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Detection of Beta-lactamase Resistance in *Klebsiella pneumoniae* Isolated from Different Clinical Sources

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Abstract

186 samples were collected from various pathogenic specimens, including 75 urine samples, 46 cerebrospinal fluid samples, 23 sputum samples, 9 body fluid samples, 14 blood samples, 4 discharge samples, 11 fluid samples from wounds and burns, and 4 bone marrow samples. Accordingly, 23 isolates of *K. pneumoniae* were identified as follows: 12 from urine samples, three isolates from cerebrospinal fluid, two each from catheters, wounds, and sputum, and one isolate from burns and bronchitis. The 23 isolates under study were tested for their susceptibility to 11 penicillin, cephalosporin, and carbapenem antibiotics, including beta-lactam and other antibiotics. The results showed high resistance of *K. pneumoniae* to piperacillin at a rate of 91%, and then to ampicillin at a rate of 86.9%, while were sensitive to both imipenem and meropenem, at a rate of 78%. The results showed that 100% of the isolates showed their ability to produce beta-lactamase enzymes as confirmed by the acid and mineral methods. The high percentage of enzymatic production indicates the presence of enzymatic resistance among bacterial isolates under study, which is why the bacteria gave high resistance to antibiotics. The results of the study showed the ability of 14 isolates to produce broad-spectrum beta-lactamase enzymes at a rate of 60%.

Keywords: *Klebsiella pneumoniae, Bacterial pathogens, Beta-lactamase, Iraq*
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I. INTRODUCTION

The importance of Gram-negative bacteria has increased not only because of their ability to cause hospital infections but also because of the increasing spread of their resistance to many antibiotics, which has become a common health problem worldwide (Villegas *et al*., 2011). *Klebsiella pneumoniae* is widespread and is considered an opportunistic pathogen and usually causes many pathological conditions, such as respiratory infection, wound infection, blood poisoning, diarrhea, and urinary tract infection (Shah *et al*., 2010). Khalili and his group found that 50-60% of hospital-acquired infections associated with health care units in Tehran hospitals (Iran) are caused by Gram-negative bacteria and that the resistance of Gram-negative bacteria to antimicrobials had increased over the past ten years (Khalili *et al*., 2012). Several studies have indicated a worrying increase in the resistance of Gram-negative bacterial species to antimicrobials, especially beta-lactam antibiotics that are commonly used in the treatment of hospital-acquired infections or community-related infections. Such studies found multiple resistance to many pathogens such as *K. pneumoniae*, *E. coli*, and *A. baumannii*. These studies also indicated that the continued use of beta-lactams to treat negative bacterial infections may be a prominent reason for the spread of resistance to these antibiotics (Shaikh *et al*., 2015). Failure to treat cases of infection with Gram-negative bacteria may be due to the intrinsic resistance and to the production of many types of efficient beta-lactamase enzymes, or the acquisition of resistance through antibiotic resistance gene transfer via bacterial conjugation. Continuous exposure of bacterial strains to a large number of beta-lactam antibiotics may stimulate the continued activity of bacteria to produce beta-lactamase enzymes and cause resistant mutations which leads to expanding their effectiveness against newly developed and therefore generate enzymes

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known as broad-spectrum beta-lactamases (EL Nobi *et al*., 2023; Mishra *et al*., 2012). Shaikh and his group (2015) pointed out the difficulty of solving the problem of the spread of broad-spectrum beta-lactamase producing strains to various reasons, including the difficulty of detection, as well as the inconsistency in the reports submitted (Shaikh *et al*., 2015). Recently, an increase in the prevalence of infections caused by strains producing these enzymes has been observed globally (Rupinder *et al*., 2013). Beta-lactam antibiotics are broad-spectrum antibiotics that include Penicillin and cephalosporins. They contain a lactam ring composed of three carbon atoms and one nitrogen atom as an amino structure. This ring is linked to a variable side chain, it is grouped by a peptide bond to form an effective antimicrobial agent. Beta-lactam antibiotics include five types: Penicillin (ampicillin, oxacillin), carbaciphems (Laracarbef), cephalosporins (cephalexin, cefaclor), penems (meropenem, Impenem), and monobactams (aztreonam) (Bashir *et al*., 2011). This group of antibiotic works to kill germs by stopping the process of bacterial cell wall synthesis (Kocsis and Szabo, 2013). These antibiotics work on the penicillin-binding proteins (PBPS), which work to bind the cell wall units N-acetylglucoseamine and N-acetylmuramic acid, that is, they work as transpeptidase, and the beta-lactam antibiotics bind with these proteins and stop the process of trans-peptidation (cross linking), as a result, bacteria will lose the barrier that protects them from external environmental fluctuations and leads to the lysis and death of the bacterial cell (Brooks *et al*., 2010). Some substances have a molecular structure similar to that of beta-lactam enzymes, but they have very weak activity against microbes, as they bind reversibly or irreversibly to these enzymes and prevent the decomposition of the beta-lactam ring, thus preventing the enzyme from working. These inhibitors include clavulinic acid, sulbactam, and tazobactam. These three inhibitors are effective against beta-lactam enzymes belonging to Gram-negative bacilli (Patel *et al*., 2010; Al-Sammak *et al*., 2009). Resistance to antibiotics is most likely a result of their possession of beta-lactamase enzymes. Which have genes located on the chromosome or plasmids, in addition to the presence on other genetic elements called transposon genes. Gram-negative bacteria, especially members of the Enterobacteriaceae family, possess beta-lactamase, which are produced in the periplasmic space. These enzymes work to decompose the beta-lactam ring and transform it into an inactive form (Mims *et al*., 2004).

II. Materials And Methods

A. Sample collection

186 samples were collected from various pathogenic specimens, including 75 urine samples, 46 cerebrospinal fluid samples, 23 sputum samples, 9 body fluid samples, 14 blood samples, 4 discharge samples, 11 fluid samples from wounds and burns, and 4 bone marrow samples during the period from June to September, 2023 from patients visiting Ibn Sina Teaching Hospital, Ibn Al-Atheer Teaching Hospital, and Mosul General Hospital Mosul city-Iraq.

B. Isolation and identification

Samples were cultured on blood agar and MacConkey agar plates and incubated for 24-48 hours at a 37°C. Individual colonies were transferred to nutrient agar slants and kept in the refrigerator for further identification, provided that they were renewed monthly (Vandepitte *et al*., 2003).

The samples were initially diagnosed based on their morphological and cultural characteristics on blood agar and MacConkey agar media in terms of the colonies, their height, shape, texture, smell, their ability to analyze blood, and the color and height of the colonies. Suspected colonies wer stained with Gram stain to observe the shape of the cells and their interaction with the dye (Khaleel *et al*., 2019)

To further confirm the identification, API 20E strips (BioMeurix, France) was used for the purpose of diagnosing some Gram-negative bacteria following the insructions by the manufacturing company. Finally, suspected strains were further identified using VITEK-2 system (Ahmed and Faisal, 2008).

C. Antibiotic susceptibility testing

The susceptibility of *K. pneumoniae* isolates towards 11 antibiotics was tested using the disk diffusion method. The antibiotics Ceftriazone 10Mg/ml, Amikacin 10Mg/ml, Gentamicin 10Mg/ml, Ciprofloxacin 10Mg/ml, Piperacillin 30Mg/ml, Augmantin 30Mg/ml, Imipenem 10Mg/ml, Meropenem 10Mg/ml, Cefotaxime 10Mg/ml, Ceftazidime 10Mg/ml, and Ampicillin 30 Mg/ml were used as disks and obtained from Bio Analyse, Turkey (Forbes *et al*., 2007).

D. Acidimetric method for detecting beta-lactamases

The basis of this method is based on the fact that the decomposition of the beta-lactam ring generates a carboxyl group that lowers the pH of the medium, which can be detected in test tubes or on filter papers. We used the tube method as follows: The reagent was prepared by adding 2mL of phenol red solution and diluted by adding (16.6) cm of distilled water, then (1.2) g of penicillin G was added, adjusted at pH (8.5) using 1M NaOH. This violet-colored reagent was stored at -20ºC until used. To perform the test, 100µL of the reagent was added to each tube. The tubes were inoculated with fresh *K. pneumoniae* culture and left at room temperature for 5 minutes. The presence of a yellow color is an evidence of the production of beta-lactamase enzymes (Livermore and Brown, 2001).

E. Detection of Extended-spectrum ß – Lactamases

For this purpose, two different methods were used as follows:

1.Detection by NCCLS Method

The NCCLS recommendations were adopted in screening for this group of enzymes based on their minimum inhibitory concentration (MIC) values for the antibiotics Cefotaxime, Ceftriaxone, Ceftazidime, and cefepime. If the MIC values for these or one of the antibiotics were higher or equal to 2 µg/mL or for the anti-cefoxim with a value of more or equal to 8 µg/mL, then the production of ESBLS enzymes is suspected and initial confirmatory screening tests are conducted for the production of these enzymes (Queenan *et al*., 2004). Initial confirmation was done as follows: A bacterial suspension equivalent in turbidity to the first tube No. (0.5) was prepared from standard McFarland tubes, and the nutrient agar medium was inoculated. The antibiotics Cephotaxim, Ceftriaxone, and Ceftazidime were distributed on the surface of the medium at equal distances, and the plates were incubated at a temperature of (37) °C for (16) days - (18) hours. The inhibition zone was then measured and compared with the NCCLs table for this test. Positive isolates were identified for this test (Samaha and Arag, 2003).

2.Detection of Metalloß-lactamase by Imipenem- EDTA Disk Method

This method is based on the inhibitory effect of EDTA solution on metal beta-lactamase enzymes. The agar medium was inoculated with Gram-negative bacteria. Two Imipenem discs (10 Mg) were placed on the surface of the Muller-Hinton culture medium and 5µL of EDTA solution with a concentration of (750 Mg) was added to one of the two tablets. The same method was repeated by adding 5 µL (292 Mg) of the EDTA concentration for the other plate instead of the concentration (750 mg). The plates were incubated at 37°C for 16-18 hours. The positive result of this test is an increase in the inhibition zone of the EDTA + Imipenem disc, which is evidence of the presence of metallo-beta-lactamase enzymes.

III. RESULTS

A. Isolation and identification

23 isolates of *K. pneumoniae* were isolated from a total of 186 pathological samples collected from various sources. The isolates identified were 12 from urine samples, three isolates from cerebrospinal fluid, two each from catheters, wounds, and sputum, and one sample from burns and bronchitis.

B. Antibiotic resistance of K. pneumoniae

The 23 isolates under study were tested for their susceptibility to 11 Penicillins, cephalosporins, and carbapenem antibiotics, including beta-lactam antibiotics and a group of other antibiotics. The results showed high resistance of *K. pneumoniae* to piperacillin at a rate of 91%, and then to ampicillin at a rate of 86.9%, while were sensitive to both Imipenem and meropenem, at a rate of 78% (Figure 1-1).

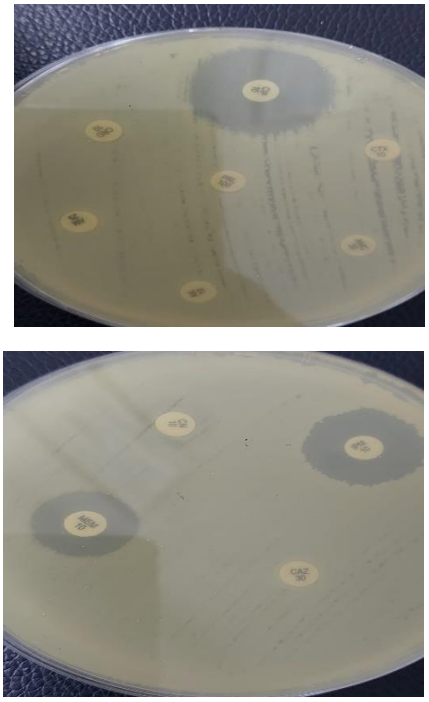

Figure 1. Antibiotic susceptibility of *K. pneumoniae*

C. Detection of beta-lactamase enzymes

Enterobacteriaceae members are known to produce beta-lactamase enzymes, and this family includes the genus *Klebsiella*. Therefore, the production of this enzyme was investigated in this genus using three methods, the acid method, the mineral method, and the broad-spectrum method. The results showed that all isolates showed their ability to produce beta-lactamase enzymes by the acid method and the mineral method figures (2 and 3).

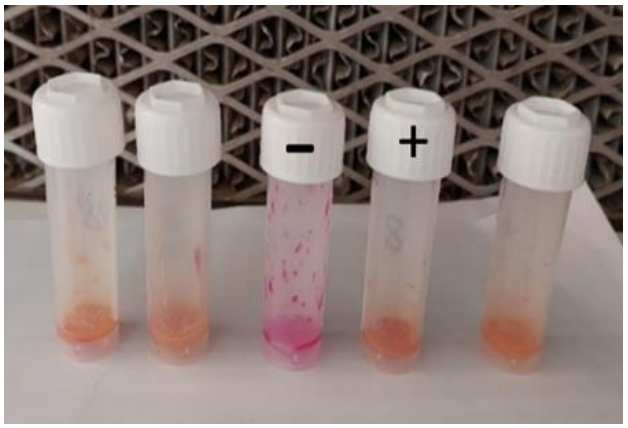

Figure 2. Production of beta-lactamase using the acid method

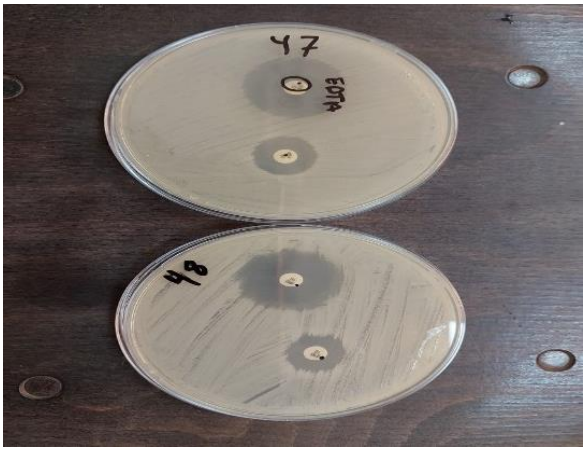

Figure 3. Production of beta-lactamase using the metallic method

The study also conducted a test to detect broad-spectrum beta-lactamase enzymes using the double-disc synthesis method (Figure 4). The study's results showed the ability of 14 isolates to produce broad-spectrum beta-lactamase enzymes at a rate of 60%.

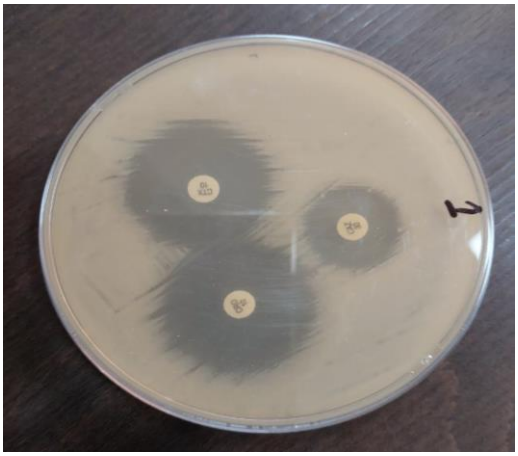

Figure 4. Production of broad-spectrum beta-lactamase

IV. Discussion

Identification of isolates was determined based on cultural experiments, and morphological, microscopic, and biochemical tests, and the diagnostic results were supported using the API20E diagnostic system and the VITEK-2 system. The study found that K. pneumoniae exhibits high resistance to piperacillin (91%), ampicillin (86.7%), and imipenem and meropenem (78%), among other antibiotics. These results agreed with (Khaleel *et al.,* 2019), where bacterial susceptibility varies in local, international, and Arab studies according to geographical regions and health awareness regarding antibiotics usage among patients. This causes an increase in resistance of bacteria causing disease, which in turn increases public health problems, as this study came close to the study of Khalili *et al.* (2012) and a study by Nabi *et al*. (2014) in Islamabad.

Five *K. pneumoniae* isolates were resistant to all antibiotics. The high level of resistance to antibiotics comes as a result of many factors, including the period of residence in the hospital, the random and wide-range use of antibiotics, and the irrational use of antibiotics, especially for isolates that possess beta-lactamase enzymes. This was confirmed by the results of the detection of beta-lactamase enzymes, as well as depending on broad-spectrum beta-lactamase enzymes (Shah *et al*., 2010).

The findings demonstrated that all isolates could manufacture beta-lactamase enzymes using both the mineral technique and the acid method figures. This percentage of enzymatic production indicates the presence of enzymatic resistance among bacterial isolates under study, which is why the bacteria gave high resistance to antibiotics, and these results were in agreement with (Khalili *et al.*, 2012) and Wenzel (2003) who pointed out that the increased resistance of bacteria to antibiotics such as penicillins and cephalosporins is due to their production of beta-lactamase enzymes, as the percentage of production of beta-lactamase enzymes varied in the study of Khaleel *et al* . (2019), reaching 50%, as well as in the study of Kand *et al*. (2005), the percentage of *Klebsiella* species producing the enzyme was 50%. The production of beta-lactamase enzymes by these isolates are useful in avoiding the use of antibiotics sensitive to these enzymes in treatment, as well as providing early evidence to doctors of the effectiveness of the antibiotic for treatment.

The findings showed that 14 isolates possessed a 60% production rate for broad-spectrum beta-lactamase enzymes. These results were close to the study of Batchoun *et al*. (2009) in Jordan. While the isolation results for broad-spectrum beta-lactamase enzymes were about 93% of the total isolates, although the dominant mechanism for antibiotic resistance in bacteria is the production of beta-lactamase enzymes, the most common of which are broad-spectrum beta-lactamase enzymes.

The production of various types of beta-lactamase enzymes is not the only mechanism by which gram-negative bacteria can resist beta-lactam antibiotics. In addition, Gram-negative bacteria can create internal resistance by reducing the amount of proteins associated with penicillin or by possessing resistance genes carried on the plasmid or acquired through mutations. Also, Gram-negative bacteria may contain other enzymes that differ from beta-lactamase enzymes, so a difference in isolation rates may occur, with some enzymes dominating in other species. The spread of bacterial strains producing different beta-lactamase enzymes in any hospital depends on the method of using antibiotics and the rate of transmission of the producing strains. Among people working

and lying in hospitals and the type of sterilization used in hospital units, especially in intensive care units, and this was confirmed by Chang *et al*. (2017), as well as confirmed by the study of Khaleel *et al*. (2019).

V. CONCLUSION

The increasing spread of bacterial resistance to many antibiotics has become a common health problem worldwide. Most infections caused by these bacterial groups are common among patients with septicemia, pneumonia, urinary tract infections, infections arising after surgical operations, and patients with burns and wounds in hospitals. The current work detected high ability of *K. pneumoniae* to produce beta-lactamase enzymes as confirmed by the acid method and the mineral method. The results of the study showed that 60% of the isolates were broad-spectrum beta-lactamase producers which reflects the danger of *K. pneumoniae* strains.

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