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Antibiogram of *Klebsiella pneumoniae* that isolated from clinical and environmental samples in Al-Diwaniyah hospitals

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Antibiogram of *Klebsiella pneumoniae* that isolated from clinical and environmental samples in Al-Diwaniyah hospitals

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ABSTRACT

Antibiotic resistance has become a worrying phenomenon in the world today, and this global threat requires implementing antibiotic management policies in all hospitals. This study aims to analyze susceptibility to various antibiotics commonly used in treatment against *K.pneumoniae* strains, isolated by using conventional methods from various clinical and environmental sites. A total number of (n=886) samples were collected from AL-Diwaniyah hospitals during the period from August 2019 to January 2020. Among the 275 organisms that were isolated of them, 70 (40%) were identified as *K.pneumoniae* by standard microbiological techniques, 68 isolates (97.14%) were distributed in clinical specimens and 2(2.85%) isolate in environmental samples, and antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion testing to 28 antibiotics and interpreted as per CLSI guidelines. The results susceptibility testing showed that frequency resistance of *K.pneumoniae* isolates to Piperacillin and Ampicillin /sulbactam was (100 %) Cefuroxime (97.14%) Ceftriaxone (84.28%) Ceftazidime (81.42%). Also, the results showed the least resistance to Gentamicin (14.28%), Netilmicin (15.71%), (12.85%) for Ofloxacin and Levofloxacin, Lomefloxacin (32.85%), Nalidixic acid (38.57%), Trimethoprim-Sulfamethoxazole (27.14%), Doxycycline (31.42%), Chloramphenicol (25.71%), Meropenem (22.85%), which could be the drugs of choice for the treatment of infections. The present study reveals the frequency of isolation of *K.pneumoniae* from a urine sample, especially female patients between (21-30) age, who are more affected and infection, and their tendency towards antibiotic resistance. The data of this study may be used to measure trends in antimicrobial susceptibilities to formulate local antibiotic policies and overall to help clinicians in the rational choice of antibiotic therapy.

1.INTRODUCTION

The bacterium *Klebsiella pneumoniae* is the most important species within the Genus *Klebsiella* spp. in 1882, Friedlander described encapsulated bacilli from the lungs of a patient who died of pneumonia (29). Although it is a common colonizer of the skin, nasopharynx, and gastrointestinal tract (43). It poses a risk to patients who are hospitalized for long periods and have weakened immune systems (13). It has adapted over the years to live well in a hospital environment around a person, so it is easy to reach and cause disease (31). *K. pneumoniae* harbors many different virulence factors for growth (51) and to overcome the immune response by the host (15). The capsule polysaccharide (CPS) is one of the most important virulence agents used by bacteria in the first place (41). Help in the adhesion process and thus the beginning of colonization to cause infections (21). Lipopolysaccharide (LPS) is an essential component of the outer membrane of Gram-negative bacteria, and it greatly contributes to structural integrity, as it is an important pathogen that contributes to many diseases, and it consists of three main parts: lipid A, core oligosaccharide and O- antigen (28). Iron-scavenging systems (siderophores) are an essential component in the growth of *K. pneumoniae* during infection (47).

K.pneumoniae is one of the most important bacteria that cause serious infections in humans, and its symptoms vary according to which part of the body is affected by the bacteria (35). Antibiotic resistance has become a worrying phenomenon in the world today, and this global threat requires the implementation of antibiotic management policies in all hospitals (42). Most *K.pneumoniae* strains are intrinsically resistant to Carbenicillin, Ampicillin (55). Over the past few decades *K.pneumoniae* with multiple antibiotic resistance and responsible for hospital-acquired infections (10). have become a major threat to public health (31). Continues to cause MDR infections around the world (44). So, it is not surprising that this pathogen is recognized as an "urgent threat to human health" by many organizations (13). The cause of resistance is the misuse of antibiotics (32). Determining an appropriate treatment protocol and analyzing the resistance status to prevent the development of resistance becomes an issue. Importantly, high mortality rates were observed in infections caused by *K.pneumoniae* strains resistant to carbapenem. (42). It is essential to understand the antimicrobial susceptibility pattern of *K.pneumoniae* which shows variation in different geographical settings, to implement effective control measures to prevent the rapid spread of drug resistance, and also to carry out a rational selection of antimicrobials in our hospital.

2.MATERIALS AND METHODS

1.2.Sample collection and processing

In the current work, 786 clinical samples (144 sputa, 132 burn swabs, 510 urine samples) were taken from patients attending AL- Diwaniyah city hospitals for both sexes and different age groups. The total of environmental samples (n=100) were investigated in operating rooms (n=30), lobbies(n=25), a central processing unit (n=15), dialysis (n=10) and ICU(n=20). All samples were collected from August 2019 to January 2020 from different hospitals and specialized health centers in AL- Diwaniyah province. Iraq. Highly aseptic conditions were followed during the sampling. The samples were then ice-box transported to a microbiological laboratory — Biology Department, College of Science, University of Al-Qadisiyah, Iraq — to performed the required tests.

2.2.Identification of *Klebsiella* spp.

The isolates under investigation were initially identified on the blood agar (Himedia, India) and MacConkey agar (Oxoid, UK) using the sterile standard loop method. The media were incubated at 37°C for 24 hours. The bacterial diagnosis was confirmed by traditional morphological (Gram stain/ Himedia-India) and biochemical tests were used, according to (18,33), and Vitek 2 Compact® (Biomérieux France) were used to recognize the identity of the bacterium.

3.2.Antimicrobial susceptibility testing (AST)

The study isolates were exposed to drug sensitivity to 28 antibiotics and was performed by Kirby-Bauer disk diffusion method (12). Recommended by Clinical and Laboratory Standards Institute (17). The antibiotics chosen for the study were Amoxicillin – clavulanate (20/10mg), Ampicillin – sulbactam (10/10mg), Piperacillin (100mg), Cefoxitin (30mg), Cefpodoxime (10mg), Cefuroxime (30mg), Cefixime (5mg), Cefotaxime (30mg), Ceftriaxone (30mg), Ceftazidime (30mg), Aztreonam(30mg), Imipenem (10mg), Meropenem (10mg), Nitrofurantoin (300mg), Nalidixic acid (30mg), Ofloxacin (5mg), Netilmicin (30mg), Gentamicin (10mg), Tobramycin (10mg), Amikacin (30mg), Trimethoprim (5mg), Trimethoprim-Sulfamethoxazole (1.25/23.75 mg), Ciprofloxacin(5mg), Levofloxacin (5mg), lomefloxacin (10mg), Tetracycline (30mg) , doxycycline(30mg), Chloramphenicol (30mg). However, the present study revealed that some *K.pneumoniae* isolates were considered to be multi-drug resistant (MDR) because they were resistant to at least three classes of antibiotics tested, Extensively drug-resistant (XDR). When it is insensitive to at least one antibiotic for all classes of antibiotics used except for one or two classes and it

reached, while the isolates that are insensitive to all classes of antibiotics used in the study were considered completely Pandrug-resistant (PDR) .

3.RESULTS

The current study included Collection of 886 samples from Hospitals in the AL-Diwaniyah governorate and it included various samples, which are the clinical samples, which amounted to 786 samples and included 510 urine samples, and 144 samples of sputum, and 132 samples from burn swabs, included the hospital environment, which amounted to 100 samples. The results of the phenotypic examination showed that the bacterial colonies were large, Pink and mucoid on MacConkey agar as lactose fermentation, large dome-shaped colonies on Blood agar. Microscopic diagnosis of bacteria showed that the bacteria were non-motile Gram-negative, short, stout, blunt rods were seen. The biochemical characters identified were negative Indole test positive, Voges-Proskauer test positive, Citrate use test positive, Urease test. In our study, only 70 isolates (40%) were identified as *K.pneumoniae*, two isolates were diagnosed (2.29%) as *K. pneumoniae* and it was the only type of *Klebsiella* spp. Diagnosed in environmental samples of the hospital, as for the isolates obtained from clinical samples, the prevailing type between them was *K.pneumoniae*, with the number of 68 isolates (97.14%) were distributed in clinical specimens according to their isolation sources to 34 isolates, at a rate of (22.51%) from urine samples, and 13 isolates, at a rate of (22.80%) of sputum samples, and 21 isolates and a rate of (24.13%) from burn swabs. As for environmental samples, it included one isolate. With a percentage (25%) of dialysis, one isolation, and (1%) of the central processing unit, as shown in Fig 1. According to the demographic data, the number of patients infected was higher in female patients, 42 (61.76%), compared to males 26 (38.23%). Most of the prevailing age groups in our current study are between (21-30) .

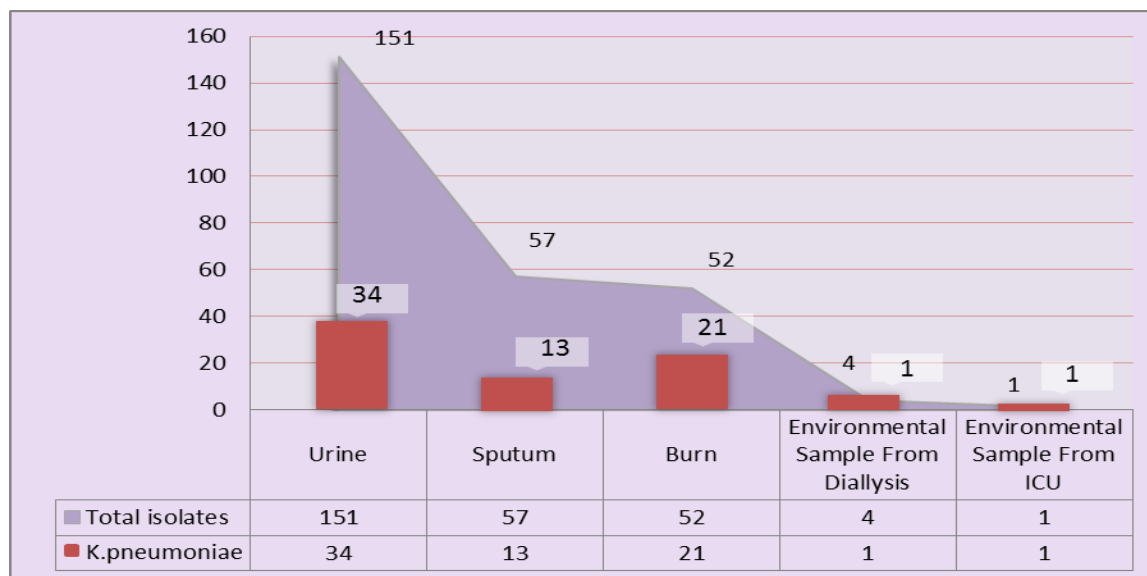


Figure 1: Distribution of *K.pneumoniae* isolates according to isolation source.

The results of the susceptibility tests showed a clear variation in the response of the bacterial isolates under study to the antibiotics used. The statistical results showed that there were significant differences in the studied bacterial response to antibiotics at a probability level of $p < 0.05$, as shown in Table 1.

Antimicrobial susceptibility testing revealed 100% were *K.pneumoniae* resistance to Piperacillin and Ampicillin/sulbactam, amoxicillin/clavulanic acid (98.57%), the isolates showed a resistance rate of 74.28% against Cefotaxime, Ceftazidime (81.42%), Cefixime and Ceftriaxone a resistance rate of 61.42% and 84.28% respectively, Cefoxitin (42.85%), monobactam antibiotic represented by the Azteronam antibiotic, the isolates showed a resistance rate of 52.85%, Imipenem (41.42%), Meropenem (22.85%), *K. pneumoniae* showed the least resistance to aminoglycosides, especially Gentamicin and Netlimicin then Tobramycin was relatively effective against *K.pneumoniae*, with a resistance rate of 14.28%, 15.71%, and 41.42% respectively and moderate resistance against Amikacin 57.14%, and also showed the least resistance to quinolones antibiotic especially, Ofloxacin, lomefloxacin, Nalidixic acid, and Levofloxacin, with the rate of 12.85%, 32.85%, 38.57%, and 12.85% respectively, but the isolates showed moderate resistance to ciprofloxacin at a rate of 50%, the percentage of resistance to Trimethoprim-Sulfamethoxazole and Trimethoprim was 27.14% and 41.42%, respectively, Tetracycline (70%), Nitrofurantoin (61.42%), Chloramphenicol (25.71%).

Table 1: Antimicrobial sensitivity of *Klebsiella pneumoniae*

Sensitive	Intermediate	Resistant	Antibiotics
1(1.42)	0(0)	69(98.57)	Amoxicillin – clavulanic acid
0(0)	0(0)	70(100)	Ampicillin – sulbactam
0(0)	0(0)	70(100)	Piperacillin
35(50)	5(7.14)	30(42.85)	Cefoxitin
21(30)	5(7.14)	44(62.85)	Cefpodoxime
2(2.85)	0(0)	68(97.14)	Cefuroxime
24(34.28)	3(4.28)	43(61.42)	Cefixime
7(10)	11(15.71)	52(74.28)	Cefotaxime
2(2.85)	9(12.85)	59(84.28)	Ceftriaxone
1(1.42)	12(17.14)	57(81.42)	Ceftazidime
29(41.42)	4(5.71)	37(52.85)	Azteronam
35(50)	6(8.57)	29(41.42)	Imipenem
48(68.57)	6(8.57)	16(22.85)	Meropenem
18(25.71)	9(12.85)	43(61.42)	Nitrofurantoin
22(31.42)	21(30)	27(38.57)	Nalidixic acid
55(78.57)	6(8.57)	9(12.85)	Ofloxacin
52(74.28)	7(10)	11(15.71)	Netlimicin
53(75.71)	7(10)	10(14.28)	Gentamicin
22(31.42)	19(27.14)	29(41.42)	Tobramycin
11(15.71)	19(27.14)	40(57.14)	Amikacin
34(48.57)	7(10)	29(41.42)	Trimethoprim
45(64.28)	6(8.57)	19(27.14)	Trimethoprim-Sulfamethaxazole
31(44.28)	4(5.71)	35(50)	Ciprofloxacin
51(72.85)	19(27.14)	9(12.85)	Levofloxacin
42(60)	5(7.14)	23(32.85)	Lomifloxacin
4(5.71)	17(24.28)	49(70)	Tetracycline
41(58.57)	7(10)	22(31.42)	Doxycycline
41(58.57)	11(15.71)	18(25.71)	Chloramphenicol
$\chi^2 = 778.5$ P value = 0*			

* There are significant differences at the 5% probability level.

4.DISCUSSION

The present study highlights the most alarming situation of highly diverse antibiotic resistance. In our study, 100% of the isolates showed resistance to Piperacillin. The cause of resistance is attributed to the ability of the bacteria to produce chromosomally encoded β -lactamases could handle this intrinsic resistance or plasmid-encoded penicillinase enzymes, and most *K.pneumoniae* isolates produce SHV-1 beta-lactamase that mediates the chromosomes, and is usually produced at low or moderate levels, but enough to protect against the antibiotic (11). An alarming finding seen in this study was that resistance was shown for the inhibitors of beta-lactamase, Ampicillin/Sulbactam and Amoxicillin /Clavulanic acid, it was resistant to these two antibiotics by 100% and 98.57%, respectively. Overall resistance was high on account of the production of extended-spectrum β -lactamases (ESBLs) by *K. pneumoniae*. The resistance may also be due to the production of metallo- β -lactamases (MBL), which can be chromosomally encoded or plasmid-mediated. The dose, as well as the incidence of toxicity, is then reduced if beta-lactamase inhibitors are used with β -lactam antibiotics (46).

Despite using Cefoxitin, the treatment of bacterial infections in Iraq is restricted, but the study showed that 42.85% of the isolates are resistant to the antibiotic. One of the main reasons for antibiotic resistance is the result of expression of the chromosomal AmpC enzyme or the acquisition of AmpC plasmid, or several other non-specialized resistance mechanisms, such as the reduction of the outer membrane permeability due to mutations that occur in the gene encoding pores (14). The reason for the high resistance to a third-generation antibiotic (Cefotaxime, Ceftazidime Cefixime, and Ceftriaxone) is these isolates often return to patients who used many antibiotics for a long period (37). Because of the production of ESBL enzymes, many studies showed that *Klebsiella* spp. are highly productive of these enzymes (16). Various previous Iraqi studies in Al-Diwaniyah province documented the multiple resistance and enzyme production of antibacterial agents in Gram-negative bacilli (6,7,5,2,3,25). As for Carbapenem antibiotics, which spread rapidly through the outer membrane of most gram-negative bacteria, the isolates showed resistance to Imipenem by 41.42%, and this result agreed with the findings of the study of the researchers (20). The bacteria resisted the antibiotic by 34.6% As for Meropenem, it recorded a resistance rate of 22.85%, which was in agreement with the results (28). As its isolates showed resistance to 19.04%, while (54). Recorded a sensitivity rate of 100% for each of them, and the reason is due to the limited use of this antibiotic on a routine basis.

As for aminoglycosides antibiotics, which have a broad spectrum of activity and a fast bactericidal effect. It binds to the 30S ribosome and interferes with protein synthesis, exerting a bactericidal action (27). We found *K.pneumoniae* showed the least resistance to aminoglycosides, especially Gentamicin and Netilmicin, then Tobramycin was relatively effective against *K.pneumoniae*, with moderate resistance against Amikacin and this result was identical to the study (58). The widespread therapeutic use of aminoglycosides results from developing resistance aminoglycoside-modifying enzymes (AMEs) are the most common mechanism of acquired resistance against these antibiotics. Other mechanisms of resistance are target modification, mutations of the ribosome target, and efflux pumps, in addition to A modification of 16S rRNA (27,56), which is a means of resistance in the *K. pneumoniae* strain bearing a plasmid encoding 16S rRNA methyltransferase (23).

Quinolone and later derivatives such as the fluoroquinolones act by exerting their antibacterial effects by inhibition of certain bacterial topoisomerase enzymes, namely DNA gyrase in gram-negative bacteria, essential for bacterial DNA replication, by binding to these targets quinolones disrupt DNA synthesis and cause cell death (48). *K.pneumoniae* showed the least resistance to quinolones antibiotics, especially Ofloxacin, Lomefloxacin, Nalidixic acid, and Levofloxacin, but the isolates showed moderate resistance to Ciprofloxacin at a rate of 50%. The cause of resistance was due to the increasing use of antibiotics in the treatment of the diseases caused by it, which helped to develop resistance mechanisms. Ciprofloxacin is the most-consumed antibacterial agent worldwide (1,49). Resistance to quinolones can be due to several different mechanisms, including topoisomerase target modification by mutation (48). The *Qnr* determinant is located on a plasmid-encoded integron, first described in *K. pneumoniae* and then in *E. coli* and *Enterobacter cloacae* (26,36,57).

The percentage of resistance to Trimethoprim-Sulfamethoxazole and Trimethoprim was 27.14% and 41.42%, respectively. The results of the study came close to what was found by (50). For Trimethoprim-Sulfamethoxazole, which recorded a resistance rate of 34.83%, the least resistance to these antibiotic due to acting by inhibiting 2 steps in bacterial folic acid synthesis. Sulfamethoxazole inhibits dihydropteroate synthetase and blocks the formation of dihydrofolate, whereas trimethoprim inhibits dihydrofolate reductase (DHFR) and blocks the formation of tetrahydrofolate. Inhibition of these two steps blocks the synthesis of pyrimidines and purines that go into making DNA (45)

As for the Tetracycline antibiotic which crosses Gram-negative bacterial membrane through one of the OmpF and OmpC porin channels, the isolates resisted it by 70%. The extensive use of tetracycline leads to the development of bacterial resistance, this resistance is caused by the Protection of the tetracycline target, the ribosome. Lowering the amount of tetracycline in the cytoplasm to prevent the tetracycline from reaching the ribosome the OmpF mutant cells show resistance to tetracycline, this is achieved by decreasing the permeability of the cell envelope and efflux of tetracycline outside the cytoplasm (19). As for the Doxycycline antibiotic, it was effective against bacteria, with a resistance rate of 31.42%. Because Doxycycline is second-generation tetracycline, which has better tissue penetration, longer half-life, and a large volume of distribution compared to the original tetracyclines (52). The Nitrofurantoin antibiotic, showed a resistance rate of 61.42%, and the results of the study agreed with what was found (8), as its isolates showed a resistance rate of 59.2%. Whereas, bacteria showed a resistance rate of 75% in a study conducted by (58) in Iran. The isolates showed sensitivity to Chloramphenicol, and the resistance rate reached 25.71%, and it agreed with the results (9) . Because chloramphenicol has side effects and toxicity and the availability of safer alternatives chloramphenicol use is limited. It is used only in life-threatening situations (53).

The isolates were considered to be multidrug-resistance (MDR) reached 40 *K.pneumoniae* isolates with a percentage (57.14%), and the isolates were also considered to be Extensively drug-resistant (XDR). reached 15 isolates with a percentage of (21.42%). Pandrug-resistant (PDR) included 15 isolates (21.42%). The driving force of antibiotic resistance is the widespread use of antimicrobial drugs. In Iraq, antibiotics are widely used and this situation is leading to the emergence of uncontrolled resistance at the hospital level, so therapeutic strategies must be formulated to control infections due to *Klebsiella* spp. Carefully.

5. Conclusion

The high resistance of *K.pneumoniae* antibiotic to commonly used antibiotics is the main cause of prolonged infection, increased hospitalization, increased treatment cost, and increased morbidity and mortality. *K. pneumoniae* infection was prevalent in females and at 21-30 age. *K.pneumoiniae* was more sensitive to Gentamicin, Netilmicin, Tobramycin, Ofloxacin, Lomefloxacin, Nalidixic acid, Levofloxacin, Trimethoprim, Trimethoprim-Sulfamethoxazole, Doxycycline, Chloramphenicol, Imipenem, Meropenem, which could be the drugs of choice to the treatment of *K. pneumoniae* infections isolated from Clinical and environmental samples. Regular surveillance of the antibiotic susceptibility pattern may help to overcome the indiscriminate use of antibiotics, which is a major reason for the emergence of drug resistance among pathogens and for developing antibiotic policies. The data of this study can be used to determine trends in antimicrobial sensitivity, to shape local antibiotic policies and to help clinicians in general in making the rational choice of antibiotic therapy.

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