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Research Article

DETECTION OF LasB AND AprA GENES OF PSEUDOMONAS AERUGINOSA ISOLATED FROM DIFFERENT INFECTION IN HILLA PROVINCE

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Abstract

Pseudomonas aeruginosa is a significant cause of bloodstream infections acquired in healthcare settings. The microorganism synthesizes two main proteases: alkaline protease A (AprA) and elastase B (LasB). This study gathered one hundred samples from clinical cases of people suffering from wound, ear, urinary tract, and blood and burn infections. The phenotypic properties of Pseudomonas aeruginosa were ascertained using the gram-staining approach, which involved observing the formation of pigments following incubation at a temperature of 37°C. Analysis of biological constituents and mechanisms. The outcome derived from Pseudomonas aeruginosa utilizing Las B: The allocation of isolates was as follows: 10% (5 isolates) were obtained from cases of otitis media, 40% (20 isolates) were obtained from burn infections, 30% (15 isolates) were obtained from wound infections, 8% (5 isolates) were obtained from urinary tract infections, and 12% (6 isolates) were obtained from blood samples. The distribution of alkaline protease A (AprA) in isolates from different sources is as follows: The allocation of isolates was as follows: The distribution of isolates was as follows: 8% from otitis media (4 isolates), 50% from infection of burn (25 isolates), 26% from infections of wound (13 isolates), 8% from urinary tract infections (4 isolates), and 8% from blood (4 isolates). **Conclusion:** Out of 100 clinical samples, analysis of the virulence gene showed that 50 samples of Pseudomonas aeruginosa contained both the AprA gene and the Las B gene. The genes provide the genetic instructions for producing the elastase enzyme, recognized for its exceptional effectiveness in protein analysis and tissue death. On the other hand, the AprA gene can increase the synthesis of pyocyanin in this bacteria

Keywords: Pseudomonas aeruginosa, Las B gene , AprA gene , clinical samples .

INTRODUCTION

Pseudomonas aeruginosa is a prevalent Gram-negative bacterium notorious for causing bloodstream infections in healthcare environments. Although there have been advancements in antibiotic treatment and hospital care, the mortality rate for P. aeruginosa bacteremia remains approximately 30% (Peña *et al.,* 2012; Peña *et al.,* 2013). The mortality rate of P. aeruginosa bacteremia is affected by multiple factors, including the pathogen's inherent virulence and certain underlying host circumstances (Chamot *et al.,* 2003; Kang *et al.,* 2005; Peña *et al.,* 2015). A significant percentage of fatalities occur within the initial 24-72 hours after contracting P. aeruginosa (Hattemer *et al.,* 2013; Peña *et al.,* 2013), indicating that the early natural defense mechanisms of the host are ineffective. Pseudomonas aeruginosa secretes many extracellular proteases to achieve this objective. Pseudomonas aeruginosa infections are frequently associated with two enzymes: the 50-kDa alkaline protease AprA and the 33-kDa Elastase LasB. These entities can be detected under controlled laboratory settings and within living organisms at different infection sites (Potempa & Pike, 2005; Upritchard *et al.,* 2008). AprA and LasB are zinc metalloproteases that demonstrate stability dependent on the presence of calcium, as demonstrated by the investigations carried out by Thayer et al. (1991) and Baumann et al. (1993).They have exhibited proteolytic activity against many substrates (Andrejkou8 *et al.,* 2013) and suppress host immunological responses. LasB has been discovered to degrade mucins and surfactant proteins, which jointly enhance the eradication of microbes (Mun *et al.,* 2009; Kuang *et al.,* 2011).

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Furthermore, Apra has been demonstrated to hinder the elimination of germs by breaking down the C2 component of the complement system, therefore obstructing complement-mediated phagocytosis (Laarman *et al.,* 2012). Apra's immunomodulatory activities extend beyond the targeting of host factors. Furthermore, it can degrade flagellin, a substance detected by toll-like receptor 5 (TLR5) and acknowledged for its ability to induce proinflammatory reactions (Bardoel *et al.,* 2011; Pel *et al.,* 2014). Pseudomonas aeruginosa synthesizes various enzymes, including Alkaline Protease and Elastase. The alkaline protease collaborates with Elastase to degrade collagen and elastin (Ulrich *et al.,* 2010). The affected area experiences necrosis due to an excessive enzymatic release, particularly in the lungs of persons with cystic fibrosis. The phenomena described by Parker et al. (2012) lead to the breakdown of epithelial cells. It can examine soft tissue and diverse molecular biologics, including Fibrinogen (Komori *et al.,* 2001) and Components of the Complementary System (Laarman *et al.,* 2015). Protease enzymes have a crucial role in enhancing the virulence of P.aeruginosa bacteria, particularly when they are secreted from the cells to inflict damage on host tissues (Seo & Darwin, 2013).

METHODOLOGY

Sampling

A total of one hundred samples were obtained from clinical instances of individuals afflicted with infections in wounds, ears, urinary tracts, blood, and burns. A solitary colony was chosen and introduced into a designated growth medium (cetrimide agar) to foster incubation. Subsequently, the phenotypic traits of P.aeuroginosa were ascertained by the

gram staining technique, involving the observation of pigment formation following incubation at a temperature of 37°C. The biochemical investigations were conducted following the methods described by Macfaddin in 2000. The research involved assessments of motility, indole synthesis, methyl red, Voges-Proskauer, citrate utilization, urease activity, oxidase activity, catalase activity, and the ability to thrive at 42°C.

Genotype identification

DNA Extraction

The DNA of Pseudomonas aeruginosa isolates was extracted and purified using a Geneaid Company (UK) Extraction and Purification kit.

Detection of the last and aprA gene:

The objective is to identify the las B gene in P. aeruginosa isolates using the lasB primer in conjunction with two sets of primers: 1200 bp F (ACA et al.), R: (GAT et al. CC), and aprA F (ACC et al. C) R (GAT et al. G), which amplify a 152 bp fragment. The gene lasB was discovered utilizing a solution concentration of 10 moles per microliter (µl). This was achieved by withdrawing 10 µl from the Stock solution and diluting it with 90 µl of distilled water. The stock solution was stored at a temperature of -20°C. Sonbol et al. (2015) thoroughly explained the methods and circumstances of the polymerase chain reaction (PCR).

PCR amplification

The ultimate result consists of reaction volumes of 30µl, which include 10 ul of a solitary 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 get the total volume. The amplification procedure was conducted using a thermo-cycler (Eppendorf) according to the following protocol: an initial step of two minutes at 94°C, followed by 40 cycles of one minute at 92°C and one minute at 75°C for the LasB Gene and C 52 for the aprA gene, and finally a concluding step of two minutes at 72°C.The process was finalized by extending the duration by 10 minutes while maintaining a temperature of 72°C. The amplified products were separated by electrophoresis in agarose gels with a concentration of 1.8% and then made visible by staining with ethidium bromide. Each electrophoresis test incorporated standard molecular markers. Photographs were taken of gels that were irradiated with UV light.

RESULTS AND DISCUSSION

Phenotypic Characteristics

Conventional diagnostic methods have revealed that P. aeruginosa exhibits the production of a green pigment and emits a distinct odor when grown on cetrimide agar. The biochemical analysis of P. aeruginosa isolates yielded positive catalase and oxidase assay outcomes. Simultaneously, it produced unfavorable outcomes for methyl red, Voges Proskauer, indole, and slow urea hydrolysis, demonstrated using Simmon's citrate. The detected biochemical features of the organism in this experiment align with the findings reported by Abro et al. (2009). Overall, biochemical methods have aided in the detection of P. aeruginosa. However, several operations are arduous and require a substantial amount of time. Moreover, supplementary morphological, biochemical, and physiological tests are usually important for precise identification. The analysis showed that the lasB and aprA genes were detected in 50 P.aeruginosa isolates. Pseudomonas aeruginosa possesses the LasB and aprA genes.

Table 1. Bacteria distrubtion from different infections

No	Sources	No	LasB (%)	No	aprA(%)
1	Burns	20	40%	25	50%
2	Otitis media	5	10%	4	8%
3	Wounds	15	30%	13	26%
4	UTI	4	8%	4	8%
5	Blood	6	12%	4	8%

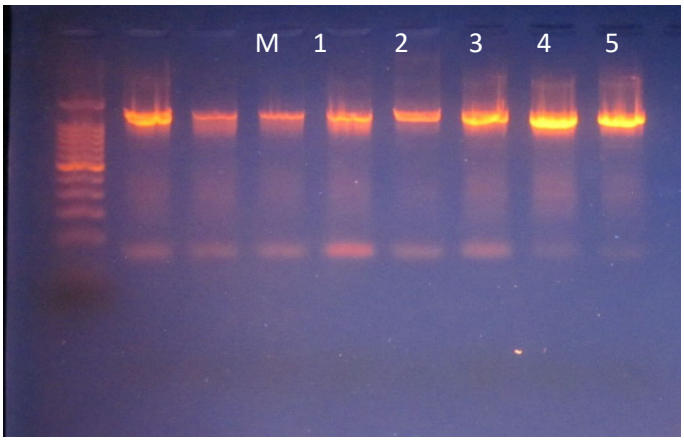

Figure 1. Agarose gel electrophoresis results with a 2% concentration, displaying the PCR products of the las B gene, which has a length of 1200 base pairs. Line M has a DNA marker called 100 bp-2000 bp ladder

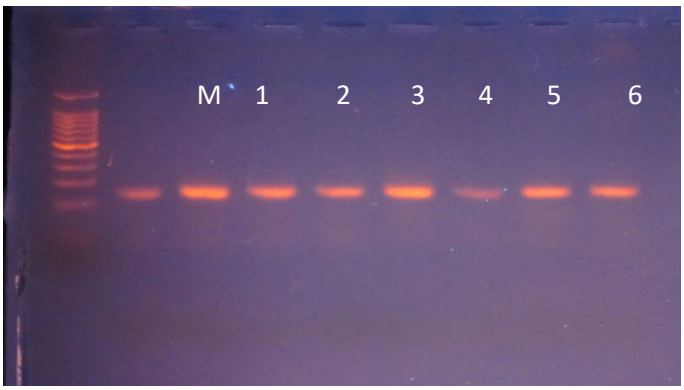

Figure 2. Agarose gel electrophoresis (2%) was performed on the PCR products of the aprA gene (152 bp), Line M has a DNA marker called 100 bp-2000 bp ladder,

This study replicated the findings of Wolska and Szweda (2009), which demonstrated that 84.6% of the P. aeruginosa bacteria that were isolated contained the last gene. The results align with the research carried out by Shi et al. (2012), which demonstrated that 80% of P. aeruginosa bacteria harbor the last gene. Furthermore, Sabharwal *et al.* (2014) discovered that 75% of the P. aeruginosa isolates examined in their study possessed this particular gene. The findings indicated that a significant fraction of clinical samples included the definitive gene, which is accountable for producing Elastase. This enzyme plays a crucial role in the pathogenicity of P. aeruginosa by degrading the Elastin protein (Cathcart *et al.,* 2011). This protein is crucial for preserving the flexibility of human blood arteries. Elastin is an essential component of the

lungs, playing a crucial role in the expansion and contraction of the lungs. Hence, the enzyme Elastase plays a pivotal role in determining the severity of P.aeruginosa infection (Bai *et al.,* 2011). The elastase enzyme significantly impacts the elastic proteins, the primary constituents of lung tissue and blood vessels in humans. Pulmonary tissue infections in humans, resulting in pulmonary hemorrhage, are caused by Pseudomonas aeruginosa strains that secrete Elastase type A and B enzymes. This enzyme exerts a significant influence on both leukocytes and cytotoxic lymphocytes. Thus, Pseudomonas aeruginosa strains that generate this enzyme demonstrate potent immunosuppressive characteristics. They not only evaluate and inhibit the immunological components of the body, such as IgG, IgA, and complement type C3 but also have a substantial ability to do so (Cotar *et al.,* 2010). The DNA amplification data of the studied Pseudomonas aeruginosa isolates were obtained using a primer to target the Alkaline protease enzyme, a crucial virulence factor of Pseudomonas aeruginosa. All isolates underwent gene amplification, producing a polymerase product measuring 152 base pairs in size. In Saleh's 2012 study, it was found that the gene was present in all burn isolates, wound isolates, and urine isolates, with a prevalence rate of 100% in each instance. However, the gene was absent in isolates collected from the ear or the surrounding environment. The variation in the prevalence of this gene in Pseudomonas aeruginosa isolates from different sources, as observed in numerous studies, can be attributed to genetic changes occurring in the bacterial genome, such as deletion mutations. Moreover, the variation is influenced by the illness's location and the origins of the isolates. The work by Taherzadeh, innan *et al.* (2011) includes citations to specimens.

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