Nat. Volatiles & Essent. Oils, 2021; 8(4): 7531-7538

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Natural Volatiles &
Essential Oils

Isolation And Molecular Detection Of Klebsiella Pneumoniae Isolated From Patients With Oral Inflammation Plus Pneumonia And Its Effect On Secretory Iga Levels

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Abstract:

The study's purpose was to find out whether Klebsiella pneumoniae could be isolated and detected molecularly from patients with oral inflammation and pneumonia, and then estimate the impact of the bacteria on IgA.The investigation was carried out at the Microbiology Department of the university. 50 samples were taken from individuals with mouth ulcers who had discomfort and redness around the ulcers, as well as symptoms of pneumonia, which were detected on X-rays. The patients were made aware of the research and gave their permission before the swabs and samples collected. Aseptically obtained samples included mouth ulcer swabs and transtracheal wash. Using a disposable 5 ml dropper, we collected the stimulated saliva and then quickly frozen it in a refrigerator (-10^{o}C). We used the ELISA technique to quantify the IgA in saliva, the kit purchased from Mybiosource company, the method was done according to the manufacturer instructions. Also, 5 ml of blood collected from patients with pneumonia for IgA estimation. PCR method done by using primers to determine the genotype of the rmpA gene. It was determined that the patients provided a total of 50 samples for this investigation. Results showed that 39 (78 percent) of the samples tested had bacterial growth, 14 of them were diagnosed as K. pneumonia , whereas 11 (22 percent) of the samples tested negative for growth. The PCR results revealed the amplification of the tested rmpA gene (85.7%) in the oral samples, whereas the percent of pneumonic patients samples was 88.9% and 92.9% of oral plus pneumonic samples. The results showed that no correlation between infection with K. pneumonia with IgA levels in patients with oral lesion , while it showed significant increase in the IgA levels in patients with pneumonia.

In conclusion, The prevalence of k. pneumonia was important pathogen detected in oral, lung or both and it cause increase in IgA levels in patients with pneumonia.

Keywords: k. pneumonia, PCR, ELISA, oral, IgA.

Introduction:

As one of the most significant Enterobacteraceae genera, Klebsiella pneumoniae is well-known. It is a Gram-negative bacillus that is nonmotile, lactose fermented, and does not produce spores. The thick margins seen under a microscope are caused by the edges curving outward and having rounded ends, and the capsule boosts the pathogenicity of the bacterium (Braun et al., 2004). During the nineteenth century, it was given this name in honor of its detected by Edwin Klebs, who made the discovery back in 1834. (Brise et al. 2005).

There are a number of opportunistic illnesses in which K.pneumoniaeis an significant causative agent. These include infections of the respiratory system as well as inflammation of the skin and wounds.

After Escherichia coli, it is the most predominantbacteria. Diabetics, drinkers, lung deficiencies, immune suppressive patients and those in critical care units are at greater risk of infection, thus early and precise diagnosis is necessary to avoid infection in hospitals (Li et al.,2012; Chiu et al.,2013; Guo et al.,2016).

Trabulsi (1991) only mentioned K. pneumoniae colonization in the nasal canals, but Burnet et al. (1978) thought it was a natural component in oral and nasal cavities. Other research has shown that vegetarians' dental plaque (Sedgley et al., 1996) and the plasma of AIDS patients are more likely to have it (Zambom et al., 1990).However, K. pneumoniae-infected mouth ulcers are rare, especially in radiation treatment patients (Finegold and Martin, 1983)

There are several virulence factors found in K. pneumoniae bacteria, including capsule,polysaccharide, iron siderophore, serum resistance, enterotoxin as well as urea synthesis. This bacterium is very aggressive, and it has developed resistance to a wide range of medications (Navan-venzia et al., 2017; Dubey et al., 2013).

It is critical to use genotyping methods to determine the genetic attractionamongisolates of bacteria, classify bacteria, as well as pinpoint the source of infection before isolating and characterizing the most virulent strain. Genotyping may be done using a variety of methods, one of which is PCR, which is a quick and simple procedure that doesn'trequired a lot of time or money (Goudarzi et al., 2011).

IgA is the predominant type immunoglobulin in the mixedsaliva and is recognized as the key secretory factor of theadaptive immunity in the mouth (Rashkovaet al., 2009).

The goal of this study was for Isolation and molecular finding of K. pneumoniae which isolated from patients with oral inflammation plus pneumonia with estimation the effects of this bacteria on IgA.

Materials and Methods:

The investigation was carried out at the Microbiology Department of the university. 50 samples were taken from individuals with mouth ulcers who had discomfort and redness around the ulcers, as well as symptoms of pneumonia, which were detected on X-rays. In the General Outpatients Department, they were given a registration number and their personal

information was taken: name, age, gender, profession (if applicable), contact information, and any other pertinent details.

The patients were made aware of the research and gave their permission before the swabs and samples collected. Aseptically obtained samples included mouth ulcer swabs and transtracheal wash. Following sample collection, the samples were promptly sent to the Microbiology Laboratory for examination. Using sterile swab sticks, researchers gathered samples aseptically. The patient's number and date of birth were clearly written on every samples.

The saliva was collected in the morning, on an empty stomach, after the salivation had been stimulated for two minutes with the chewing of neutral chewing gum standard (from the test "Saliva Chek" GC. Using a disposable 5 ml dropper, we collected the stimulated saliva and then quickly frozen it in a refrigerator (-10^{o}C). We used the ELISA technique to quantify the IgA in saliva, the kit purchased from Mybiosource company, the method was done according to the manufacturer instructions. Also, 5 ml of blood collected from patients with pneumonia for IgA estimation.

As detailed by Markey et al., (2013), oral swabs and transtracheal wash taken from the chosen patients were investigated microbiologically using a culture method and direct microscopy.

DNA extraction kit was used to extract the DNA of bacteriain accordance with the manufacturer's instructions (Promega,USA).

K.pneumoniae was genotyped using the PCR method using the following primers to determine the genotype of the rmpA gene:

F ACT GGG CTA CCT CTG CTT CA

R CTT GCA TGA GCC ATC TTT CA **536 bp**

This experiment's outcomes included the creation of bands of varying sizes. 2 µ of template DNA, Master Mix10µ, 2 µ of each primer as well as deionized distilled water6 µwere used to make the reaction mixture. According to manufacturer's specifications (Promga, USA), reaction conditions were programmed as follows (Mehr et al., 2017):

To begin, a single cycle of denaturation at 94° C for three minutes was used.

DNA amplification using a single 94° C cycle. Primer attachment to DNA templet took 35 cycles with each cycle including these steps:

A- Anneling stage: 1 minute of cycling at 48° C. Stage

B- Expansion: 2 minutes at 72° C.

C-A single cycle at 72° C for 5 minutes is the last extension stage

It took 80 minutes to separate the reaction products using an agarose gel (2 percent) with 5 µl of Ethidium bromide, and then UV light was used to image the DNA ladder (100-1500) base pair.

The computer application SPSS 16 was used to do the statistical analysis.

Results and discussions:

It was determined that the patients provided a total of 50 samples for this investigation. Table 1 shows that 39 (78 percent)of the samples tested had bacterial growth,14 of them were diagnosed as K. pneumonia, whereas 11 (22 percent) of the samples tested negative for growth(Fig. 1, 2).

Table 1. Klebsiella pneumonia among oral and lung samples

Type of sample	No growth	Bacterial growth (%)	K. pneumonia
Oral lesion	11 (22%)	7(17.9%)	4 (57.1%)
Pneumonia		18 (46.1%)	7 (38.9%)
Oral lesion + pneumonia		14 (35.9%)	3 (21.4%)
Total	11 (22%)	39 (78%)	14 (35.9%)

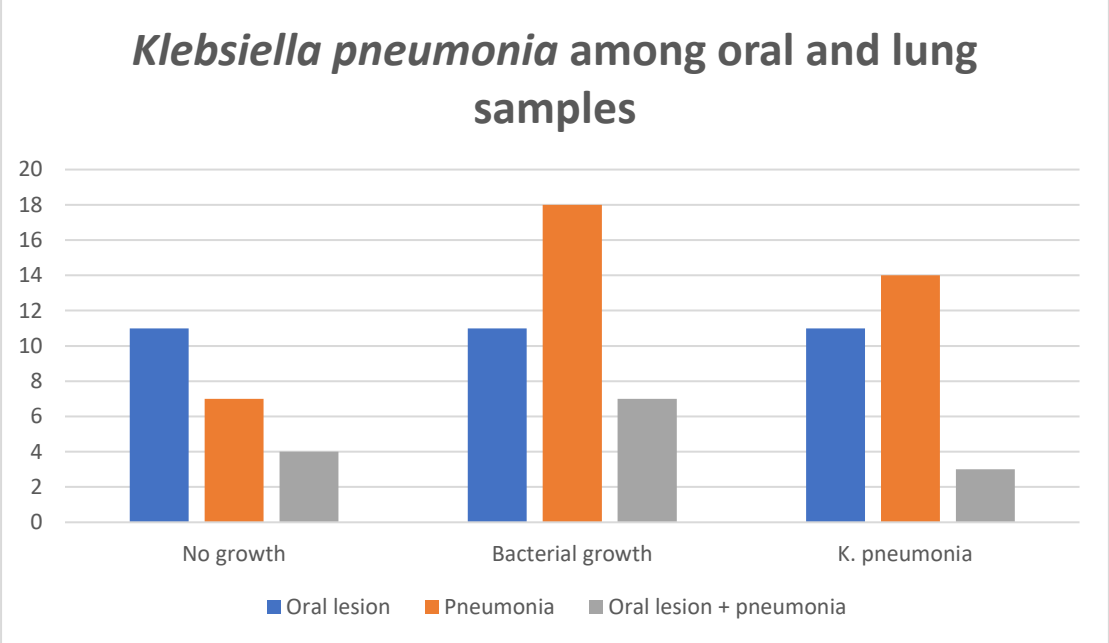

Figure 1.Klebsiella pneumonia among oral and lung samples

Figure 2. K. pneumonia on MacConkey agar

Klebsiella pneumoniae, owing to its frequency of isolation, according to its pathogenic and resistance mechanisms, is regarded an opportunistic pathogen that emerges as accountable for the etiology of respiratory infections acquired in the community (Expósito et al., 2018).

The current result was partially in agreement with results of Ikeda et al., (2018) who showed that the prevalence of Klebsiella pneumoniae isolated from clinical cases of pneumonia about 40-50%, also it was agreed with results of Anitha et al. (2016) who reported that prevalence of Klebsiella pneumoniae from oral lesion was high.

The PCR results revealed the amplification of the tested rmpA gene (85.7%) in the oral samples, whereas the percent of pneumonic patients samples was 88.9% and 92.9% of oral plus pneumonic samples(Table 2, figure 3).

Table 2. PCR results for rmpA gene of K. pneumonia among samples

Sample	No. of positive for rmpA gene	Percentage
Oral lesion	6	85.7
Pneumonia	16	88.9
Oral lesion + pneumonia	13	92.9

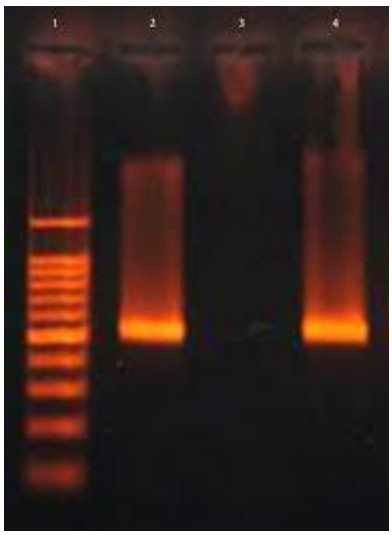

Figure 3. Agarose-gel image of rmpA at 536bp, gene amplification. M is the ladder

It has been reported that rmpA was used for confirmative identification
of the bacterium (Mohammed et al., 2020), Also,Al-aajem, 2020 reported that **rmpA** was very important gene for detection of K. pneumonia.
We followed DMF (T+t) by categorizing the patients studies in two ways to evaluate the relationship between IgA dependency and K. pneumonia infection.According to IgA, there are three distinct groups:

IgA has three levels of IgA: low (100 g/ml), medium (100-300 g/ml), and high (>300 g/ml).The results showed that no correlation between infection with K. pneumonia with IgA levels in patients with oral lesion , while it showed significant increase in the IgA levels in patients with pneumonia (Table 3).

Table 3. Correlation between IgA levels with infection by K. pneumonia

Sample	Mean ± SE
Oral lesion	**153.4±3.1 C**
Pneumonia	**308.6±7.5 A**
Oral lesion + pneumonia	**278.3±2.8 B**

Capital letters denotes significant differences between groups

Children's secretory immunity was not linked to dental caries, according to the different research, Because of their capacity to specifically link with plaque germs and so hinder their colonization on the enamel surface, SIgA play a role in the development of dental caries. As a result, they work against all other modes of microbe attachment and prevent plaque biofilm from forming. S.mutans and antigens of its enzymes and metabolic products are mostly associated with secretory IgA.However, in the actual oral environment, this very significant preventative activity of SIgA against dental caries is not as effective as it seems owing to the frequent washing off action of saliva and the inability to maintain a sufficient concentration of SIgA on the enamel surface (Rashkovaet al., 2009).

Rodríguez (2005) found that the processes by which IgA protects against infection require both antigenic specificity of antibodies in secretions andeither blocking or stimulating the pathogen's entry into the lungs, or bothUsing the lungs, the immune system is best able to develop locally, these results were in agreement with current results.

Conclusion:

The prevalence of k. pneumonia was important pathogen detected in oral, lung or both and it cause increase in IgA levels in patients with pneumonia.

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