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Research Article

MOLECULAR DETECTION FOR lytA AND PLY GENES IN STREPTOCOCCUS PNEUMONIAE ISOLATED FROM OTITIS MEDIA INFECTION

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Abstract

The precise mechanisms that contribute to the long-term persistence of otitis media (OM) in patients remain incompletely comprehended. Comprehending the molecular mechanisms that assist bacteria in persisting within the middle ear during otitis media (OM) could unveil the essential pathways necessary for the condition. The objective of this study is to isolate and identify Streptococcus pneumoniae from patients with otitis media in Hilla governorate. In this study, certain individuals were identified using standard phenotypic approaches, while others were identified using both phenotypic and genotypic methods, specifically the monoplex-PCR methodology. All isolates showed positive amplification when specific primers for S. pneumoniae were used to detect the lytA and ply genes at the molecular level. **Conclusion:** Out of 100 clinical samples, analysis of the virulence gene showed that all samples of *Streptococcus pneumoniae* contained both the lytA and ply gene. The genes provide the genetic instructions for producing the capsule , recognized for its exceptional effectiveness in protein analysis and tissue death .

Keywords: *Streptococcus pneumonia,lytA* , *ply* gene, monoplex-PCR, otitis media.

INTRODUCTION

Otitis media (OM) is a disorder that affects 80% of individuals aged 3-50 years (Vergison *et al.,* 2010). It commonly presents as a chronic condition and is typically unresponsive to antibiotic treatment or the insertion of tympanostomy tubes (Steele *et al.,* 2017). The cause of OM is multifaceted, although S. pneumoniae is recognised as a significant causative agent (Chan *et al.,* 2016). Streptococcus pneumoniae, also known as the pneumococcus, is a major cause of illness and death globally (Udan *et al.,* 2008). Typically, it colonises the human nasopharynx, nose, and throat without causing any symptoms. This colonisation is seen as necessary for the later development of disease in persons who are vulnerable (LeMessurier, *et al.,* 2006). This bacterium is a highly significant pathogen on a global scale, responsible for causing a diverse array of diseases including pneumonia, meningitis, otitis media, bacteraemia, as well as less common infections such as endocarditis and arthritis (Liang *et al.,* 2009). The symptoms of pneumococcal infection vary based on the specific site of infection inside the body. Symptoms may encompass chest pain, elevated body temperature, coughing, rigidity in the neck, cognitive impairment, disorientation, ear discomfort, photophobia, insomnia, arthralgia, chills, and irritability. Pneumococcal illness can lead to severe consequences such as hearing impairment, cerebral injury, and mortality (Wen *et al.,* 2015). In 1994, Weiser *et al.,* reported the presence of spontaneous and changeable changes in the appearance of pneumococci colonies on transparent agar surfaces, known as intrastrain phase variation in opacity (Weiser *et al.,* 1994). The visible strains with reduced amounts of capsular polysaccharide demonstrate superior colonization of the nasopharynx in animal models of pneumococcal carriage (Briles, *et al.,* 2005). On the other hand, opaque variants that have higher levels of capsular polysaccharide are not good at colonising animals and are more difficult for phagocytes to eliminate. However, these opaque variants also have reduced ability to stick to host cells (Kim *et al.,* 1999). Hence, it is postulated that the intrastrain phase shifts of pneumococci represent adaptations to various stages in the development of illnesses (Weiser, 2010). The polysaccharide capsule (CPS) is a key factor that contributes to the pathogenicity of this bacteria. The purpose of this structure is believed to be the safeguarding of the bacterium from detrimental environmental circumstances, while also displaying features that prevent phagocytosis (Cleavers *et al.,* 2000). The CPS consists of saccharide repeating units that undergo polymerization to form a polysaccharide chain. A total of 90 distinct capsule serotypes have been found so far. In this study, we aimed to identify the genotypes of some virulence factors, including the capsule capsA and the autolysin lytA, and their function in the pathogenicity of *S. pneumoniae*.

MATERIALS AND METHODS

Samples

A total of 100 samples were obtained from both outpatients and inpatients diagnosed with otitis media at the Hilla Teaching Hospital for Children in Babylon province. The samples were taken between February 2023 and April 2023. The study participants consisted of individuals of both genders (male and female) spanning an age range of 1 to 80 years.

Bacteria Diagnosis

The identification of S. pneumonia was accomplished by examining its cellular appearance, culture characteristics, and biochemical reactions as described in Macfaddin (2000).

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Table 1. Primer sequences for lytA and ply genes

Target gene	DNA sequence(5—3)	Annealing TM	Size product	References
lytA	F5- GACGCTGAAATAACTCCCCACT-3 R 5- ACAGCTAAAGCGGCTGATACA-3	62	212	Design
ply	F 5- GAAATCGTCCGCTTACGCAC-3 R 5- ACTCTTGACCCATCAGGGAGA-3	60	1305	Design

Extraction and Isolation of DNA

The process of DNA extraction involved the utilisation of the Genomic DNA Extraction Kit from Geneaid. The purity of a DNA solution is evaluated using the OD 260-280 ratio, which is ideal to be within the range of 1.8 to 2.0 for DNA of exceptional purity. A polymerase chain reaction (PCR) technique designed for execution on a thermocycler. The PCR products and the ladder marker are separated by separation on a gel with a 1.2% concentration Agarose gel(Guido *et al.,* 2005).

(PCR) Technique

The work utilisedmonoplex PCR to identify specific genes responsible for encoding virulence factors in S. pneumoniae isolates. The lytA and ply genes were detected using Monoplex PCR (Table 1).

PCR amplification

The end result consists of reaction volumes of 30µl, consisting of 10 ul of a single primer, 12.5 ul of Green Master Mix, 5 ul of Genomic DNA, and the remaining 2.5 ul filled with Nuclease-free water to reach the total volume.The amplification process was performed using a thermo-cycler (Eppendorf) following this convention: an initial step of 2min at 94°C, then 40 cycles of 1min at 92°C and 1 min at 62°C for the lytA gene and C 60 for the ply gene, and then an end step of two minutes at 72°C. The operation was concluded by prolonging the stay by 7 min while keeping the temperature at 72°C. Each electrophoresis test incorporated well-established molecular markers. Photographs were taken of gels that were subjected to ultraviolet (UV) radiation.

RESULTS AND DISCUSSION

The association between the pathogenicity of S. pneumoniae and several virulence factors is widely recognised. In this investigation, certain individuals were identified using standard phenotypic approaches, while others were identified using both phenotypic and genotypic methods, including monoplex PCR techniques.

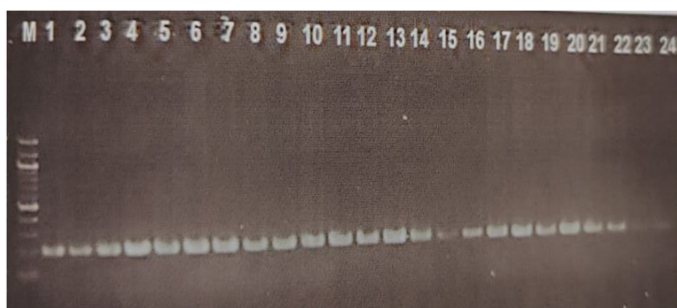

Figure 1. Agarose gel electrophoresis results with a 2% concentration, displaying the PCR products of the *lytA*gene, which has a length of 212 base pairs. Line M has a DNA marker called 100 bp-2000 bp ladder

A PCR primer was particularly designed for the purpose of detecting the autolysin gene (lytA), as illustrated in Figure 1. The findings indicated that, in general, of the examined isolates possessed the lytA gene. Autolysin is phenotypically evident in all strains of pneumococcus. The lytA gene sequence has been proposed as a more precise diagnostic tool for the detection of pneumococcal illness [Carvalho *et al.,* 2007; Abdeldaim *et al.,* 2010]. The bile solubility test, extensively researched, is frequently employed to precisely detect S. pneumoniae; nevertheless, there is no universally acknowledged benchmark for this objective [Wessels *et al.,* 2012]. Nevertheless, there are recorded cases of organisms that display genetic and physical similarity to S. mitis, but possess the lytA gene often found in pneumococci [Seki *et al.,* 2005].

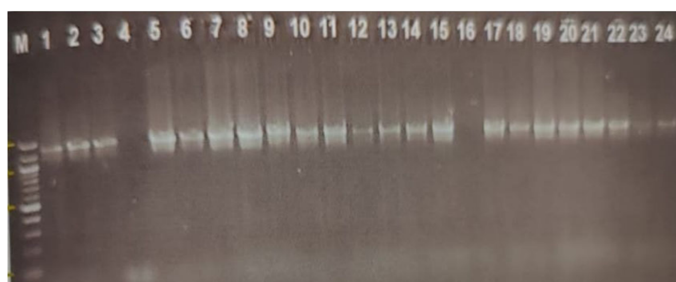

Figure 2. Agarose gel electrophoresis results with a 2% concentration, displaying the PCR products of the *ply*gene, which has a length of 1305 base pairs. Line M has a DNA marker called 100 bp-2000 bp ladder

Moreno *et al.,* [2005] conducted PCR to analyze the autolysin gene lytA. They also used a multiplex PCR to amplify specific parts of the capsular genes related to serogroups as 6 and 18. The lytA gene is commonly employed as a dependable marker for the identification of S. pneumoniae [Kawamura *et al.,* 1999]. The lytAgene exhibits higher specificity compared to the gene for S. pneumoniae, as previously observed [Neeleman *et al.,* 2004]. Several studies have confirmed that amplifying a specific portion of the lytA gene can be successfully used to detect S. pneumoniae [Hassan-King *et al.,* 1994; McAvin *et al.,* 2001]. Nevertheless, other researchers have shown evidence that lytA is also present in individuals belonging to the Smit group [Whatmore *et al.,* 2000]. Hence, Arbique *et al.,* [2004] recommended exercising caution when identifying S. pneumoniae by lytA detection. The lytA gene is generally regarded as unique to S. pneumoniae. However, whereas both the lytA PCR method previously published by McAvin et al (2001) and the lytA PCR method described by Carvalho et al (2007) focus on the same gene, only the latter is exclusive to S. pneumoniae. The difference can be attributed to the fact that all S. pneumoniae isolates have conventional lytA alleles, but the lytA alleles found in isolates from other members of the mitis group were uniformly abnormal [Llull *et al.,* 2006]. The PCR primer was used to molecularly detect the ply gene in S. pneumoniae isolates (Figure 2). Pneumolysin is an essential virulence factor that plays a significant role in several phases of the pathogenic process and triggers an immunological response from the host. Pneumolysin's cytotoxic properties

exacerbate disease progression by impeding ciliary motility in the respiratory epithelium, hence facilitating the migration of infections to the lungs. Furthermore, it functions by interfering with the robust connections between epithelial cells and offering an alternate route via which the pneumococcus germs can penetrate the bloodstream [Steinforte *et al.,* 1989]. Moreover, multiple inquiries have confirmed the significance of pneumolysin in the progression of sepsis. Empirical evidence has demonstrated that mutants that lack pneumolysin have diminished reproductive and survival abilities when present in the bloodstream of diseased animals [Benton *et al.,* 1995; Berry *et al.,* 1999]. PCR amplification from patient specimens indicates the existence of invasive pneumococcal infection. Several scientific articles have detailed the application of pneumococcal PCR, a technique that includes amplifying the pneumolysin gene. A particular report focused on utilizing PCR to identify S. pneumoniae DNA in samples that yielded negative results in culture tests, despite being diagnosed with meningitis. The ply gene has been identified and the Ply protein has been detected in direct detection techniques utilizing clinical samples. The purpose of these tests is to differentiate S. pneumoniae from other streptococci in the viridans group. The success rates of these tests have exhibited variability [Wheeler, *et al.,* 2000]. While DNA amplification techniques that focus on the ply gene initially demonstrated potential as a dependable and effective means of verifying pneumococcal isolates. According to Whatmore *et al.,* (2000), the ply gene is found in both S. mitis and S. oralis strains. Furthermore, while latex agglutination approaches that focus on the protein produced by the ply gene have displayed potential [Kearns *et al.,* 2000], their utilization is also discouraged due to the discovery of the ply gene in additional members of the Smit group, in addition to S. pneumoniae[Arbique *et al.,* 2004].

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