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# Molecular Isolation of Bacteria Pathogenic from Lung Disorder Patients

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***Abstract: This study aimed to determine the bacterial species colonizing the Lung Disorder Patients in Central Al-Dewaniyah, Iraq on a microbiological and molecular level. Nasal swab samples were obtained from 20 samples attendants in the period of time extending from June to November 2020 in Al-Dewaniyah city, Iraq. Microbiological identification techniques were utilized to identify the bacterial species isolated. Molecular identification techniques based on PCR. Two bacterial species were isolated from both the nasal cavity and the oropharynx including, S. pnemonaie and H. influenza. The results of the current study showed after collecting 20 samples from patients suffering from respiratory symptoms, where samples were taken by cotton swab from the upper respiratory tract and cultured in the laboratory, where we found that 10 samples are positive S. pnemonaie and 7 positive samples to H. influenzae. The virulence genes for each of the two diagnosed bacteria were also examined, and it was found that there were 6 positive samples for each of the virulence genes for the bacteria S. pnemonaie. The results of the virulence genes for bacteria were H. influenzae 3 positive for a gene HMW2 and 4 positive for a gene hifA as shown in Table 1 and fig.1,2,3,4,5 and 6.***

## 1. INTRODUCTION

Respiratory infections are one of the most frequent syndromes in children at the community level (Bello et al.,2003). Respiratory tract infections can be divided into two large groups: upper respiratory tract infections and lower respiratory tract. Studies carried out in patients with upper respiratory tract infections have revealed that the main pathogen in this area is S. pyogenes, while in lower respiratory tract infections it is more common to find S. pneumonaie and H. influenzae (Bulter and Schawartz,1998 ; Preben et al.,1996). Normally, the upper respiratory tract is colonized early by relatively avirulent bacteria, such as Streptococcus of the viridans group, non-hemolytic Streptoccoccus, and diphtheroids and, transiently, by potential pathogens such as H. influenzae type b (Hib) and H. influenzae non typeable (Hint) (García et al.,2001). Upper respiratory tract infections associated with H. influenzae, especially nontypeable ones, are considered a cause of morbidity and mortality in infants and children under 10 years of age in developing countries. H. influenzae type b can also colonize the respiratory tract of healthy children, but its prevalence is lower. The prevalence of this microorganism in a healthy population is related to factors such as: a history of recent respiratory infection, previous antimicrobial therapy, attendance at nurseries and pre-schools, as well as a decrease in local nasopharyngeal defenses (cytokine deficiencies) (Guevara et al.,2004, Pittman,1999). Among the infections that affect the lower respiratory tract, pneumonia is the main cause of medical consultation with an incidence of 10 to 12 cases / 1 000 people per year. Pneumonia is an infection of the lung parenchyma that occurs in the extra-

hospital setting and, despite the introduction of new diagnostic methods and the important therapeutic arsenal that is available, it continues to be an entity that leads to a mortality that would globally be between 5, 00 and 7.00% (González and Semiología,1994). This disease, generally infectious, causes inflammation of the lung parenchyma and is characterized by the etiological agents that produce it (S. pneumoniae in 60.0%, H. influenzae serotype b, M. pneumoniae and K. pneumoniae) and the type of patient in which they take place (Culasso et al.,2001). The choice of a treatment plan for respiratory tract infections, especially for pneumonia, is empirical, based mainly on clinical and radiological studies, a fact that brings as a consequence a global concern about the growing resistance to antimicrobials, which is registered both in agents that infect hospitalized patients as well as those who acquire the disease in the community (Liñares et al.,1992, Molina et al.,2000). In the last two decades, a resurgence of bacterial infections has been observed at the community level, although it is a general biological phenomenon, the acquisition of resistance genes by practically all bacterial pathogens is one of the causes that contribute to this phenomenon (Tenover et al.,1997).

## 2. MATERIALS AND METHODS

### Collection of samples

Twenty samples collected in the group of patients with signs and symptoms of upper respiratory tract infection were: pharyngeal secretions in the group of patients with lower respiratory tract infections, sputum.

### Sample processing

A macroscopic study was performed on the sputum samples where the quantity, color and appearance were evaluated. A smear was performed on these, which was colored by the Gram method, to observe the presumptive morphology of the microorganisms (Koneman et al.,1999). All the collected samples were sown immediately, in the culture media, blood agar (AS) and Gelosa Chocolate base agar (GC) with 2% isovitalex, which were incubated in microaerophyll (3 to 5% C02) at 37 ° C for 24 hours and MacConkey agar which was incubated at 35 ° C for 24 hours in aerobiosis (Koneman et al.,1999). After incubation, macroscopic observation of the colonies in the different culture media was carried out by observing the characteristics and relative number of each type of colony recovered in the media; as well as changes in the environment that surrounds the colony that showed specific metabolic activities of the bacteria. Once the macroscopic study was carried out, a smear was made to color with the Gram method and establish the morphology and staining of the microorganism.

### DNA extraction and sequence analyses

DNA was extracted from isolates using the CTAB (N-cetyl-N, N,N trimethyl ammonium bromide) method described by Murray (The Human Microbiome Jumpstart Reference Strains, 2010) respectively:

| Primer | Sequence (5'-3') | | Product Size |
|---|---|---|---|
| **S. pnemonaie** | **F** | **5-AGTTTGAAACCTCGCGCAAC-3** | **515bp** |
| | **R** | **5-GTCGCTATCCTGATGCCGAA-3** | |
| ***entB*** | **F** | **5-GTCAACTGGGCCTTTGAGCCGTC-3** | **400bp** |
| | **R** | **5-TATGGGCGTAAACGCCGGTGAT-3** | |
| ***rmpA*** | **F** | **5-CATAAGAGTATTGGTTGACAG-3** | **461bp** |

| | | | |
|---|---|---|---|
| | **R** | **5-CTTGCATGAGCCATCTTTCA-3** | |
| **H. influenzae** | **F** | **5-TGCCGAACGAGTATCACAATTA-3** | **330 bp** |
| | **R** | **5-TCTTGCCACTGAAGATCTGTAA-3** | |
| **HMW2** | **F** | **5-GTCGCCCAGGGCACTGTAACCATT-3** | **731bp** |
| | **R** | **5-CCGCCCAGAATGGATATGTTGTAG-3** | |
| **hifA** | **F** | **5-ATGAAAAAAACACT(AT)CTTGGTAGC-3** | **650bp** |
| | **R** | **5-TTAT(CT)CGTAAGCAATT(GT)GGAAACC-3** | |

All PCRs and sequencing reactions were performed on a GeneAmp PCR System 9700 (Applied Biosystems).

**Analysis of the results**

The results obtained in the present study were expressed as a percentage through tables and graphs (Dawson and Robert,1997).

## 3. RESULTS

The results of the current study showed after collecting 20 samples from patients suffering from respiratory symptoms, where samples were taken by cotton swab from the upper respiratory tract and cultured in the laboratory, where we found that 10 samples are positive S. pnemonaie and 7 positive samples to H. influenzae. The virulence genes for each of the two diagnosed bacteria were also examined, and it was found that there were 6 positive samples for each of the virulence genes for the bacteria S. pnemonaie. The results of the virulence genes for bacteria were H. influenzae 3 positive for a gene HMW2 and 4 positive for a gene hifA as shown in Table 1 and fig.1,2,3,4,5 and 6.

Table 1: PCR results to S. pnemonaie and H. influenza and virulence factor

| Samples | S. pnemonaie N(%) | *entB* N(%) | *rmpA* N(%) | H. influenzae N(%) | HMW2 N(%) | hifA N(%) |
|---|---|---|---|---|---|---|
| Positive | **10(50)** | **6(60)** | **6(60)** | **7(35)** | **3(30)** | **4(40)** |
| Negative | **10(50)** | **4(40)** | **4(40)** | **13(65)** | **7(70)** | **6(60)** |
| Total | **20(100)** | **10(100)** | **10(100)** | **20(100)** | **10(100)** | **10(100)** |

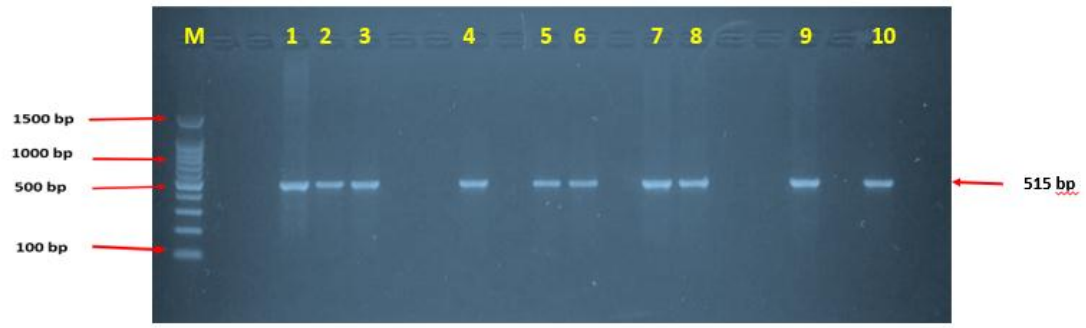


**Figure (1): Agarose gel electrophoresis image that showed the PCR product analysis of 16S ribosomal RNA gene in *S. pnemonaie* isolates. Where Marker ladder (1500-100bp), 16rRNA gene in *S. pnemonaie* isolate at 515bp PCR product size.**

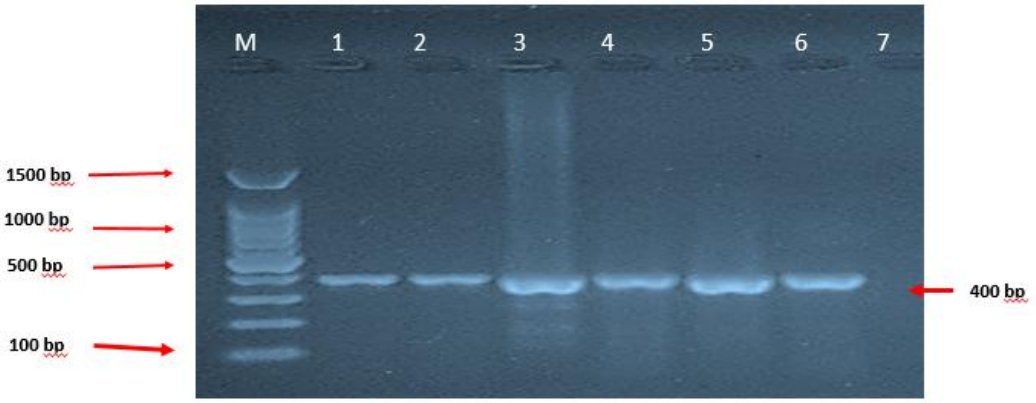


**Figure (2): Agarose gel electrophoresis image that showed the PCR product analysis of virulence factor gene(*entB)* in *S. pnemonaie* isolates. Where Marker ladder (1500-100bp), at 400bp PCR product size.**

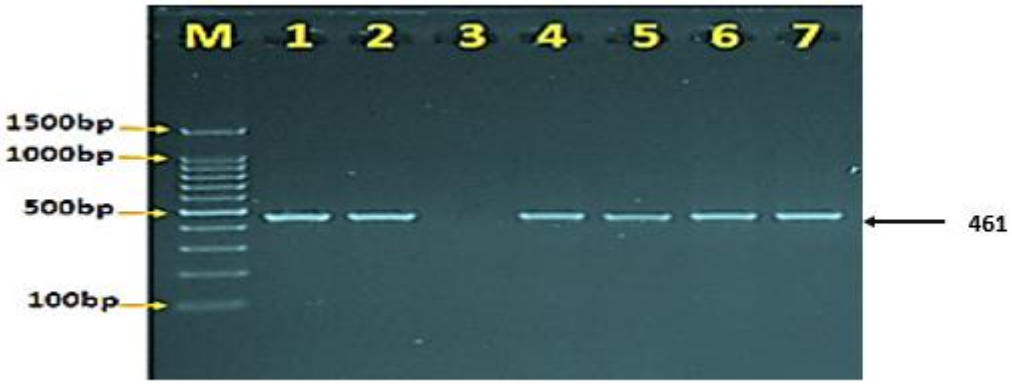


**Figure (3): Agarose gel electrophoresis image that showed the PCR product analysis of virulence factor gene(*rmpA)* in *S. pnemonaie* isolates. Where Marker ladder (1500-100bp), at 461bp PCR product size.**

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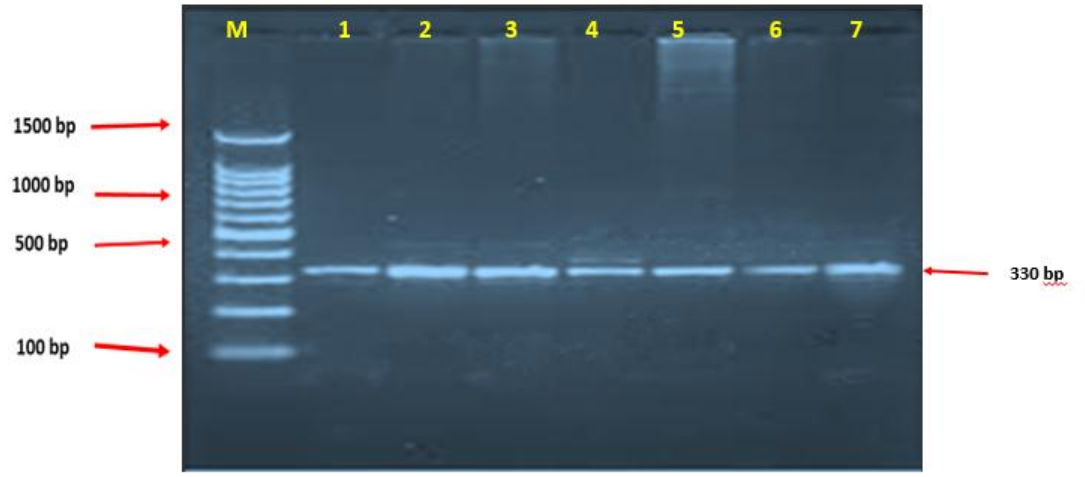


**Figure (4):**
**Agarose gel electrophoresis image that showed the PCR product analysis of 16S ribosomal RNA gene in *H. influenzae* isolates. Where Marker ladder (1500-100bp), at 330bp PCR product size.**

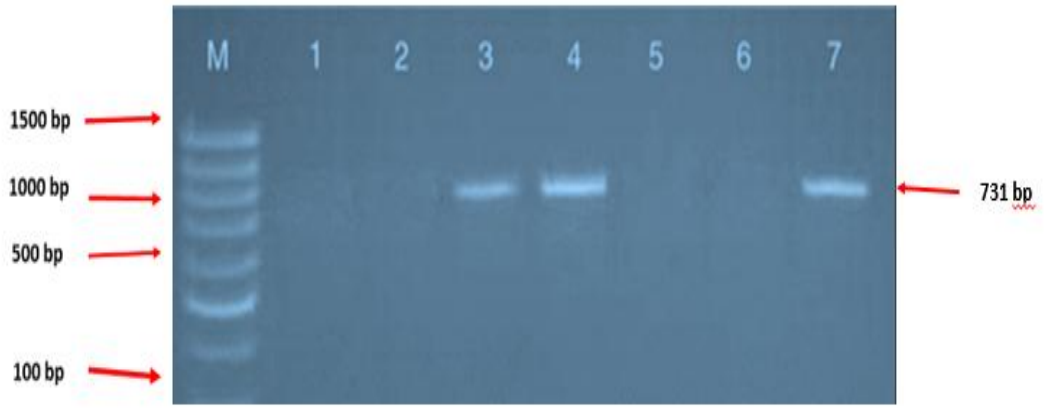


**Figure (5): Agarose gel electrophoresis image that showed the PCR product analysis of virulence factor gene(HMW2*)* in *H. influenzae* isolates. Where Marker ladder (1500-100bp), at 731bp PCR product size.**

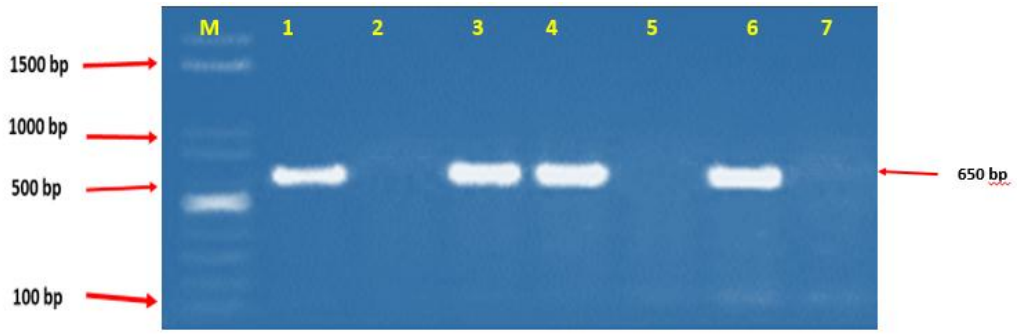


**Figure (6): Agarose gel electrophoresis image that showed the PCR product analysis of virulence factor gene(hifA*)* in *H. influenzae* isolates. Where Marker ladder (1500-100bp), at 650bp PCR product size.**

## 4. DISCUSSION

Respiratory disease is quite possibly the most regular disorders, enveloping a heterogeneous gathering of clinical pictures that incorporate sinusitis, otitis, pharyngotonsillitis, bronchitis, and pneumonia (Bulter and Schawartz,1998). In most respiratory diseases, the specialist doesn't have microbiological results, recommending the treatment observationally as prompt treatment, which is set up dependent on the most likely microorganisms and information on the example of affectability to anti-toxins in each geographic region. (García et al.,2001, Soriano et al.,2000).

In the current work, a high number of cases clinically analyzed as upper respiratory plot diseases were found, with pharyngotonsillitis and intense otitis media being distinguished all the more as often as possible. This recurrence could be identified with the presence of solid transporters, who might introduce in their standard vegetation microorganisms equipped for creating respiratory diseases because of advantage. S. pyogenes is the fundamental microorganism causing pharyngotonsillitis (García et al.,2001). In the current examination it was discovered that during the period June 2020 to November 2020 this was the most separated microorganism at the level of the upper respiratory lot. These outcomes diverge from those detailed by Guevara et al. (Guevara et al.,2004) and Rodríguez et al. (2003) who report a recurrence of 0.70% and 15.74% for this microorganism. Nonetheless, our rates concur with Bisno et al. (1997) who point out a recurrence of the microorganism in offspring of 30%. A few creators (Culasso et al.,2001, Soriano et al.,2000) avow that S. pnuemoniae, H. influenae and M. catarrhalis are the fundamental microbes that cause intense clinical pictures in the upper respiratory framework, in any case, in the current work it was discovered that S. aureus and P aeruginosa were the principle etiological specialists causing analyzed otitis. These outcomes are like those found by Trigoso et al. (18) who revealed in a review study completed in Bolivia, S. aureus and P. aeruginosa as the primary driver of otitis. S. pneumoniae is an irresistible specialist that can be discovered causing otitis media as an auxiliary condition and pneumonia as the fundamental condition in lower respiratory diseases in kids (Bulter and Schawartz,1998). The consequences of the current examination show that during the testing time the commonness in the lower respiratory parcel was low (3.96%) contrasted with different investigations where prevalences higher than this figure have been found. Notwithstanding, the consequences of this work are connected as far as colonization, since a higher commonness was found in the lower respiratory parcel. The outcomes acquired in the current examination show that H. influenzae was disconnected from the lower respiratory lot and H. influenzae in diseases of the upper respiratory lot, despite the fact that H. influenzae biotype I was disconnected all the more oftentimes in the upper respiratory plot. lower respiratory plot and pneumonia, it can't be construed about the relationship of a specific biotype of H. influenzae in a particular clinical picture because of the low number of strains found, notwithstanding, in such manner Ulloa et al. (1993) call attention to that there is no connection between's the conveyance of a specific biotype of the microorganism and the clinical analysis. With respect to recurrence of S. pneumoniae and H. influenzae discovered, this is viewed as low since the writing reports these microorganisms as the fundamental causative specialists of local area pneumonia (Morant et al.,1998, Soriano et al.,2000). One of the causes that recommends the low level of these two microorganisms is the way that these days there are antibodies against them and that is the reason in certain nations the rate of these microbes has been decreased. With respect to defenselessness, this investigation tracked down a 31.25% protection from oxacillin in S. aureus disconnects from local area contaminations. Quite a while back, it was

accepted that microscopic organisms causing nosocomial diseases were recognized from local area based microbes by their protection from anti-toxins (Goodman et al.,1991, Salmelinna et al.,2000). Of the instruments that H. influenzae utilizations to dodge β-lactams, the most exceptional from a clinical and microbiological perspective is the creation of β-lactamases, which have the property of hydrolyzing the β-lactam ring and dropping the medication activity (Calderón et al.,1999). The system of opposition of S. pneumoniae to this gathering of antimicrobial specialists isn't because of the creation of these compounds, however because of changes in the penicillin-restricting proteins, an interaction that forestalls the porousness of the anti-microbial.

This low pace of articulation of pili by nontypeable H. influenzae might be ascribed to the changed bacterial articulation of haemagglutination pili through an interaction called stage variety which is interceded by slipped-strand mispairing recommending a mean by which H. influenzae may quickly adjust to changing conditions (Cerquetti et al.,2000). Scientists likewise showed that non capsulated strain separated from fundamental site commonly express fimbriae, if a fimbriae quality group is available and they uncovered that no relationship between articulation of fimbriae and the clinical show of disease (Saikia et al.,2012). The hifA quality encodes for the significant subunit of haemagglutinating fimbriae of H. influenzae. In another investigation (Rahman,2009) they tracked down that around (4, 65%) showed the presence of fimbrial quality hifA and of these secludes they tracked down that 13% was of type b and 15% was non sort b and nontypeable H. influenzae. Concerning H.influenzae,the results showed that this quality (hia) is communicated by the non b capsulated H. influenzae and from the detaches that gave adverse outcome with respect to HMW protein from NTHi. For the most part, the presence of Hia in typeable H. influenzae can be clarified by the arrangement homology between Hia in NTHi and Haemophilus surface fibrils (Hsf) adhesin of typeable H. influenzae where numerous investigations showed that Hia has 72% amino corrosive personality and 81% comparably to the Hsf adhesin communicated by Hib and other typeable H. influenzae recommending that they address an allelic variations (Ogilvie et al.,2001). While HMW2 The investigation uncovered that solitary NTHi express these two adhesins as non pilus adhesins called HMW1and HMW2 proteins and they are not communicated by typeable H. influenzae whether type b or typeable non b H. influenzae. with respect to, they just communicated by 3 disengages out of 10, they are distinguished in all disconnects aside from secludes from sputum. A homolog of HMW2 recently answered to be available just in NTHi was found in 13.2% of the obtrusive non sort b epitomized H. influenzae however further examinations done on these segregates and they found that they neglect to hybridize [30]. On the other hand, these hmw+ strains might have begun from a non capsulated forerunner by procurement of the embodiment locus or antecedent, hmw-inadequate strains might have lost the hmw locus and separated from hmw+ epitomized forerunners at a beginning phase in cloned advancement (Feil et al.,2001).

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