolate, followed by *Pseudomonas aeruginosa* (12%) and *Klebsiella pneumonia* (17%). Ten antimicrobials showed high resistance, especially cephalosporins. The three bacteria only responded well to tigecycline, carbapenem, and aminoglycosides. The majority of bacterial isolates exhibit multi-drug resistance and high resistance to ceftriaxone, the empirical therapy used. The chosen empirical treatment consisted of a combination of aminoglycosides (amikacin) and carbapenem (imipenem, meropenem, and doripenem) (20).

The investigation of *K. pneumoniae* isolates from Iraqi hospitals in Baghdad. 45 *K. pneumoniae* isolates were found to be strong biofilm producers (25/55.5%) and 35 (77.7%) to be multi-drug resistant (MDR). It was discovered that 100% of the K. pneumoniae isolates were resistant to ampicillin and cephalexin, and that these isolates also showed some degree of resistance to imipenem, tigecycline, and meropenem (21).

Molecular identification of *K. pneumoniae*

For every extracted DNA sample, a polymerase chain reaction has been performed to identify the presence of the *K. pneumoniae 16S rRNA* gene (95 bp), a gene used for *K. pneumoniae* identification. The PCR products were validated through band analysis using gel electrophoresis and molecular size comparison with a 100 bp DNA ladder. The *16S rRNA* gene PCR reaction results are displayed in Figure (4-6). Ninety clinical samples were found to contain *K. pneumoniae*, confirming the VITEK2 system's results.

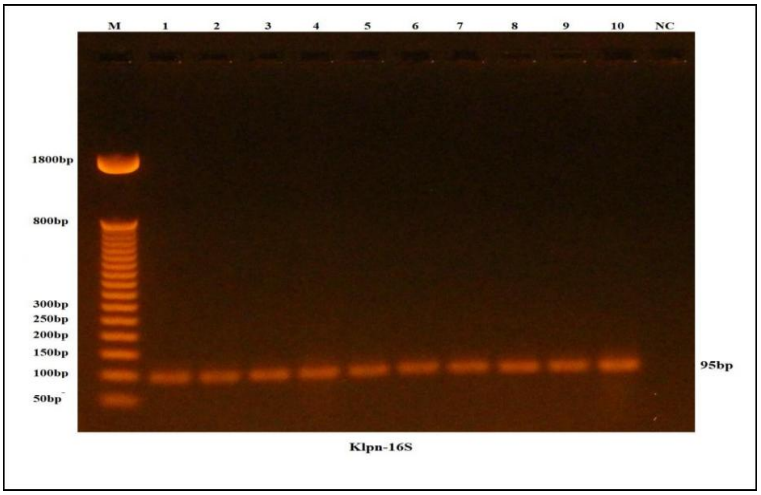

Figure:(4-6): Agarose gel electrophoresis of PCR products of *16S rRNA* gene (95bp) in 10 selected isolates of *Klebsiella pneumoniae.* Lane L :100 bp DNA ladder, lane N : negative control ,Lanes (1-10) PCR produt for *16S rRNA* . (80 V for 120 min).

Molecular Detection of Efflux pumps genes

To find the efflux pump genes, look for *cepA* (1051 bp), *smvA* (81 bp), and *smvR* (101 bp).The PCR products were validated through band analysis using gel electrophoresis and molecular size comparison with a 100 bp DNA ladder. The *cepA* gene (1051 bp) PCR reaction results are displayed in Figure (4-7). Out of the 90 clinical samples that were found to be *K. pneumoniae*, 72 isolates (80%) had this gene.

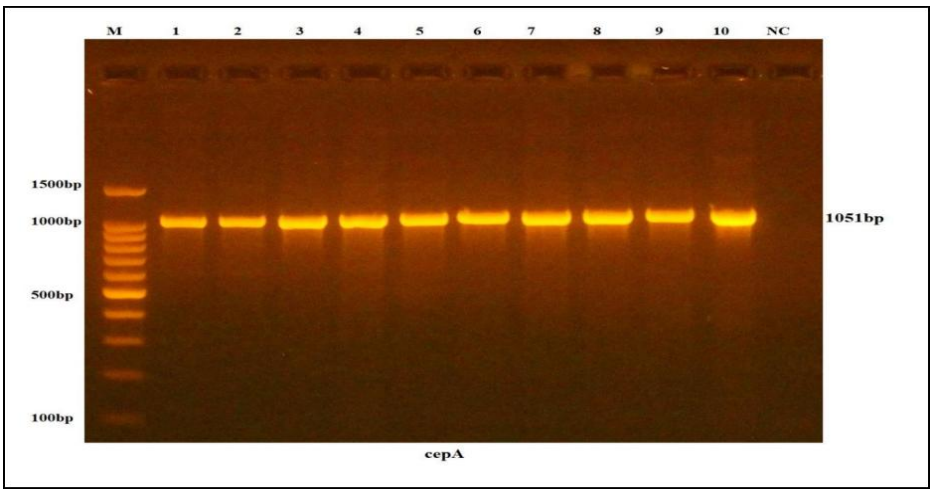

Figure (4-7) Agarose gel electrophoresis of PCR products of *cepA* gene (1051bp) in 10 selected isolates of *Klebsiella pneumoniae.* Lane L :100 bp DNA ladder, ,Lanes (1-10) PCR produt for , *cepA* . (80 V for 120 min).

Based on the *smvA* gene PCR reaction results displayed in Figure (4-8), it was determined that 90% of the 90 clinical samples contained the *K. pneumoniae* gene. Additionally, 90 clinical samples were identified as *K. pneumoniae* by the PCR reaction for *smvR* genes shown in Figure (4-9), which supported the findings from the VITEK2 system.

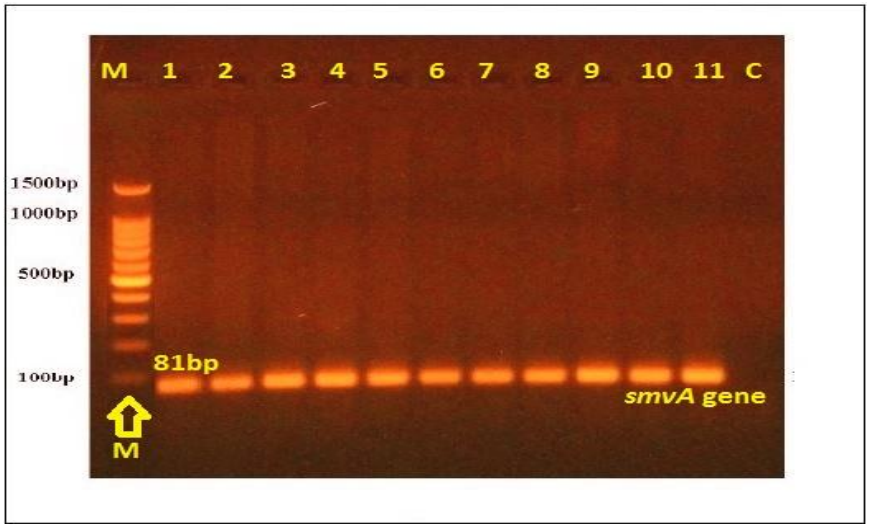

Figure (4-8) Agarose gel electrophoresis of PCR products of *smvA* gene (81bp) in 15 selected isolates of *Klebsiella pneumoniae.* Lane L :100 bp DNA ladder, ,Lanes (1-10) PCR produt for *smvA* . (80 V for 120 min).

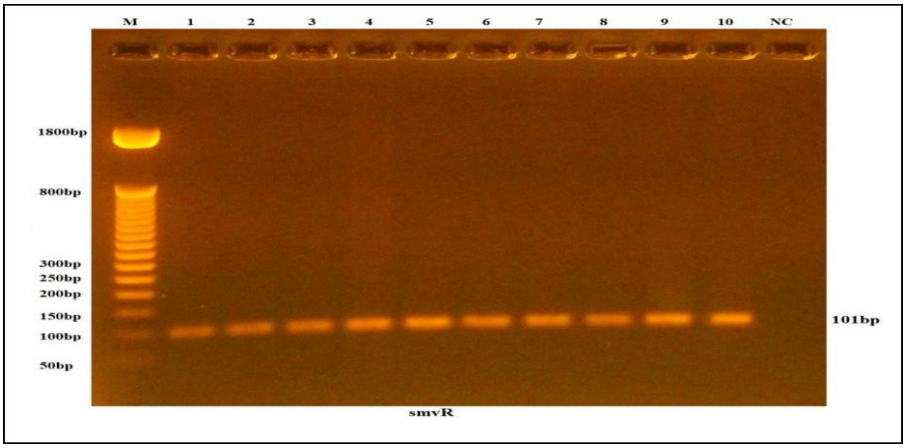

Figure (4-9) Agarose gel electrophoresis of PCR products of *smvR* gene (101bp), in 10 selected isolates of *Klebsiella pneumoniae.* Lane L :100 bp DNA ladder, ,Lanes (1-10) PCR produt for *smvR* . (80 V for 120 min).

The PCR detection of efflux pump gene in *K. pneumoniae* isolates revealed that the efflux pumps gene *cepA* (1051bp) was found in 80% of the isolates, while other genes *smvA* (81bp), and *smvR* (101bp) were present in all isolates (100%).

SmvA is an efflux pump of the Major Facilitator Superfamily, a membrane transport protein, that is chromosomally encoded. Chlorhexidine tolerance has been linked to increased expression of *SmvA*, which is a result of deletions in the regulator *SmvR* (22). Wand *et al.* (2019)(9), study revealed that Enterobacterales lacking SmvR were less susceptible to chlorhexidine , whille strains harboring *smvA* showed a two-fold increase in susceptibility to hexadecylpyridinium chloride monohydrate, cetrimide, and chlorhexidine.

The *cepA* gene was found in 40 isolates (71.4%) of the 56 strains of *K. pneumoniae* that were isolated from blood specimens from patients in intensive care units at Suez Canal University Hospital in Ismailia, Egypt. The study's findings suggest that the presence of the efflux pump gene *cepA*

influences the activity of chlorhexidine on *K. pneumoniae* infections associated with intensive care units (22). Enterobacter's transcriptional regulator SmvR functioned as a repressor of *smvA* expression, which encodes an efflux pump belonging to the MFS family. On the other hand, the resistance to CHX seen in certain clinical isolates might be the result of *smvA* gene. The *smvRA* locus seems to be a significant opportunistic trait even though it did not appear to be connected to the antimicrobial resistance of ECC. To prevent the formation and spread of virulent isolates, it is crucial to characterize and investigate the mechanisms by which this pathogen responds to biocides, particularly in a hospital setting (23).

Sequencing of PCR products (efflux pump genes)

Applied Biosystem (AB) capillary system (Macrogen Research, Seoul, Korea) was used to sequence the PCR products for the *cepA* (1051) genes. Direct sequencing was applied to the PCR products, and an automatic sequencer was used to sequence both strands of the product. The National Center for Biotechnology Information (NCBI) website's Basic Local Alignment Search Tool (BLAST) was used to analyze DNA sequences and perform similarity searches (http://www.ncbi.nlm.nih.gov).

The accuracy of the PCR-identified *cepA* genes was verified by comparing the obtained sequences to reference strains in GenBank. Additionally, these sequences were examined to determine whether any variations (differences in nucleotides) were present in these genes.

The outcomes of aligning the carbapenem-resistant local isolate K85's *cepA* sequence with the *K. pneumoniae cepA* gene (Accession no. GenBank's AB073019.1) (24) indicated 99% identity and the existence of some variations in the query's nucleotides in the positions 97, 101, 307, 394, 580, 583,656,798 (delesion), and 922 of the subject as shown in the Figure (4-10).

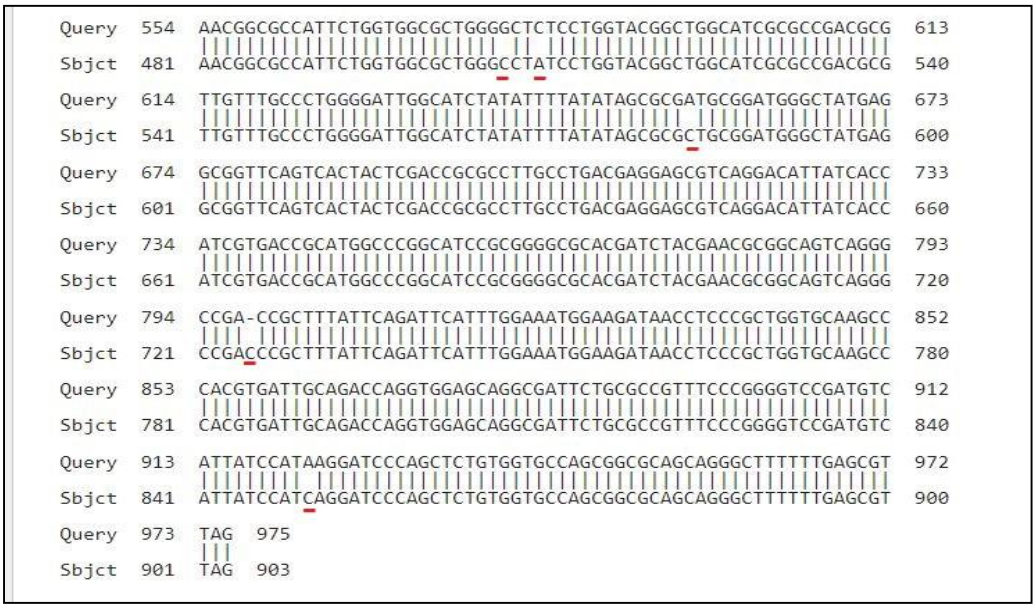

Figure (4-10): Alignment of carbapenems-resistant *K. pneumoniae cepA* gene sequence from this study with gene *cepA* from *K. pneumoniae* (Accession no. AB073019.1) available in GenBank.

The results of alignment of the local isolate which exhibited sensitivity to the carbapenems antibiotics revealed 100% identity with the reference strain from NCBI with accession number NC_022082.1 (*K. pneumoniae* JM54).

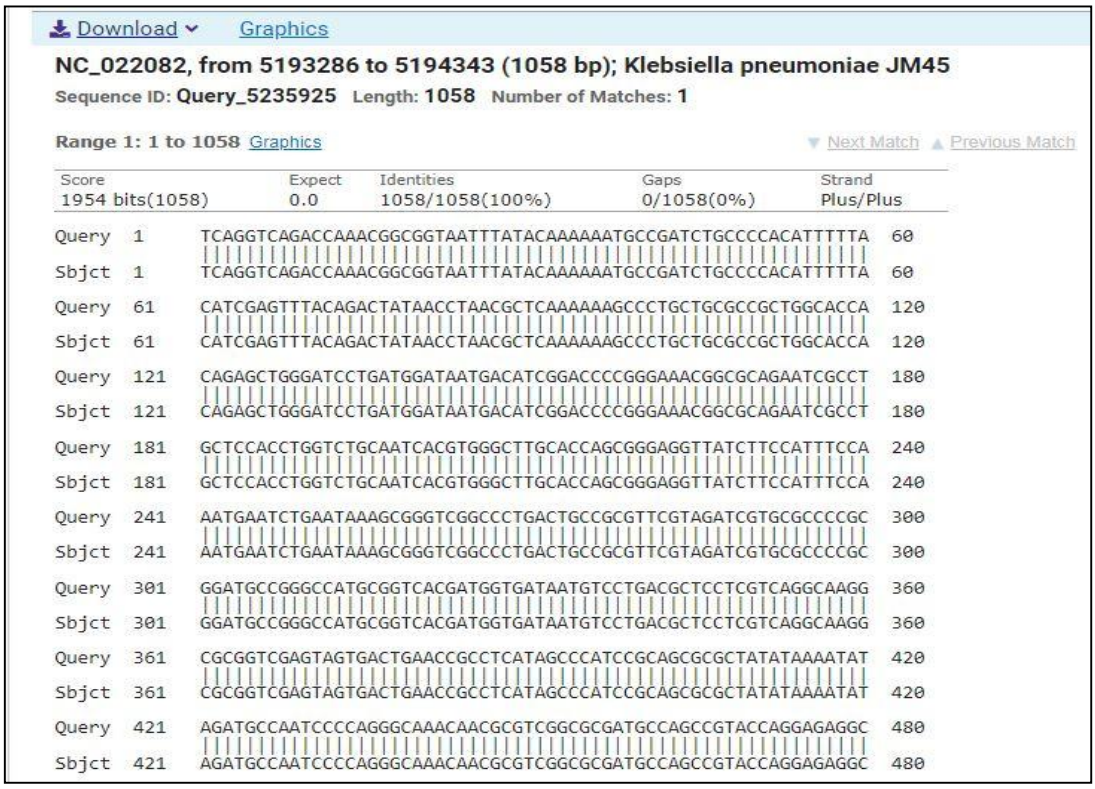

Figure (4-10): Alignment of carbapenems-sensitive *K. pneumoniae cepA* gene sequence from this study with gene *cepA* from *K. pneumoniae* (Accession no. NC_022082.1**) available in GenBank**.

In the current study, the isolate *K. pneumoniae* exhibited high resistance to carbapenems and biocides. Although the exact mechanisms underlying Gram-negative bacteria's resistance to chlorhexidine remain unknown, *K. pneumoniae* has shown evidence of a correlation between the *cepA* gene and resistance to chlorhexidine. With 88% of the isolates having the *cepA* gene, it was far more common than the *qacEdelta1* gene. The findings demonstrate that *cepA* expression rises with MIC. A significant portion of the *K. pneumoniae* population may be resistant due to the nearly universal carriage of the *cepA* gene, whose expression is correlated with the chlorhexidine minimum inhibitory concentration (MIC) (10, 22).

NCTC7427, a ST86 strain (a hypervirulent *K. pneumoniae* strain) with inactive AcrAB-TolC, was characterized by Wand *et al.* (2022)(25). The strain had regulators for SmvA, oqxAB, and CepA DNA sequences, but its susceptibility to triclosan, chlorhexidine, and benzalkonium chloride increased by a factor of more than four. Previous study demonstrated that the rates of carbapenem resistance among the 74 CRKP isolates were high and 64.9% (48/74) and 93.2% (69/74) of the 74 isolates tested positive for *cepA* and *qacEΔ1*, respectively. Only 4.1% (3/74) of the isolates had no genes related to disinfectant resistance, while 46 isolates (62.2%) had both *QacEΔ1* and *cepA* detected concurrently. The study revealed that CRKP isolates had high frequencies of *qacEΔ1* and *cepA*, as well as high rates of resistance to the majority of antibiotics (7). Figure 3, showed the phylogenetic relationships (by using NCBI tools) for *cepA* gene of the resistant isolate with other *cepA* gene sequences from NCBI with identity 99%.

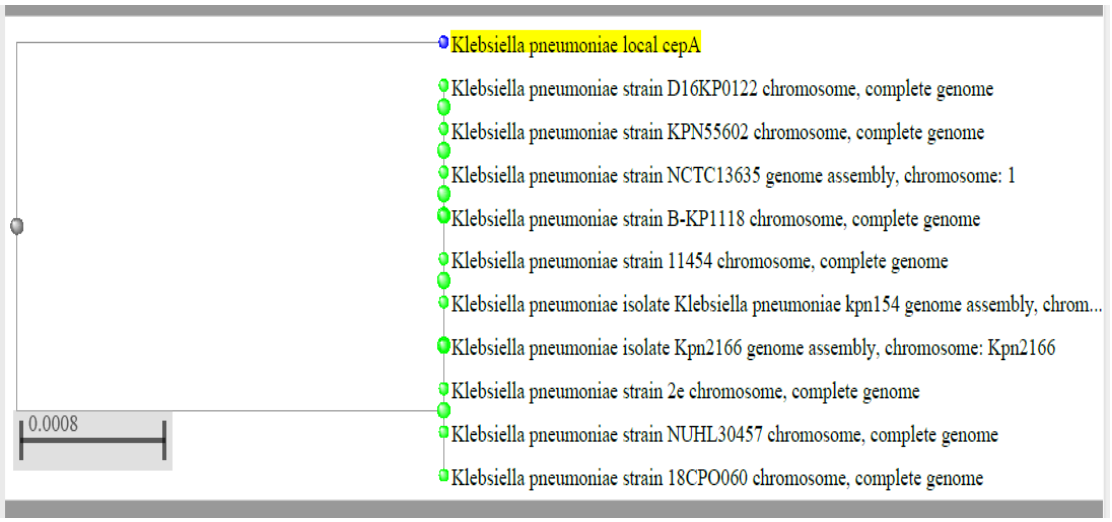

Figure (5): Phylogenetic relationships based on complete nucleotide sequence of the *cepA* gene carbapenems-resistant local isolates of *K. pneumoniae* (K85) using NCBI tools..

Wand *et al.* (2015) (9) found that a *blaSHV* gene caused penicillin resistance in several Murray isolates and that a short (9- or 18-bp) insertion in the *cepA* (*fieF*) gene was linked to high susceptibility to antiseptics, especially chlorhexidine. The disinfectant resistance genes *cepA*, *qacE*, and *qac E* were searched for in the Murray isolates in order to comprehend antiseptic resistance. A closer look at the *cepA* sequences showed that multiple isolates had either 9- or 18-bp insertions. All isolates tested positive for the *cepA* gene, but none of them had *qacE*.

The previous findings demonstrated a strong correlation between the *qacED1* and *cepA* genes and rising antimicrobial resistance in some CRKP strains, including piperacillin, ciprofloxacin, and levofloxacin. Although there is no concrete evidence to support this theory, the widespread presence of cepA genes in CRKP and their association with resistance to carbapenems and antiseptics raise the possibility that strains that are resistant to antibiotics may be chosen for by disinfectants. Mutations in the efflux pump genes may be the cause of the connection between the presence and expression of disinfectant genes and their resistance to bacteria (7, 26).

Conclusion

The most of the local isolates from burn patients were multi-drug resistant with high resistance to cephalosporins. The findings revealed a predominant of efflux pump genes *smvA*, *smvR*, and *cepA* among the local isolates. The sequence analysis of *cepA* gene indicated to the present of different variations among *cepA* gene among the carbapenem resistant local isolates from burn patients.

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