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Antibiotic Susceptibility of Klebsiella pneumoniae isolated from clinical and environmental samples in Baqubah Teaching hospital / Diyala

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ABSTRACT

Clinical samples (278) from people with various disorders and aquatic environmental samples (50) were also taken. Clinical samples included urine samples (130) sputum samples (83), wound samples (36), burns (15) and blood samples (14), fourteen samples were gathered. Samples from the Ba'aqubah Teaching Hospital in Diyala for the months of December 2021 to May 2022. The results of all samples' bacterial culture showed that (62.9%)175 samples were found to be positive for bacterial culture and 103 samples (37.1%)to be negative while (39.4) 69% of Klebsiella pneumoniae bacteria the isolates were identified using microbiological and molecular techniques, K. pneumoniae bacteria were found in urine (32.31%)42, sputum (20.5%)17, , wounds (19.44%) 7 burns (13.33%)2 blood (7.14%)1, water (5%)5 According to the Kirby-Bauer disc, different isolates of K. pneumoniae bacteria are resistant to the various antibiotics used in the study, including (Amoxicillin + Clavulanic Acid 87.80%, Amikacin 48.78%, Trimethoprime-sulfamethoxazole 80.48%, Azithromycin 68.29%, Levofloxacin 43.90%, Ciprofloxacin46.34%, Ceftazidim 100% Ceftriaxone 92.68% Ticarcillinclavulanate 92.68% Pipracillintazobactam 60.97% Merpenem 95.12% Imipenem 46.34(%The findings of the antibiotic sensitivity test revealed that 32 (78%) of the K. pneumoniae isolates are multidrug resistant (MDR), and that 9 (22%) of them are pandemic multi-drug resistant (PDR) to all of the medicines utilized.

Key words: Klebsiella pneumoniae, Nosocomial infection, Antibiotic resistance

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INTRODUCTION

K. pneumoniae is one of the most important species of the genus Klebsiella which is Gram negative, Fermented Lactose, Non motil, Facultative anaerobic and Capsule [1]. *K. pneumoniae* is naturally present in the human digestive system and can cause many opportunistic infections, such as hospital-acquired infections (Nosocomial infection), such as urinary tract infection, Pneumonia, bacteremia, and liver abscess. Purulent liver abscess, Diarrhea, wound infections, and burns can therefore be isolated from different pathogens [2]. *K. pneumoniae* possesses many virulence factors that make it capable of invading the body and causing disease. Capsule) is composed of polysaccharides, fimbriae, iron transport systems (sidero phores) and lipopolysaccharides, which are endotoxins [3]. The genomes of *K. pneumoniae* are very diverse and the strains differ from each other by 0.5% according to different nucleotides [4]. It is important to note that the majority of the genome is made up of additional genes (Accessory genome), which includes chromosomally carried genes and plasmid genes. The genome of *K. pneumoniae* typically consists of (6000–5000) genes and is made up of a set of genes that are found in all types of these bacteria. Bacteria can adapt to specific sites of infection or colonization thanks to mobile genetic elements (MGEs) and the genes encoded in genomic islands associated with pathogenicity [5]. Antibiotic resistance to most or all of the regularly used antibiotics has developed as a result of the widespread use of antibiotics to treat *K. pneumoniae* and has become a major issue in contemporary medicine [6]. Due to the lack of available treatments, multidrug resistant *K. pneumoniae* (MDR) has emerged as a prominent cause of hospital acquired infections with high infection rates [7]. *K. pneumoniae* developed due to the accumulation of antibiotic resistance genes through horizontal gene transfer (HGT) and genetic mutations (mutation). *K. pneumoniae* is able to produce extended

spectrum -lactamase (ESBL) enzymes [8]. Because of the importance of *K. pneumoniae* bacteria in causing many diseases and the lack of studies in Diyala governorate about it, this study came to aim at: Identifying the pattern of resistance and sensitivity to antibiotics of the different clinical isolates of different source sources of *K. pneumoniae* isolated bacteria in Diyala Governorate, and determining the extent of the spread of MDR isolates and Multiple PDRs among themselves by following these steps.

Isolation and identification of *K. pneumoniae* bacteria from different clinical and environmental samples in Diyala Governorate.

Examining the sensitivity and resistance of bacterial isolates to antibiotics and determining the resistance pattern of the isolates.

Diagnosis of MDR and PDR isolates resistant to multiple antibiotics.

MATERIALS AND METHODS

Collection of samples and culture

Clinical isolates

(278) samples were collected and distributed among (130) urine samples for people suffering from urinary tract infection, (83) sputum samples taken from people suffering from respiratory infection, (36) samples from wound swabs and (15) samples from burns swabs In addition to (14) blood samples taken from patients, these samples were collected from Ba'aqubah Teaching Hospital, Burns and Wounds Hall, Chest Diseases Hospital, educational laboratories for the period from December 2021 until May 2022. One hour, the bacteria were identified phenotypically with the observation of mucosal lactose-fermenting bacteria on the McConkey medium.

Environmental isolates

Fifty water samples were collected from the sewage water of the city of Ba'aqubah, where 10 ml of each sample was taken and placed in sterile tubes, then stored at a low temperature for transportation and planted within 4 hours [9]. 1 mL of each aqueous sample was added to 5 mL MacConkey broth with a Durham tube for gas production and incubated at 42 °C for 18–24 h [10] and then one loop of broth was grown on MacConkey agar and EMB agar in order to obtain a colony isolated one.

Identification of bacterial isolate

Cultural diagnosis

The bacteria were identified by studying the general characteristics of the colonies growing on MacConkey aquariums and observing the appearance of colony shapes in terms of mucous texture, color, and edges [11].

Microscopic diagnosis

Several colonies of the developing bacteria were taken and placed on a glass slide with Normal Saline solution, spread on a glass slide and left to dry and then fixed by heat by passing quickly over the flame two to three times and then dyed with a cream dye and examined under the microscope to observe the color, shape and clusters of bacteria [12].

Antibiotic sensitivity test

A sensitivity test was conducted for all isolates using a group of different antibiotics using the Kirby–Bauer disc method mentioned as follows: The bacterial suspension of the isolates was prepared with the turbidity of the suspension equal to the McFarland constant 0.5, which is equivalent to1.5*108 cells / ml, as tubes containing 3 mL of physiological saline solution of the colonies of bacterial isolates growing on MacConkey solid medium at 24 hours of age, then sanitized in the solution until the turbidity of the solution was equal to the turbidity of the standard McFarland constant and the bacterial suspension was used after 15 minutes of preparation. The dishes containing Mueller-Hinton solid medium were inoculated with A sterile cotton swab dipped in the bacterial suspension, taking into account the removal of excess moisture by pressing it on the walls of the tube from the inside, and distribute the vaccine all over the dish. The spaces between the discs on one side and the edge of the plate on the other. The plates were incubated at 35°C for 20 hours. The area of inhibition was measured in milliliters using the ruler around each disc and the results were compared with the standard values mentioned [13].

RESULTS

Klebsiella pneumoniae is sensitive to antibiotics

The sensitivity of (41) isolates of *K. pneumoniae* bacteria towards a group of (12) antibiotics has been studied for the purpose of knowing the extent of sensitivity or resistance of the isolates to these antibiotics. Infections, the sensitivity or resistance of isolates to antibiotics was determined based on measuring the diameter of the inhibition zone surrounding the antibiotic tablets and the results were compared with the standard tables contained in (13). Against the antibiotics that were used in the study as follows: the resistance rate to Amoxicillin+Clavulaincacid (87.80), Amikacin (48.78%), Trimethoprime-sulfamethoxazole (80.48%), Azithromycin (68.29%), Levofloxacin (43.90%) and Ciprofloxacin (46.34%) Anti-Ceftazidim (100%), Anti-Ceftriaxone (92.68%), Anti-Ticarcillinclavulanate (92.68%) Anti-Pipracillintazobactam (60.97%) Anti-Merpenem (95.12%) Imipenem 46.34% antagonist (Table 1).

Multiple antibiotic resistances

K. pneumoniae isolates showed different and multiple resistance to the antibiotics used in the study as shown in Table 2. The results of the antibiotic sensitivity test examination revealed that 32 isolates of *K. pneumoniae* isolates (78%) are multi-drugresistance (MDR) and

that 9 isolates of bacteria (22%) have resisted all the antibiotics under the current study. resistant (PDR) and Table 3 shows this. Table 4 shows the distribution of multi -resistance *K. pneumoniae* bacteria (MDR) and (PDR), according to the source.

DISCUSSION

The differences in the resistance ratio between this study and other studies are due to reasons such as the number of isolates, different working conditions, health

Table 1: Percentage of resistance of *K. pneumoniae* isolates to antibiotics.

Antibiotic	Percentage	Antibiotic	Percentage
Amoxicillin+Clavulanic acid	87.80%	Ceftazidime	100%
Amikacin	48.78%	Ceftriaxone	92.68%
Trimethoprimesulfame thouxazole	80.48%	Ticarcilline clavulanate	92.68%
Azithromycin	68.29%	Piperacillin tazobactam	60.97%
Levofloxacin	43.90%	Meropenem	95.12%
Ciprofloxacin	46.34%	Imipenem	46.34%

Table 2: Multiple antibiotic resistance of isolates of *K. pneumoniae*.

No. of antibiotics	No. of resistant isolates	% percentage
4	2	4.87%
5	3	7.31%
6	4	9.75%
7	4	9.75%
8	7	17.07%
9	8	19.51%
10	1	2.43%
11	3	7.31%
12	9	21.95%
Total	41	

Table 3: Antibiotic resistance pattern of *K. pneumoniae.*

Resistance	No.of isolates	Percentage
MDR	32	78%
PDR	9	22%

Table 4: Resistance insulation (MDR) and (PDR), according to the source.

Isolate	Source	Isolate	Source
1	wounds	21	urine
2	burns	22	urine
3	urine	23	urine
4	burns	24	urine urine
5	sputum	25	urine
6	urine	26	urine
7	wounds	27	urine
8	wounds	28	urine
9	Urine	29	urine
10	sputum	30	urine
11	sputum	31	urine
12	Sputum	32	wounds
13	Sputum	33	burns
14	Sputum	34	burns
15	Sputum	35	wounds
16	Sputum	36	wounds
17	Sputum	37	wounds
18	Sputum	38	burns
19	Urine	39	burns
20	Urine	40	wounds
		41	burns

status of the patient as well as the misuse of antibiotics and long-term exposure to antibiotics. The ability of *K. pneumoniae* bacteria to resist many antibiotics is due to The ability of bacteria to produce beta-lactam enzymes and a change in the permeability of the outer wall or by flow systems, whereby reducing the number of channels in the cell membrane that allow antibiotics to enter through them accelerates the active flow (pumping to the periphery) of the antibiotics as well as changing the location of the target (the site of activity of the antibiotic There is a group of genes regulated by 38 operon genes in bacteria and 19 of these genes are directly responsible for the development of resistance to antibiotics [14]. The problem of the ability of K. *pneumoniae* bacteria to produce beta-lactam enzymes comes in the first place, which at present constitutes a health problem that defines the health care sector, public health and society all over the world [15]. The multi-resistance of *K. pneumoniae* bacteria is one of the most important problems that we face from a medical point of view, and this is in the difficulty of choosing the appropriate treatment for the patient, and one of the reasons that led to the emergence of multi-resistance is the indiscriminate use of antibiotics without consulting a doctor and without conducting an antibiotic sensitivity test, which This led to the adaptation of bacteria and increase their resistance to the antibiotics used in treatment [16] as some of the antibiotics previously used in the treatment of these bacteria became useless, which caused many challenges to find the appropriate treatment all over the world, most of the infection with *K. pneumoniae* bacteria talked about Hospitals, especially after surgical operations or in the case of burns, which caused the high death rates, so the availability of appropriate treatment at the right time ensures the treatment and survival of the injured [17-24].

CONCLUSION

Through the current study, the following conclusions were reached.

The highest percentage of bacterial growth in the samples under study was in the urine samples.

Some isolates of *K. pneumoniae* possessed the trait of multi-resistance (MDR) and some of them possessed the trait of total resistance (PDR) to the antibiotics under study.

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