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Plesiomonas Shigelloides: Unraveling the Mysteries of a Neglected Enteric Pathogen

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Abstract. The oxidase-positive, motile, Gram-negative bacteria Plesiomonas shigelloides is found all across the natural world. Additionally, it is a major pathogen that mostly causes disorders of the intestines in humans. Most of these illnesses are characterised by diarrhoea, which may be watery, invasive, or chronic in nature. There have been reports of intestinal illnesses caused by Plesiomonas that were spread by food and water. There are a number of extraintestinal diseases caused by P. shigelloides, the most prevalent of which are sepsis and meningitis, both of which are associated with significant fatality rates. Phylogenetically closely related to other Enterobacteriaceae species, P. shigelloides differs biochemically from them. A single biovar, with over a hundred serovars documented. Some have proposed P. shigelloides as a "natural" vaccination against shigellosis since it is thermo-, alkali-, acido-, and halotolerant. There are some intestinal media that are known to limit the development of Plesiomonas, and in the lab, it looks barely there on the surface of many agar plates. The antibiotic sensitivity patterns of Plesiomonas are somewhat peculiar, and the amount of the inoculum determines how susceptible the bacteria are to certain drugs. One distinguishing feature of high bacterial densities in the presence of certain β-lactam antibiotics is the development of significant cell filamentation

Highlights:

1. Pathogen: Causes diarrheal diseases, sepsis, and meningitis in humans.
2. Characteristics: Gram-negative, motile, tolerates heat, alkali, acid, and salt.
3. Antibiotics: Susceptibility varies; β-lactams cause cell filamentation in high densities.

Keywords: Plesiomonas shigelloides, bacteria, diarrhea

Introduction

In 1947, a patient's feces were examined, and rod-shaped bacteria were found to be associated with gastroenteritis. According to Ferguson and Henderson [15], her first assessment placed her in the category of enteropathogenic bacteria C27. Although Bader [7] did not place this bacterium in the genus Shigella, he did find that it agglutinates with serum that is typical of this species. This finding adds to the description of this bacterium. He named the bacterium Pseudomonas shigelloides and put it in the

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Pseudomonadaceae family. Then there was this one bacterium that, according to morphological features, was reclassified to the family Aeromonadaceae, genus Aeromonas [14]. The cytosine and guanine (CG) content of bacteria of the genus Fergusonia of the family Vibrionaceae was the subject of a second paper two years later [5]. Hendrie et al. [14] put out biochemical traits, CG percentage in DNA, and vibriostatic sensitivity of 0/129 as reasons to transfer organism C27 to the genus Vibrio. A new genus Plesiomonas was established in the 1980 Approved Lists of Bacterial Names [5] in response to the existing taxonomic discrepancies in all suggested classifications. New suggestions for more appropriate taxonomic categorization of this bacterium continued to emerge even after that. Sequence homology with P. mirabilis led researchers to place the species in the genus Proteus and, therefore, the family Enterobacteriaceae [12].

P. mirabilis and P. vulgaris were shown to have a closer association than P. shigelloides and P. mirabilis. Based on 16S sequencing rDNA, it was indicated by Martinezmurcia et al. [23] that it should be included in the family Enterobacteriaceae. They came to the same results in their 1994 study [19], and they also suggested the potential of a new family Plesiomonadaceae; however, more data is needed to confirm this.

General characteristics of the species P. shigelloides

Bacteria of the species P. shigelloides are short Gram-negative rods without apparent curvature, approximately 3 µm in length and less than 1 µm in diameter [10]. After exposure to certain stressors (e.g., presence of certain antibiotics in the medium), elongated cells or filaments may occur in culture [10]. No endospore formation was observed. Cells do not produce capsules [10].

The vast majority of strains are motile, and the distribution of flagella can be twofold: younger cultures form flagella all over the cell surface, while older cultures tend to have one to five flagella at one pole [10]. Another publication states that the location of the flagella is conditioned by the consistency of the nutrient medium: in a liquid medium, flagella grow from one pole; in a less viscous medium, flagella grow from the entire surface of the cell [17].

For the cultivation of samples where the presence of the bacterium P. shigelloides can be expected, blood agar (KA), MacConkey agar, Endo agar, Hektoen agar, or xylose, lysine, and deoxycholate agar (XLD) can be used [2]. Some of these media can help

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identify P. shigelloides. A colony of P. shigelloides on Hektoen agar appears dark green without brightening the surrounding medium; on MacConkey and XLD agar, colonies are not pigmented. For detection and isolation of P. shigelloides strains, a medium containing brilliant green and bile salts was specially designed [5]. Plesiomonas agar is also used for its isolation [5].

The temperature range for the growth of P. shigelloides bacteria is between 8–45 °C, with the optimum temperature usually given as between 37 and 38 °C. It tolerates 1–4% NaCl for its growth; at higher concentrations, growth is limited to certain nutrient media. The tolerable pH range is 4–9; under more extreme conditions, growth was not recorded [2, 5].

The formation of electrodense granules by P. shigelloides cells has been recorded. In the logarithmic phase of growth, these granules can be observed with a light microscope. With an electron microscope, smaller granules can be captured already in the early stages of growth, and as the cells age, they gradually increase in size. Granules are typically located at the poles of the cells [12]. Based on analysis using X-ray microprobes in an electron microscope, these granules were found to contain phosphates, potassium, and magnesium. The concentration of these elements in the granules was noticeably higher than in the cytoplasm of the cell [12]. Additionally, electrotransparent vacuoles were observed in P. shigelloides cells [10].

The species P. shigelloides has a rather complex serological system. Aldová, Schubert, Shimada, and Sakazaki contributed significantly to its classification. Due to the presence of flagella in most strains, both O (somatic) and H (flagellar) antigens are often identified. By 2000, a total of 102 O antigens and 50 H antigens had been described [4]. Some antigens are more common in the P. shigelloides population, such as O17, while others were observed in only one strain. It was found that several P. shigelloides antigens are shared with other bacteria, mainly of the genus Shigella. The most common antigen O17 is identical to the somatic antigen of S. sonnei; serovar O11 agglutinates with the same serum as S. dysenteriae serovar 8; serovar O23 corresponds to antigens of S. boydii serovar 13, etc. [4].

Occurrence of the bacterium P. shigelloides

Common sources from which P. shigelloides bacteria are isolated include: especially water and the gastrointestinal tract and other surfaces of animal bodies. Some

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studies they also mention isolation from soil, but these are samples from river banks or sludge, so they are associated with the aquatic environment. At the same time as soil samples, water samples were taken from the respective waterways, and the bacterium was isolated from both sediments and water [1], [5].

Water samples from which the bacterium P. shigelloides has been isolated in the past, comes from diverse geographic locations. Due to the high optimum temperature growth is most often in tropical or subtropical regions, but it is relatively abundant occurrence even in the temperate zone of Europe, Asia and America [16]. Between the most extreme locality from which the bacterium P. shigelloides was isolated undoubtedly belongs to the Swedish lake Vettasjärvi, which is located north of the Arctic Circle [18].

Another common source is aquatic animals, primarily fish, particularly commercially bred species such as cichlids (Cichlidae) or carp (Cyprinidae) [8], but also wild species like eels (Anguillidae) [13]. Other sources from which P. shigelloides has been isolated include bivalves, amphibians, crustaceans, reptiles, birds, and often mammals [10].

Isolation or detection of P. shigelloides bacteria is quite common in the gastrointestinal tract and [3]. These seizures are often associated with diarrhea diseases, although the role of P. shigelloides in the etiology of these diseases is not yet satisfactorily established clarified. In many cases, the bacterium P. shigelloides is isolated as one of several potential pathogens [9].

We rarely encounter the presence of P. shigelloides bacteria in extraintestinal human samples. Typically, in such a case, it is sepsis. Till this day more than 20 such cases have been recorded. Next we meet with meningoencephalitis, often as an accompanying phenomenon of sepsis. Rarer cases have been described biliary tract infection [6], pancreatic abscess, osteomyelitis (Ingram et al. 1987) or eye infections [10]. Patients with extraintestinal infection with P. shigelloides are usually immunocompromised individuals. These are, for example, newborns, patients with chronic disease or patients debilitated after surgery [6]. Rarely, extraintestinal infections have been reported in the immunocompetent individuals [10].

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Methods

Methods of identification of P. shigelloides bacteria

The bacterial species P. shigelloides is characterized by consistent biochemical traits across the species. Characteristic biochemical features include indole formation, nitrate reduction, negative test for acetoin production, negative test for the presence of phenylalanine deaminase and urease. It is relatively homogeneous across species also the ability to ferment some sugars, e.g. D-glucose, m-inositol, maltose and trehalose [1]

Based on the characteristics described above, P. shigelloides bacteria can be identified according to biochemical characteristics. Outside of conventional tests in some cases panel identification tests, e.g. API 20E System (BioMérieux), have also proved their worth. However, the ability to reliably identify the species of P. shigelloides is unfortunately not a manufacturer sufficiently tested [2], [3]. To identify this species, the automatic system VITEK 2 [4], [5]. Biolog system according to Miller and Rhoden (1991) after 24 hours correctly identified only one strain of P. shigelloides out of three [6].

There are two media that have been directly designed for the cultivation and isolation of bacteria P. shigelloides from the sample. The first of them is agar containing inositol, a brilliant green and bile salts (IBB), which was proposed in 1977 by Schubert [7]. Modification of IBB resulted in Plesiomonas agar [8]. In the first study showed that PL agar was more sensitive than IBB, but a subsequent study showed that IBB agar has fewer false negatives [9]. It turned out that even heat-damaged cells can grow and multiply on IBB agar compared to PL agar [10]. While VonGraevenitz and Bucher (1983) recommend the use of alkaline peptone waters for the isolation of P. shigelloides bacteria, Millership and Chattopadhyay (1984) rates this medium as inappropriate. In the current literature, usually IBB appears as the selective medium of choice for P. shigelloides [13]–[15].

Molecular identification methods mainly include 23S gene sequencing and 16S rRNA [16], [17]. A method was proposed to quantify P. shigelloides in samples by PCR amplification of the 23S rRNA gene, detecting a minimum of four cells in one PCR reaction [18].

Loh and Yap (2002) reported a rapid and accurate method for the detection of bacteria P. shigelloides based on real-time PCR (qPCR). It included the use of fluorescent labeled probes for a species-specific segment of the 23S rRNA gene. The method was

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optimized for stool samples and allowed a diagnosis to be obtained within three hours. The detection limit was approximately 100 fg of DNA, corresponding to approximately 20 cells in the reaction volume [9].

Another molecular method of identification is the detection of the hugA gene for the heme receptor, which is specific to the P. shigelloides species. The method was described by Herrera et al. (2006) and they successfully tested it on fish meat from the supermarket. Presence of bacteria P. shigelloides was detected in 23% of the fish meat samples tested, a similar result obtained by cultivation on an enriched selective medium. The specificity of the reaction was verified on representatives of the genera Vibrio, Aeromonas, Pseudomonas, Shewanella, Edwardsiella, Listeria, Proteus, Yersinia, Salmonella and Escherichia. Amplification only occurred in the case of one strain of E. coli. The resulting E. coli amplicon was approximately 200 bp in length, while the target sequence of the hugA gene is 435 bp in length. It was on the electrophoretic gel so at first glance it is obvious that this is not the expected amplicon and the sample was evaluated as false positive. For its simplicity and effectiveness, this method was chosen to confirm the relevance of the tested isolates to the P. shigelloides species in this work [20].

Result and Discussion

Pathogenicity of P. shigelloides bacteria

The bacterium P. shigelloides is often described as an opportunistic human pathogen, but and other animals, especially fish. Infection of the gastrointestinal tract with the presence P. shigelloides are usually characterized by watery stools, abdominal pain, sometimes and fever, nausea and vomiting [3]. As risk factors for acquisition infections appear traveling to tropical and subtropical countries, drinking water and aquatic animals from contaminated sources [5].

Isolation of the bacterium P. shigelloides from the stool of healthy patients is quite common, some sources report the frequency of isolation as often as symptomatic even asymptomatic patients [3]. Of which it follows that the presence of the bacterium P. shigelloides in itself is not necessary in a given individual cause symptoms of the disease. Virulence factors and the mechanism of infection therefore have become a key aspect in a number of researches. These researches mainly focus on hemolytic,

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gelatinase, elastase or DNase activity, production enterotoxins and cytotoxins, invasiveness into animal tissues, biofilm formation and others mechanisms of animal tissue damage [11]. Some studies focus on the analysis of genes for virulence factors [11].

Gonzalez-Rey et al. (2011) used four different ones to typify 24 strains molecular-biological methods. It is thus the most complex molecularly biological study of the bacterial species P. shigelloides that has been published so far. The following methods were used: RAPD-PCR, rep-PCR using ERIC and REP primer and PFGE. Using these methods, it was possible to prove the connection between human and animal isolates. This is the first molecular biological evidence that bacteria P. shigelloides can be transmitted zoonotically [8].

Susceptibility of P. shigelloides strains to antibiotics

Due to the clinical importance of P. shigelloides bacteria, the susceptibility to antimicrobial substances the subject of a large number of studies, although often only as a complementary method. These studies mainly use two approaches: testing minimum inhibitory [11] and the disc diffusion method [2]. Some studies have looked for a connection between serotypes, isolate origin and resistance pattern [9].

A number of P. shigelloides isolates have been shown to produce different types β-lactamase. β-lactamase producers are resistant to a wide range to varying degrees penicillin antibiotics. In the case of the P. shigelloides species, this is mainly the case o ampicillin and carbenicillin.

Bravo et al. (2009) report a high proportion of multiresistant strains among by clinical isolates of P. shigelloides from patients with diarrhea. Approximately 22% of those studied strains were resistant to three or more antibiotics. The most common combination was resistance to ampicillin, tetracycline and kanamycin, occurred in 7.4% studied strains. Current sources in clinical medicine recommend the treatment of infections caused by bacteria P. shigelloides combination of penicillins with beta-lactamase inhibitors, e.g. amoxicillin with clavulanic acid or trimethoprim with sulfamethoxazole (co-trimoxazole). Broad-spectrum cephalosporins or fluoroquinolones are also recommended [9].

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In general, antibiotic treatment is not recommended in most cases of gastrointestinal infections required, supportive treatment is applied if necessary. Severe extraintestinal infections require rapid deployment of effective treatment [6].

A remarkable phenomenon that has been described in P. shigelloides bacteria is the influence initial inoculum density on susceptibility to some antibiotics. This phenomenon in detail described by (Wiegand and Burak 2004). In this study, they tested using the MIC method whether this phenomenon is affected by inducible production of β-lactamases. The hypothesis was that with increasing density inocula, the production of β-lactamases by individual bacteria increases due to quorum sensing. Susceptibility to several cephalosporins was tested in 5 producing strains of β-lactamase and one strain for which no β-lactamase was known to be produced.

Inoculum density-dependent resistance occurred in all strains. It was also found that the concentration of the active form of the antibiotic did not change significantly during cultivation. A significant influence of β-lactamases was thereby ruled out. However, in the same study, significant filamentation of P. shigelloides cells was observed in the presence of an antibiotic. Filamentation in the presence of cephalosporins has also been described in other literature in several cases members of the Enterobacteriaceae family [12]. Wiegand and Burak (2004) further state that the reason for filamentation is receptor blocking similar to PBP3, which is responsible for septum formation during cell division. That's it resulting in the formation of filaments with multiple copies of the chromosome. They further state that thus damaged cells can regain the ability to divide when transferred to antibiotic-free

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