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Hypervirulent (hypermucoviscosity) *Klebsiella pneumoniae* isolated from clinical samples in Al-Diwaniyah Hospital

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Abstract---*Klebsiella pneumoniae* is not only one of the major pathogens acquired from hospital, but it is also one of the pathogens transmitted through urine samples that causes the liver Abscesses, blood poisoning, diarrhea. The study aimed to isolate the Hypervirulent *Klebsiella pneumoniae* (Hvkp) bacteria from clinical samples in Al-Diwaniyah city / Iraq. And evaluation of the efficiency of some antibiotics in inhibiting isolates. The study extended from January to mid-April. A total of 50 isolates identified as *Klebsiella pneumoniae* using traditional biochemical tests, Of them, 13 isolates were hypervirulent, with a percentage of 26% of the studied isolates. A drug sensitivity test was conducted for 10 antibiotics and they were as follows (Amoxicillin – clavulanat, Cefotaxime, Ceftazidime, Ceftriaxone, Cefoxitin, Imipenem, Meropenem, Azteronom, Amikacin and Gentamicin) The ratio of resistance to an antibody Amoxicillin – clavulanat have reached 50% While the percentage of resistance to Cefotaxime 46% While the percentage of resistance to Ceftazidime 56% While the percentage of resistance Ceftriaxone 60% While the percentage of resistance to Cefoxitin, Imipenem, Meropenem, Azteronom, Amikacin and Gentamicin Straight (56%, 20%, 26%, 40%, 46%, 56%)

Keywords---Hypervirulent *Klebsiella pneumoniae*, These isolates are detected by String method, Drug sensitivity test was conducted by disk diffusion to antibiotics.

Introduction

Klebsiella pneumoniae is a common opportunistic bacteria that can cause septicemia, liver abscesses, diarrhea, and pneumonia in humans. (Guo et al.,2017) . It is one of the most well-known pathogens acquired in hospitals, and it

has been linked to increased morbidity and mortality in patients. (Cabral et al., 2012). In addition, *K. pneumoniae* has the potential to induce community-acquired infections in healthy people, such as liver abscess, endophthalmitis, and meningitis. In addition, *K. pneumoniae* has the potential to induce community-acquired infections in healthy people, such as liver abscess, endophthalmitis, and meningitis. (Russo et al., 2015)

In the 1980s, a rare case of *K. pneumoniae*-induced liver abscess with endophthalmitis was recorded in Taiwan, and the causative organism was identified as hypervirulent *K. pneumoniae* (hvKP) (Liu et al., 1986). Since then, hvKP has been recognized as another circulating pathotype in addition to classical *K. pneumoniae* (cKP), associated with high pathogenicity and mortality due to hypervirulence (Lan et al., 2020).

Factors contributing to the hypervirulence mainly include capsule, siderophores, lipopolysaccharide (LPS) and fimbriae (Parrott et al., 2020). The string test based on hypermucoviscous phenotype (string ≥ 5 mm) was widely used as a marker for hvKp, with ~90% predicted accuracy for clinical hvKp strains (Russo et al., 2018). This semi-qualitative assay is easily influenced by colony conditions and the user's technique (Tan et al., 2014). Also, cKP strains with mucoviscosity and hvKP strains without hypermucoviscosity have been identified (Catalán-Nájera et al., 2017). Another mucoviscosity assay was quantified by comparing the absorbance of supernatants collected by low-speed centrifugation because hypermucoviscous strains cannot sediment sufficiently, leaving the supernatants turbid (Walker and Miller, 2020).

Methods

This study was conducted from (15/1/2022) to (1/4/2022). It included taking samples from clinical samples of urine, sputum, wounds and burns in Al-Diwaniyah city.

Isolation and diagnosis

The samples were initially planted on the medium of MacConkey by Streaking method and the plates were incubated at 37°C for 24 hours. This medium was used to distinguish between fermented and non-fermenting bacteria of lactose (Where the colonies appear pink) While the non-fermenting bacteria had colorless or light beige colonies.

Morphological Characterizations

It was observed that the growth of the developing colonies, their shapes, their distinctive smell, and their fermentation of lactose sugar on the medium of the MacConkey agar, and the pattern of hemolysis on the medium of the blood agar.

Microscopical Characterizations

The colonies were initially diagnosed based on their morphological and cultural characteristics on the Macconkey agar in terms of their ability to ferment

lactose sugar, the size of the colonies and their strength in terms of their ability to produce and their total inputs of the substance

Testing Susceptibility Antibiotic

The antibiotic sensitivity of *K.pneumoniae* isolates was tested on MHA using the Bauer–Kirby method diffusion disc.. The bacterial suspension of *K.pneumoniae* isolates was prepared by transferring 3-4 colonies at 24 hours of age growing on the medium of Macconkey aquarium to 5 ml of physiological saline solution. Then a sterile cotton swab was dipped in the bacterial suspension solution and by diffusion method the sample is spread throughout the dish and evenly after that the tablets are placed evenly on the medium and then incubated at a temperature of 37 for 24 hours.

Test String

This test was carried out by growing the bacteria at a temperature of 37, then using a lube to extend the bacterial colonies. Any sticky formed with a length of more than 5 mm was considered to give a positive result. Otherwise, it is a negative result. The positive bacteria were counted as HvKp, while the negative result was a bacteria. Classic Ckp

Positive results Any sticky formed with a length of more than 5 mm

Negative results Non formed

Result

A total of 50 isolates identified as *Klebsiella pneumonia* using traditional biochemical tests, Of them, 13 isolates were hypervirulent, with a percentage of 26%.

Table (1): Percentage (%) of Hypervirulent *K.pneumoniae* in Al-Diwaniyahcity

type of isolation	No.	(%)
Hypervirulent <i>K.pneumoniae</i>	13	26%
Total	50	100%

*Of the 50 isolates clinical samples, 13 samples were diagnosed with Hypervirulent *K.pneumoniae*.

Table (2): Explain the ages of Patients infected with Hypervirulent *K.pneumoniae*

Age	Hypervirulent <i>K.pneumoniae</i> positive	Total (%)
21-30	3	23.7%
31-40	6	46.2%
41-50	4	30.1%
51-60	0	0%

* The largest recorded cases of hypervirulent *K.pneumoniae* infection were for patients aged 31-40, while no infection was recorded in patients aged 51-60.

Table (3): The number of isolates that were resistant to antibiotics with percentages in the studied isolates

Name antibiotic	The number of resistant isolates	(%)
Amoxicillin – clavulanat	25	50%
Cefotaxime	23	46%
Ceftazidime	28	56%
Ceftriaxone	30	60%
Cefoxitin	28	56%
Imipenem	10	20%
Meropenem	13	26%
Azteronam	20	40%
Amikacin	23	46%
Gentamicin	28	56%

* The results show the highest percentage of resistance to the antibiotic Ceftriaxone while the lowest percentage of resistance to the antibiotic appears Imipenem

Discussion

Fifty isolates were diagnosed as *Klebsiella pneumoniae*, of which 13 (26%) were hypervirulent. These results are in contradiction to the study .(Kim et al. 2017) The study recorded 13% of isolates This difference is explained by the different sources and places of sample collection While these results are similar to the study (El-Mahdy et al.,2018) The study recorded 26% of isolates. Through table 2 The largest recorded cases of highly virulent K.pneumoniae infection were in patients aged 31-40 years, while no infection was recorded in patients aged 51-60 years. These results were similar to the study (Shon et al.,2013) Where the rate of infection in this bacterium was in the first degree between the ages of 31-40.

While these results contradiction to the study.(Cubero et al.,2016) The study recorded the rate of infection in this bacterium was in the first degree between the ages of 51-60 This difference is explained by the difference in the place of study, as it is known about Barcelona that its climate is cold and temperate throughout the year. (Rodríguez Algeciras & Matzarakis, 2016).

During the 3 table, The results show the highest percentage of resistance to the antibiotic Ceftriaxone while the lowest percentage of resistance to the antibiotic appears Imipenem These results were similar to the study (Bakhtiari et al.,2021) The study recorded the lowest percentage of resistance to the antibiotic appears Imipenem 20% and highest percentage of resistance to the antibiotic Ceftriaxone 60%

While these results contradiction to the study (Mohamed et al.,2018) The study recorded highest percentage of resistance to the antibiotic Amoxicillin – clavulanat and lowest percentage of resistance to the antibiotic appears Meropenem This difference is attributed to the places where the samples were isolated in addition

to the age of the patients isolated from them, according to what was mentioned in (Sprang & Silman , 2013).

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