



Biological Properties of *Streptococcus pluranimalium* as the New Human Pathogen

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Abstract

BACKGROUND: The limited amount of information available today does not fully reflect the biological properties of *Streptococcus pluranimalium* as a pathogen new to humans, its pathogenicity factors, and, as a consequence, the pathogenesis of diseases, which it causes

AIM: The aim of this research was to study the biological properties of *S. pluranimalium*, its sensitivity to antibiotics and antiseptics, as well as its adhesive properties.

METHODS: Two hundred samples were collected from the coronal pockets in patients with acute purulent pericoronitis during 2019–2021 years. Among them, five clinical strains of *S. pluranimalium* were isolated. Final identification was carried out using a Vitec-2compact bioMérieux automatic bacteriological analyzer. The sensitivity of the studied microbial strains to antibiotics of various groups was determined by the disk diffusion method. The adhesive properties of *S. pluranimalium* were determined according to the standard Brilis method.

RESULTS: It possesses typical morphological and cultural properties characteristic of the genus *Streptococcus* representatives. This microorganism virtually does not break down carbohydrates, but it produces arylamidases that enables it to be differentiated from other streptococci. *S. pluranimalium* demonstrates variable sensitivity to antibiotics; the lowest sensitivity has been found out to the second-generation fluoroquinolones. In addition, the clinical isolates studied show high adhesive properties to human red blood cells.

CONCLUSIONS: *S. pluranimalium* is increasingly acting as the causative agent of human infectious diseases. The information available today fully reflects the biological properties of a pathogen new to humans, its pathogenicity factors.

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Introduction

The genus *Streptococcus* includes more than 60 species; nevertheless, up till now, their taxonomic classification has not been clearly defined [1]. Along with significant phylogenetic diversification, representatives of this genus occupy a number of ecological niches for residing. Most species make up the normal microbiome of various biotopes, while some species have long been known as the leading pathogens for humans or animals [2], [3]. However, with the advent of new laboratory technologies, changes in environmental conditions triggered by industrialization, and the global production and consumption of genetically modified products, there have been registered not only changes in the composition, but also a surge in the identification of new pathogens that are not characteristic of human microbiota [4], [5], [6], [7].

The fact that streptococci pathogenic for fish, broiler chickens, and cattle are being increasingly

registered as causative agents of severe infectious in humans is one of the current challenges [8]. *Streptococcus pluranimalium* is known as an atypical representative of this genus, which is extremely rarely isolated from humans [9]. However, recently, according to the few available reports, cases of its isolation in patients with sinusitis, endocarditis, and brain abscesses are both primary and secondary in nature [10], [11], [12], [13], [14], [15], [16]. Moreover, the occurrence of infections caused by *S. pluranimalium* is not always associated with immunosuppressive states of patients and covers cases in different countries and continents [8], [9], [12].

In turn, the problem of rapid acquisition of antibiotic resistance by microorganisms also concerns representatives of this species. Genetic analysis of *S. pluranimalium* of animal origin revealed the *mef(A)*, *msr(D)* and *Inu(C)* genes. The *mef(A)* gene encodes an efflux pump exhibiting resistance to macrolides, and susceptibility to lincosamides and streptogramin B antibiotics. The *msr(D)* gene, one of the ABC-F

subfamily of ATP-binding cassette proteins, mediate macrolide resistance through ribosomal protection [17]. However, this issue needs further research.

The limited amount of information available today does not fully reflect the biological properties of a pathogen new to humans, its pathogenicity factors, and, as a consequence, the pathogenesis of diseases, which it causes [9].

The aim of this research was to study the biological properties of *S. pluranimalium*, its sensitivity to antibiotics, as well as its adhesive properties.

Materials and Methods

Two hundred samples were collected from the coronal pockets in patients with acute purulent pericoronitis during 2019–2021 years. Among them, five clinical strains of *S. pluranimalium* were isolated [18]. The material was inoculated on 5% of blood agar and yeast hydrolyzate was added as a growth stimulator for microorganisms, then a pure culture was isolated. The microorganisms were cultured for 24 h at 37°C with 8–10% CO₂. Final identification and biochemical properties of the studied microorganisms were detected using a Vitek-2compact bioMérieux automatic bacteriological analyzer (France), according to the manufacturer's instructions [19]. The morphological and tinctorial properties of the microorganisms were determined by an immersion microscope MICROMed XS - 2610, cultural properties were assessed by the nature of growth on a nutrient medium.

The sensitivity of the studied microbial strains to antibiotics of various groups was determined by the Kirby-Bauer disk diffusion method according to the standard technique. The results of the pathogen sensitivity to antibiotics were evaluated by the size of the diameter of the growth inhibition zones of the microorganism around the drug disk (mm) [20], [21].

The adhesive properties of *S. pluranimalium* were determined according to the well-known standard Brilis method [22]. Human erythrocytes of O (I) group Rh (+) were used as a universal model for studying the adhesion of a microorganism. Red blood cell suspensions and microorganisms were mixed, followed by incubation and staining by standard techniques. The count was carried out under an immersion microscope on at least 50 red blood cells, taking into account no more than 5 red blood cells in a field of view. Adhesive properties were assessed by the microbial adhesion index (MAI), that is, the average number of microbial cells per a red blood cell involved in the adhesive process. Microorganisms were considered as non-adhesive with MAI = 1.75, as low-adhesive with

MAI = 1.76–2.5, medium-adhesive with MAI = 2.51–4.0 and highly adhesive with MAI ≥4.1).

Each study was repeated 5 times. Statistical analysis of the findings obtained was carried out by using standard software packages "STATISTICA +" and "Microsoft Excel 2010." The presence of differences between the studied parameters was evaluated by *Student's t-test*. The difference between the results was considered statistically significant when the $p < 0.05$.

Every patient had signed an informed consent for sampling of biological material and processing personal data before the start of the study. The study was performed in accordance with the Helsinki Declaration on Ethical Principles for Medical Research involving Humans and approved by the Bioethics Commission of Poltava State Medical University (#188, 25.11.20).

Results

According to the results of our investigation, *S. pluranimalium* has been isolated as a causative agent of acute purulent pericoronitis in 2.5% of individuals. The studied microorganisms possessed morphological and tinctorial properties typical of representatives of the genus *Streptococcus*. When stained with Gram stain, *S. pluranimalium* was seen as medium-sized Gram-positive cocci, arranged in chains or in pairs. When cultivated on blood agar with 8–10% CO₂, it formed small (1.0–1.5 mm) gray colonies with a smooth surface surrounded by an alpha hemolysis zone (Figure 1).

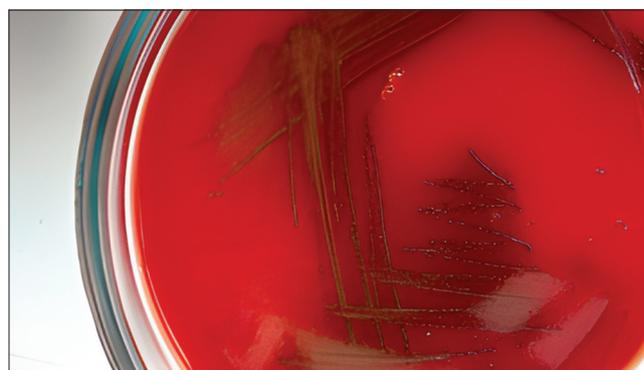


Figure 1: The character of *S. pluranimalium* growth on 5% of blood agar with using the yeast hydrolyzate

The results obtained have demonstrated *S. pluranimalium* possesses no pronounced biochemical activity. The microorganism does not break down sucrose, lactose, mannitol, mannose, maltose, trehalose, galactose, pullulan, ribose, raffinose, xylose, and sorbitol. The studied clinical strain is not capable of producing urease, it is sensitive to novobiocin and bacitracin, but at the same time it shows resistance to optokhin and polymyxin B. Production of beta

galactopyranosidase and a number of proteolytic enzymes of the arylamidase group: Leucine arylamidase, alanine arylamidase, and tyrosine arylamidase, has been found out as an important differential feature among the *S. pluranimalium* biochemical properties.

According to the results of the study, the clinical isolates of *S. pluranimalium* showed variable sensitivity to various groups of antibiotics (Table 1).

Table 1: *S. pluranimalium* sensitivity to antibiotics, mm, n = 5, M ± m

S. No.	Name of antibiotic	Zone of growth inhibition, d	N° n/n	Name of antibiotic	Zone of growth inhibition, d
1	Ofloxacin	17 ± 0.58	7	Ciprofloxacin	21 ± 0.81
2	Clindamycin	34 ± 0.93	8	Cefotaxime	36 ± 0.86
3	Cefazolin	44 ± 0.88	9	Lincomycin	34 ± 0.93
4	Doxycycline	33 ± 0.62	10	Furazidone	24 ± 0.94
5	Vancormycin	23 ± 0.71	11	Ceftazidime	35 ± 0.64
6	Tetracycline	34 ± 0.33	12	Cefuroxime	34 ± 0.57

S. pluranimalium: *Streptococcus pluranimalium*.

The studied microorganisms were the most sensitive to cephalosporins. At the same time, the diameter of the growth inhibition zones around the disk with cefazolin belonging to the first generation cephalosporins was in 1.3 times higher than this indicator around the third-generation cephalosporin antibiotics. Furthermore, the *S. pluranimalium* clinical strains were sensitive to the action of lincosamides (clindamycin and lincomycin) and tetracycline, whose effect was not inferior to the third-generation cephalosporins.

The studied microorganisms showed the lowest sensitivity to the second-generation fluoroquinolones (ofloxacin and ciprofloxacin) as indicated by the smallest diameters of the zones of bacterial growth inhibition around the discs with the antibiotics mentioned.

The results of the study have demonstrated that the *S. pluranimalium* clinical strains possess high adhesive properties to human red blood cells (Figure 2). The average MAI was ± 4.35. It should be noted that 64% of red blood cells had adherent bacteria on their surface.

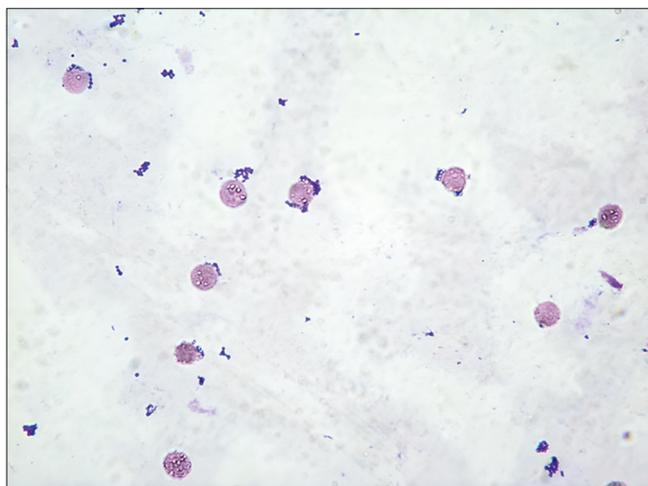


Figure 2: Microscopic picture of *S. pluranimalium* adhesion on red blood cells by Brilis. Gram stained species. Magnifying lens system ×100; Ocular lens ×10

Discussion

S. pluranimalium was first isolated from phylogenetically unrelated animal species and was described by Devriese *et al.* in 1999. Moreover, this pathogen colonized various biotopes of the animal organism: The genitourinary system, the udder and tonsils of mammals, feathers, skin, and the respiratory tract of birds [23], [24], [25]. For several years since its first description, *S. pluranimalium* was identified as the causative agent of mastitis in milk cows, septicemia, and endocarditis in chickens, as well as vaginitis, metritis, and abortion in cattle [12], [25], [26]. Nevertheless, since 2013, one or two clinical cases of human diseases caused by this microorganism are recorded annually in the world [9]. *S. pluranimalium* has been also known to cause brain abscesses, septicemia, and endocarditis in both immunocompromised and healthy patients [9], [10], [11], [12]. Moreover, the case of isolation of this microorganism from subgingival plaque in patients with upcoming tooth extraction due to periodontitis has been reported [1]. Most likely, infection occurs when consuming thermally unprocessed dairy products obtained from infected cows, or as a result of direct contact when handling animals [26]. Yet, a limited number of clinical cases impede to reliably determine a causal relationship, which would indicate the pathway of pathogen transmission to humans.

We might suggest there are several reasons for this pathogen to colonize human body. Possessing a certain set of virulence factors, this microorganism is quite capable of causing pathological processes in the body of not only animals, but humans as well. We can presuppose that the trigger mechanism for colonization and the further development of the pathological process is the high adhesive properties of *S. pluranimalium*. Recent works prove the presence of fibronectin-binding protein on the surface of the bacterial cell of *S. pluranimalium*. These proteins promote adhesion to other microorganisms and cells of the human body, acting as invasins [17]. Moreover, based on phylogenetic studies of 16S rRNA, it is revealed that *S. pluranimalium* has close relationship with *Streptococcus salivarius* group with production of similar hemolysins [9], [17]. It provides alpha hemolytic activity, that pathogen demonstrated in our study. At the same time, despite the low enzymatic activity with respect to carbohydrates, this pathogen produces several arylamidases. They cleave the terminal amino group from leucine, alanine, and tyrosine that, presumably, can be considered as factors of aggression of the microorganism that contribute to its penetration and spread into the tissues.

The results regarding the highest sensitivity of the isolates to cefazolin appeared to be quite natural. After all, the first-generation cephalosporins show significantly higher activity against Gram-positive microorganisms, while non-inherited generations of antibiotics of this group

are more active against Gram-negative pathogens [27]. It should be noted that vancomycin is the drug of choice for the treatment of infections caused by resistant Gram-positive cocci; however, it was not sufficiently effective against *S. pluranimalium* strains. Unlike the vast majority of related streptococci, *S. pluranimalium* does not carry genes responsible for resistance to tetracyclines, which is why the rather high result obtained in this study follows. Along with this, *S. pluranimalium* showed low sensitivity to peicillins, which, presumably, can be connected with the presence of the *pbp2b* gene inherent in *Streptococcus* spp. However, this needs further investigation [28].

Conclusions

S. pluranimalium is increasingly acting as the causative agent of human infectious diseases. It possesses typical morphological and cultural properties characteristics of the genus *Streptococcus* representatives of. This microorganism virtually does not break down carbohydrates, but it produces arylamidases that enables it to be differentiated from other streptococci. *S. pluranimalium* demonstrates variable sensitivity to antibiotics; the lowest sensitivity has been found out to the second-generation fluoroquinolones that excludes the medicines of this group for therapy. In addition, the clinical isolate studied shows high adhesive properties to human red blood cells.

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