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Distribution of *Klebsiella pneumoniae* Isolated from Different Clinical Samples in Al-Diwaniyah City

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ABSTRACT

Klebsiella spp. mainly Klebsiella pneumonia has been medically recognized as common cause of community and nosocomial acquired diseases all over the world . The Klebsiella associated with infections is suffering a great level of anti-agents resistance bacteria strains mostly those involved in hospital infections due to evolution and modifications in their genomes .So, the aim of this study was to detect of Klebsiella pneumoniae bacteria from different clinical sources of human and identification by VITEK 2-Compact system. . The results show that Klebsiella pneumoniae were isolated and identified 55 (62.5%) Out of the 1 total number of (150) different clinical samples from patients in Al-Diwaniyah Teaching Hospital and Maternity and Children Hospital in Al-Diwaniyah city during the period from July to December 2019 .The number of K. pneumonia isolates from burns and wounds was 6 (60%) ,urine samples 11(34.37%) sputum samples 32(80%) , and blood samples 6 (100 %). The results of the statistical analysis showed very significant differences indicating a higher level of Klebsiella pneumoniae in sputum.

1-INTRODUCTION

The genus Klebsiella placed in the group of klebsiellae, it is a member of the Enterobacteriaceae family, it was in the 19th century (1834), that this bacterium was discovered by the scientist German Edwin Klebs and named after that. Also, the scientist Von Frisch was the first diagnosed it at 1882, he isolate this immotile bacteria surrounded by a capsule from the lungs of lobed pneumonia people. Then in 1883 (one year later), the same bacilli has been isolated by Friedlander from patient whom died due to pneumonia, thus this species called (Calfeea, 2017).

Klebsiella pneumonia has been medically recognized as one of the most important opportunistic pathogens, causing healthcare- associated infections (HAIs) worldwide such as urinary tract, pulmonary, blood and soft

tissue infections, is part of the Klebsiella sp. as described previously. It considered as a saprophyte in humans and other mammals colonizing the gastrointestinal tract, skin, and nasopharynx, soil, waters and on medical devices. (Paczosa and Mecsas, 2016). It is the second pathogen bacteria, following to Escherichia coli that causes urinary tract infection, It usually affects people with weakened immune systems such as hospital patients, diabetes patients and people with chronic lung disease, alcoholics (Moore et al., 2016). Klebsiella pneumonia is provided virulence by several factors that lead to antibiotic resistance and infections. The polysaccharide capsule of this bacteria is the most important virulence factor and allows the bacteria to evade opsonization and phagocytosis and killing by the host (Li, 2014).

Lipopolysaccharides is the second virulence factor that covering the surface of a this bacteria. It is the major factor in septic shock (Hentschke et al., 2010). Moreover, fimbriae is other virulence factor allows the organism to attach to host cells. Furthermore, siderophores are another virulence factor that cause infection in the host, it is acquire iron from the host to allow spread the infection (Wyres and Holt, 2016) .So, the aim of this study was to detect of Klebsiella pneumoniae bacteria from different clinical sources of human and identification by VITEK 2-Compact system.

The genus Klebsiella pneumonia:

General features of bacteria:

Klebsiella sp. is immotile gram-negative bacteria. They are shorter and thicker in comparison with other Enterobacteriaceae family. They typically occur as rods with rounded ends, they can be found in short chains singly, in pairs, or single cells. In vivo commonly found the forms Diplobacilli (Jump up et al., 2004). They are facultative anaerobes $0.6 - 6.0 \mu m$ in length and $0.3 - 1.0 \mu m$ in width, Characteristically, Klebsiell sp. colonies show a pink large smooth mucoid on the MaCconkey medium, and they has ability to ferment lactose, sucrose and manitol, in addition to produce CO2 gas and acid, Klebsiella sp. are indole and methyl red negative, and positive reaction of voges proskauer test with the ability to utilization of citrate as the only source of carbon, it is also capable to reduce nitrates to nitrite, and does not produce H₂S, it is that is non-hemolytic on blood agar and some of them have the ability to consume urea by production urease (Goldman and green . 2009)

Classification of Klebsiella pneumonia

Klebsiella sp. Classified in to several Genuses according to the variation of biochemical reactions and difference at the DNA level, where some species of Klebsiella is derived from the disease that caused by each species when it is discovered, there are three species of *Klebsiella* that associated with humans illness: *K*. pneumonia, Klebsiella oxytoca, and K. granulomatis. (Brenner et al., 2005)

2-Materials and Methods

Samples collection:

A total of (150) samples were collected during the July to December of 2019 from the Al-Diwaniyah Teaching Hospital and Maternity and Children Hospital patients . These samples included: burns and wounds, Urine, sputum and blood samples. Burn and wound samples were collected using sterile cotton swabs, while the urine and sputum samples were collected in sterile plastic containers.

Culturing of isolates

After collecting the samples have been cultured directly on the blood agar media, even activated and allowed most of the bacterial genus to grow and incubated at 37°C for 24 hrs., also cultured at same time on MacConkey agar media to be considered selective media allowed growth gram negative bacteria and it is also considered differential media by which to differentiate between bacteria fermented and non-fermented lactose, and incubated for 24 hrs. at 37°C. The colony were translated and colonies characterized by pink and viscosity to other MacConkey agar media for purification, and continued recultured on this media for getting axenic culture(MacFaddin, 2000).

Identification via VITEK 2-Compact:-

VITEK-2 Compact is the next generation of rapid identifications of gram- negative and gram- positive bacteria in system of clinical that has been implemented according to manufacturer's instructions.

3-Results and Discussion Isolation and diagnosis of *Klebsiella pneumonia*

In this study 150 samples were collected, as were as following: wounds and burns (15) samples, urine (55) samples, sputum (55) samples, and blood (25) samples. The results shown in Table (4-1) represented that samples 107 (71.33%) of the total samples gave a positive result for bacterial growth. These samples were distributed among burns and wounds samples 12 (80%), urine samples 40 (72.72%), sputum samples 45(81.8%), and blood samples 10 (40%). The bacteria *Klebsiella sp.* which isolated and diagnosed in the present study, all the samples from different sources were collected in July to December of 2019 from Maternity and Children Hospital and Al-Diwaniyah Teaching Hospital. From the total growth positive; *Klebsiella sp.* number was88(88.24%), out of this number; this bacteria was distributed among different sample, burns and wounds were 10 (83.33%), urine 32(80%), sputum 40(88.88%) and blood 6(60%). Out of the total number of *Klebsiella sp. Klebsiella pneumonia* was 55 (62.5%), which isolated and identified, in burns and wounds *K. pneumonia* was 6 (60%), urine samples 11(34.37%) sputum samples 32(80%), and blood samples 6 (100%). the highest percentage of *K. pneumoniae* was in the samples of sputum when it reached 80%, Compared with the isolating these bacteria from the urine, burn and wounds samples.

The positive samples were diagnosed according to culture characteristics and biochemical tests via VITEK 2-Compact System it was found that (55) isolates belong to *Klebsiella pneumonia* bacteria. The colonies were large, pink and the lactose fermentation, shiny with diffusing red or pink pigment on MacConkey agar indicating fermentation of glucose producing acid (Fig - 1), oxidase negative, Indole negative, Methyl-Red test negative, Voges- Proskauer Test positive, Citrate Utilization Test positive, Urea Hydrolysis Test positive and catalase positive, accetate utilized unproductive of H_2S and the results of microscopy showed that the bacteria were bacilli and Gram-negative, non- using sporulating Edwards & Ewing (1986).

Table (1) Distribution of study samples according to their source, number and percentage of samples that gave a positive result for bacterial growth.

Source of sample	No. of samples	No. & % of Positive growth	No. & % of Klebsiella sp.	No. & % of K. pneumonia from total samples	% of K. pneumonia from total Klebsiella isolates
Burns	15	12(80)	10(66.66)	6(40)	60
and					
wounds					
Urine	55	40(72.72)	32(58.18)	11(20)	34.37
Sputum	55	45(81.8)	40(72.72)	32(58.18)	80
Blood	25	10(40)	6(24)	6(24)	100
Total	150	107(71.33)	88(58.66)	55(36.66)	62.5
		15.56	17.27	19.34	19.65
Calculate d X ²					
P value		0.004(HS)	0.002(HS)	0.001(HS)	0.001(HS)



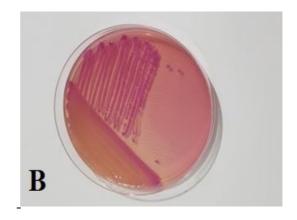




Fig -1 (A,B): Show colonies of Klebsiella pneumonia grow blood and MacConkey agar.

(C): Show mucoid appearance of Klebsiella pneumonia on MacConkey agar plate

4- Conclusion

The results of this study conclude that the *Klebsiella pneumonia* bacteria is a higher level in sputum samples associated with *pneumonia* infections is suffering a great level of anti-agents—resistance bacteria strains particularly those involved in nosocomial diseases due to development and alterations in there genomes.

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