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Isolation and identification of *Klebsiella pneumonia* from wound infections and study of the synergistic effect of carpenems and cefepime

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***Abstract*— Surgical site infection (SSI) is a major source of morbidity and mortality in developing countries despite recent advances in sterile techniques. We studied antibiotics through their effect on bacterial pathogens isolated from wound infections singly and synergistically, which were observed in hospitals in Dhi Qar Governorate. These bacteria, *Klebsiella pneumoniae* 300 clinical samples were collected from Al-Hussein Teaching, Nasiriya Teaching and Al-Haboubi Teaching Hospitals in Dhi Qar Governorate for the period from September - December 2023. The results showed the presence of 16 bacterial isolates belonging to *K. Pneumonia*. Statistical and descriptive analysis was performed using chi-square. The rates of antibiotic resistance varied through the use of 4 types of carpenems and one type of cephalosporins. The resistance rate to meropenem alone was 6 (37.5%), while the resistance rate was higher for meropenem synergistically with Imipenem, Ertapenem, Doripenem, and cefepime, 7 (43.8%), respectively. In case of synergy the resistance rate was as to Imipenem, 7 (43.8) alone, while in synergy, the resistance rate was lower for Imipenem with cefepime, Ertapenem, and Doripenem, 5 (29.4%), 5 (31.3%), 6 (37.5%), respectively the resistance rate for synergy was highest than cefepime which was 11 (68.8) alone, while synergistically the resistance rate was lower for cefepime with Ertapenem and Doripenem 7 (38.9%), 7 (43.8%) respectively. While resistance to Ertapenem was higher than Doripenem 9 (56.3) and 7 (43.8), respectively, alone and synergistically, the resistance rate was lower for Ertapenem with Doripenem 5 (31.3%). This study revealed that taking two synergistic treatments is better than taking one treatment to combat *Klebsiella pneumoniae*.**

***Keywords*— wound infections, *Klebsiella pneumonia*, Antibiotic synergistic.**

I. INTRODUCTION

The genus *Klebsiella* belongs to the *Enterobacteriaceae* family is considered a pathogen, opportunistic means sometimes a type of natural flora is endemic to the intestine and other places in the body, and it seizes the opportunity of her presence here to take advantage of the weakness of the body in certain special cases during infection with other diseases. So, it can be isolated from different infections [1].*Klebsiella pneumoniae* is a pathogenic Gram-negative bacterium, in MacConkey agar medium the polysaccharide capsule aligned with the bacterial outer membrane appear a mucoid phenotype that ferments lactose. Interest in these bacteria has increased due to the raise in the percentage of infections caused by them, and due to its high resistance to antibiotics, including broad-spectrum antibiotics. This bacterium is considered one of the most antibiotic-resistant strains of Enterobacteriaceae and it is a good host for resistant plasmids [2]. .*Klebsiella pneumoniae* has many resistance and virulence loci that code for Siderophores, capsules, lipopolysaccharides, pili efflux pumps, enzymes, and adhesions that may contribute to its successful adaptation to the hospital environment. This made it the main pathogen responsible for injuries occurring in hospitals [3] The dramatic increase in the occurrences of multidrug-resistant (MDR) and extremely drug-resistant (XDR) infections belonging to the *Enterobacteriaceae* family is a huge economic issue since these organisms are widespread natural inhabitants of human and animal micro biomes, such as *K. pneumonia* [4]. Carbapenems are the final therapeutic choice for treating infections caused by MDR Enterobacteriaceae. In spite of this, the overuse of carbapenems has resulted in the evolution of several resistance mechanisms and diminished their efficacy [5]. *K. pneumonia* resistance to carbapenems represents a major challenge to the global delivery of health services [6].The virulence factors of *K pneumoniae* are encoded by genes across its (core) chromosomal gene loci and accessory genomes. The accessory genome is essential for *K. pneumoniae* antibiotic resistance, such as Carbapenemases β-lactamase *Klebsiella pneumonia* emergence of antimicrobial resistance isolates are a significant global issue in human medicine because they enhance the likelihood that antibiotics may fail to cure people. Community-acquired and nosocomial infections brought on by multidrug-resistant bacteria are challenging to treat with current treatments [7].

II. METHODS

A. Isolation & diagnosis of Klebsiella spp

1) Collection of specimens

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During the period extending from September 2023 to December 2023, 300 samples were collected from surgical patients from Al-Hussein Teaching Hospital. Nasiriya General Hospital and Al-Haboubi Teaching Hospital in Dhi Qar. The samples were transferred using preservation medium sterile transport medium swab.

2) Isolation & diagnosis of Klebsiella spp

The bacterial isolate was diagnosed on the basis of microscopic and bacteriological characteristics and biochemical tests according to [8,9]. Initial identification of *K. pneumoniae* isolates based on morphological characteristics of colonies on MacConkey agar K. pneumoniae isolates appeared mucous, large, and pink in color on MacConkey agar due to lactose fermentation, as shown in Figure 1. All isolates were identified by microscopic examination as well as using IMVC chemical tests for Indole and methyl red. The results were negative, while the results using Voges-Proskauer and citrate were positive. The VITEK 2 system has also been used to verify the diagnosis of *Klebsiella* at the species level and to prevent discrepancies in biochemical test results. Gram-negative bacteria are short or double-stranded bacilli *K. pneumoniae* [10].

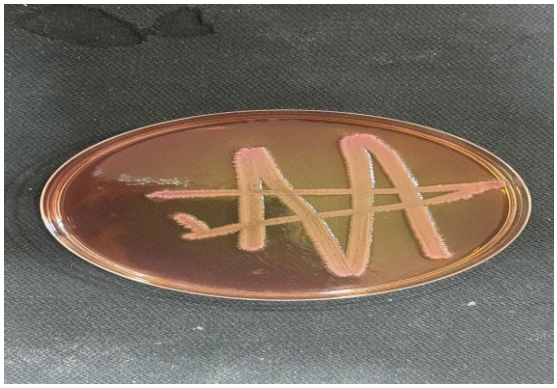

Fig. 1-:*Klebsiella pneumoniae* colonies on MacConkey agar

3) Antibiotic susceptibility& synergistic effect of Klebsiella pneumoniae isolates

All *K. pneumoniae* isolates used in this study were subjected to susceptibility testing with five different antibiotics (carbapenems and cephalosporins) (according to CLSI 2022) as shown in Figure (2) using the Kirby-Bauer method For individual and synergistic antibiotic tablets of *Klebsiella* specimens on Mueller Hinton agar incubate for 24 hours, then read the results.

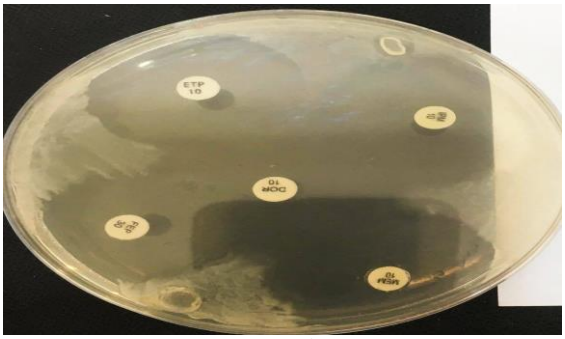

Fig. 2-: Antibiotic susceptibility test of *Klebsiella pneumoniae* isolates

III. RESULTS

A. *Isolation and Identification of Klebsiella pneumoniae*

Sixteen isolates of *K. pneumoniae* bacteria were examined from a total of 300 samples obtained from patients in different hospitals in Dhi Qar Governorate. Imam Hussein Teaching Hospital, Nasiriya Teaching Hospital, and Al-Haboubi Teaching Hospital. From September 2023 to December 2023, 16 (5.3%) of *K. pneumoniae* isolates were collected, and 150 (50%) of the *E. Coli* isolates were collected. 50 (16.6%) isolates of *Pseudomonas* bacteria were collected, 10 (3.3%) isolates of *Enterobacter* bacteria were collected, 4 (1.3%) isolates of *Proteus* bacteria were collected, and 70 (23.3%) There is no growth as shown in Figure (3).

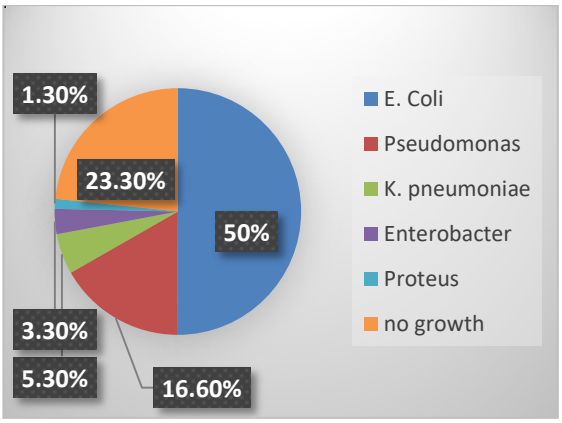

Fig. (3): Percentages of bacterial types isolated from wounds :

B. *Isolate samples according to sex*

300 samples were isolated from wounds, which differed in a higher percentage in males than in females, 170 (56.66) and 130 (43.33), respectively. As shown in Figure (4).

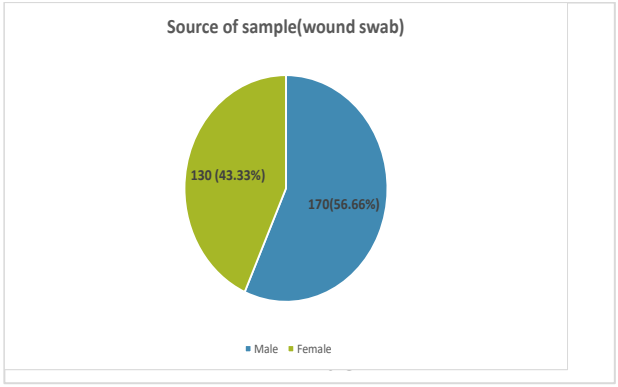

Fig. (4) : Distribution of Specimens according to sex

C. *Antibiotic susceptibility and synergistic effect on Klebsiella pneumoniae isolates*

1) Antibiotic susceptibility and synergistic effect of meropenem on Klebsiella pneumoniae isolates

There were no statistically significant differences (P > 0.28) in the rate of antibiotic resistance, as shown in Table 1. *The isolates under study showed low resistance to the antibiotic meropenem, which belongs to the group of carbapenems,* as the resistance rate reached 37.5%. While the rate of resistance was higher for the other antibiotics Imipenem, Ertapenem, Doripenem, and cefepime, as their resistance rate reached 43.7%, respectively. The resistance rate was higher when two synergistic treatments were used, while the resistance rate was lower when meropenem was used alone According to Figure (5).

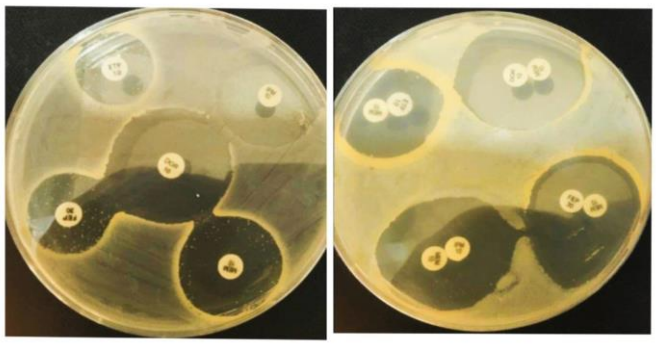

Fig. (5):Synergistic effect of meropenem with other antibiotics

Table (1) : Relationship between Meropenem with other drugs

Antibiotic		Sensitivity			Total
		S	INT	R	
Meropenem	Count	10	0	6	16
	%	62.5%	0.0%	37.5%	100.0%
M+Imp	Count	9	0	7	16
	%	56.3%	0.0%	43.7%	100.0%
m+ Cefepime	Count	9	0	7	16
	%	56.3%	0.0%	43.7%	100.0%
M+Erta	Count	6	3	7	16
	%	37.5%	18.8%	43.7%	100.0%
M+Dora	Count	8	1	7	16
	%	50.0%	6.3%	43.7%	100.0%
Total	Count	42	4	34	80
	%	52.5%	5.0%	42.5%	100.0%

Cal.x^2:9.71 Tab.x^2:20.09 df:8 p-value:0.28

D. Antibiotic susceptibility and synergistic effect of Imipenem on Klebsiella pneumoniae isolates

The current study indicated that there were no statistically significant differences (P > 0.85) in the rate of resistance to antibiotics shown in Table (2). The isolates under study showed high resistance to the antibiotic Imipenem, which belongs to the group of carbapenems, as the rate of resistance to treatment with Imipenem for Klebsiella pneumoniae samples reached 43.7%, while the rate of resistance to the two synergistic treatments with cefepime, Ertapenem, and Doripenem, respectively, reached 29.4% and 31.2%. and 37.5%, indicating that the two synergistic treatments had less resistance than Imipenem alone. This is according to Figure (6).

Table (2) : Relationship between Imipenem with other drugs

Antibiotic		Sensitivity			Total
		S	INT	R	
Imp	Count	9	0	7	16
	%	56.3%	0.0%	43.7%	100.0%
Imp+Cef	Count	10	1	5	16
	%	64.7%	5.9%	29.4%	100.0%
Imp+Erta	Count	10	1	5	16
	%	62.5%	6.3%	31.2%	100.0%
Imp+D orip	Count	10	0	6	16
	%	62.5%	0.0%	37.5%	100.0%
Total	Count	39	2	23	64
	%	61.5%	3.1%	35.4%	100.0%

Cal.x^2:2.63 Tab.x^2:16.81 df:6 p-value: 0.85

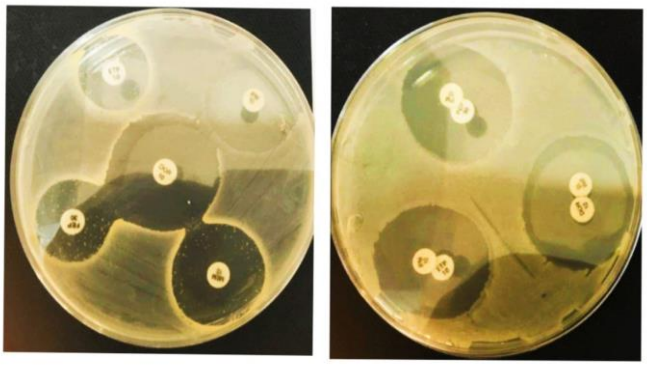

Fig. (6):Synergistic effect of Imipenem with other antibiotics

E. Antibiotic susceptibility and synergistic effect of Ertapenem and Doripenem on Klebsiella pneumoniae isolates

The current study indicated that there were no statistically significant differences (P > 0.12) in the rate of antibiotic resistance, as shown in Table (3). *The isolates under study showed high resistance to the antibiotic Ertapenem, which belongs to the group of carbapenems,* and the resistance rate in *Klebsiella pneumoniae* samples reached 56.2%, and the percentage of Resistance to Doripenem was 43.7%. Respectively, while the resistance rate to the two synergistic treatments, Ertapenem and Doripenem, was 31.2%, which indicates that taking the two synergistic treatments together is less resistant than taking Ertapenem or Doripenem alone. According to Figure (7).

Table (3) : Relationship between Ertapenem with other drugs

Antibiotic		Sensitivity			Total
		S	INT	R	
Erta	Count	4	3	9	16
	%	25.0%	18.8%	56.2%	100.0%
Dora	Count	7	2	7	16
	%	43.8%	12.5%	43.7%	100.0%
Erta+Dorip	Count	11	0	5	16
	%	68.8%	0.0%	31.2%	100.0%
Total	Count	22	5	21	48
	%	45.8%	10.4%	43.8%	100.0%

Cal.x^2:7.30 Tab.x^2:13.28 df:4 p-value:0.12

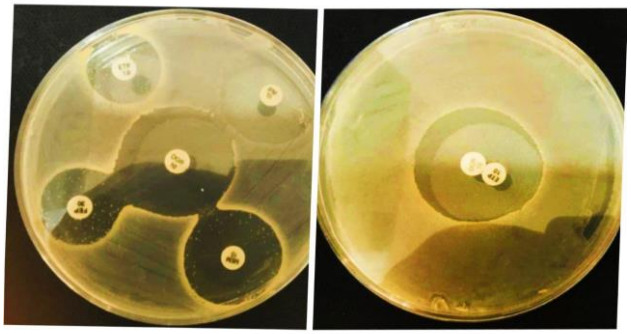

Fig. (7):Synergistic effect of Ertapenem with other antibiotics

F. Antibiotic susceptibility and synergistic effect of Cefepime on Klebsiella pneumoniae isolates

The current study indicated that there were no statistically significant differences (P > 0.36) in the rate of antibiotic resistance shown in Table (4). *The isolates under study showed high resistance to the antibiotic cefepime, which belongs to the group of cephalosporins,* and the rate of resistance to treatment with cefepime in *Klebsiella pneumoniae* samples was 68.8%, while the rate of resistance to treatment with Ertapenem and Doripenem in synergy was 38.9% and 43.8%, respectively, which indicates that taking two synergistic treatments is less resistant than taking cefepime alone, according to Figure (8).

Table (4) : Relationship between Cefepime with other drugs

Antibiotic		Sensitivity			Total
		S	INT	R	
Cefepime	Count	5	0	11	16
	%	31.2%	0.0%	68.8%	100.0%
Cefepime+Erta	Count	8	2	6	16
	%	50.0%	11.1%	38.9%	100.0%
Cefepime+Dorip	Count	8	1	7	16
	%	50.0%	6.2%	43.8%	100.0%
Total	Count	21	3	24	48
	%	44.0%	6.0%	50.0%	100.0%

Cal.x^2:4.30 Tab.x^2: 13.28 df:4 p-value: 0.36

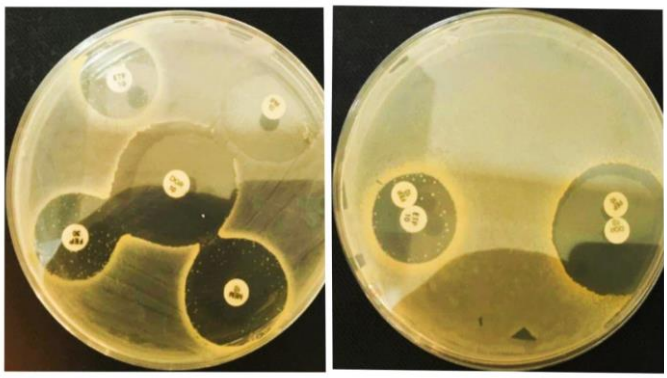

Fig. (8): Synergistic effect of Cefepime with other antibiotics

G. Statistical analysis

The statistical analysis proceeded in all groups of study, descriptive statistics analyzed by using (Chi-square). (p-value ≤ 0.01) was considered to be significant. All analyses were performed with statistical Package for the social sciences SPSS for Windows (version 23.0 SPSS Inc, Chicago, 111).

IV. Discussion

Klebsiella pneumoniae is known to be a major causative agent of both nosocomial and community infections [11]. This species has recently gained notoriety as an infectious agent due to the scarcity of effective treatments and the increasing number of severe infections due to the emergence of isolates that have acquired additional genetic traits and become either resistant to multiple antibiotics or highly virulent in this context, the current study aims to isolate and diagnose clinical *K. pneumoniae* isolates in Dhi Qar hospitals. During the monitoring period, 300 clinical samples were collected from three major teaching hospitals in the city of Dhi Qar and subjected to bacteriological examination, and 16 samples of *K. pneumoniae* bacteria were isolated and diagnosed. As the prevalence of *Klebsiella spp* bacteria *pneumoniae* is considered the most medically important *Klebsiella* species the VITEK 2 system was used to verify *Klebsiella* identification with species level and to prevent variability in biochemical test results. The result showed that all *Klebsiella spp* isolates were diagnosed as *K. pneumoniae*. This survey confirmed the spread of *K. pneumoniae* disease in Dhi Qar Hospitals. Infection with *K. pneumoniae* was greater in males than females Figure (4). This result was consistent with that reported by [12]. Who isolated *K. pneumoniae* as being more common among males (61.8%) than females (38.2%) Susceptibility tests were performed for 16 *K. pneumoniae* isolates. Resistant to the carbapenem group, but each antibiotic differs from the other in the zone of inhibition formed around the colony, where resistance was highest to Ertapenem (50%), then Imipenem, then Doripenem. and meropenem, respectively [13]. This study is consistent with the current study *K. pneumoniae* isolates were 23 (15.9%) resistant to Imipenem and 17 (11.8%) to meropenem, which is not consistent with the current study because the resistance rate is higher than in the previous study The rate of resistance to meropenem was 6 (37.5%) and to Imipenem 7 A study conducted by [14]. compared to the present study, where the resistance was 7 (43.8) higher for Imipenem and 6 (37.5) for meropenem. Although meropenem and Imipenem are expensive antimicrobial agents, sales in Iraq have increased significantly with better sensitivity. Interestingly, the present study revealed that 37.5% and 43.8% of *K. pneumoniae* isolates were resistant to meropenem and Imipenem, respectively. In the past few decades, *K. pneumoniae* has been steadily recognized as one of the major causes of hospital-acquired infections [15]. Over the past few years, the use of Imipenem and meropenem has been most successful against *K. pneumoniae* isolates. According to previous study [16]in Iran, it was found that (13.9%) of *K. Pneumoniae* strains were resistant to the drug Imipenem. but in the current study the rate of resistance was higher than in previous studies. This antimicrobial agent has been administered in recent years and use against different infections in Iraq. But when than if the treatment is used alone. In this study, the rate of resistance was lower in the

case of synergism through the use of Imipenem with Ertapenem and Doripenem, respectively. With the exception of meropenem, the resistance rate was higher when used synergistically, while the susceptibility rate was higher when used alone., However, the resistance rate to cefepime (68.8%) was quite high in this study, which correlates with other local studies conducted by [17]. where they found that 64.9% and 80.2% of *K. pneumoniae* isolates showed resistance to cefepime. This relatively high resistance to these antibiotics may be a result of widespread expansion and use of these antibiotics by people without proper medical advice. But when cefepime is used synergistically with the carbapenem group, the rate of resistance is lower. Therefore, it is preferable to use it synergistically because the rate of sensitivity is higher than the resistance. carbapenems resistant *K. pneumoniae* represents a major public inaccurate administration of antibiotics and absence of appropriate infection control strategies may be the possible causes behind enhancing the resistance of K pneumoniae to commonly used antimicrobial agents Multiple antibiotic resistance *K. pneumoniae* carry a variety of resistant determinants, are becoming a significantly worldwide problem [18].

V. CONCLUSION

Klebsiella pneumoniae bacteria are considered one of the most important types of bacteria that cause hospital-acquired wound infections in Nasiriyah Governorate. The emergence of drug-resistant isolates that threaten antimicrobials. Most clinical isolates of K. pneumoniae showed multidrug resistance. Susceptibility to antibiotics, especially to the cephalosporin cefepime, was tested while carbapenems were the most effective antibiotic against K. pneumoniae isolates. This study shows that taking two synergistic treatments is better than taking one treatment to combat Klebsiella pneumoniae.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest

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