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Does *Staphylococcus lugdunensis* Pose any Infection Risk After a Cataract Surgery ?

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Abstract

Purpose: The purpose of this study is to present the current risk and to examine the biofilm formation ability and the antibiotic sensitivity of *S.lugdunensis* that were isolated from the cataract surgeries and to minimize the risks that are likely to occur due to *S.lugdunensis* following cataract surgeries

Material and Methods: The bacteria that had been isolated from previous cataract surgeries and stored at the Microbiology Laboratory of Eskisehir Technical University, Faculty of Science, Department of Biology were used for the study. The isolates grown on blood agars were tested with gram stain, catalase, coagulase and oxidase tests. The strains were identified with ID 32 Staph and VITEK II system (BioMerieux). The RiboPrinter® Microbial Characterization System (Dupont Qualicon) and the standard EcoRI DNA preparation kit were used for Automated EcoRI Ribotyping. The isolates were assessed for biofilm production according to a modified microtiter plate method and cultivation on Congo Red Agar (CRA) plates. The antibiotic sensitivity of the isolates was tested by the disc diffusion method. Vancomycin and methicillin were assessed by microdilution method.

Results: We identified 12 *S.lugdunensis* isolates from ocular surface of patients who underwent cataract surgery. They all produced beta hemolytic rough white colonies in the blood agar. Regarding antibiotic sensitivity results, all of the tested isolates were found to be sensitive to vancomycin and levofloxacin. Cefuroxime resistance was found in two third of the strains. While 1 isolate produced strong biofilm, 4 isolates produced a moderate biofilm, with CRA method. Six isolates did not produce any biofilm. As for microtitration method, 3 isolates produced strong biofilm, while 5 isolates did not produce any biofilm.

Conclusion: Vancomycin provided a consistent coverage for *S. lugdunensis* and should be selected as the first line of treatment for acute endophthalmitis caused by coagulase-negative staphylococcus. In our study, two thirds of the *S.lugdunensis* isolates were multi drug resistant, and these isolates were resistant to cefuroxime which is used as intracameral antibiotic. This should be kept in mind in endophthalmitis in vulnerable patients.

Introduction

Staphylococcus lugdunensis is a member of coagulase-negative staphylococci (CNS). *S.lugdunensis* is a bacteria colonized in the skin and causing skin/soft-tissue infections, endocarditis, arthritis and prosthetic joint infections.(1, 2) *S.lugdunensis* was isolated as a cause of soft tissue and skin infection in the past. It also caused significant infections threatening the life, such as meningitis, chronic osteomyelitis and endocarditis. (3)

Coagulase-negative staphylococci (CNS) are gram positive el-

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ements that are frequent causes of bacterial endophthalmitis and are isolated in 62% of vitreous samples after a cataract surgery or an intraocular lens (IOL) implantation. *Staphylococcus epidermidis* and *S.lugdunensis* are the most isolated CNS species from acute endophthalmitis. (4,5) It is known that *S. lugdunensis* is an aggressive coagulase negative staphylococcus. Bacterial endophthalmitis is among the most devastating complications of intraocular surgery after a cataract operation with an estimated incidence between 0.07% and 0.3%, and can cause a significant loss of vision.(4, 6-9) Several studies were reported regarding intravitreal injections of patients with endophthalmitis caused by *S.lugdunensis* after cataract surgeries. (10-12) *S.lugdunensis* was reported to play role in acute blepharitis, anterior segment infections such as corneal ulcers and infectious keratitis. (13,14)

Most of bacterial endophthalmitis cases reported to be caused by *S.lugdunensis* in ophthalmic surgery cases are characterized with an insidious onset approximately 1 week after the intraocular surgery, accompanied by a severe, painless loss of vision. (10)

The virulence of *S.lugdunensis* is very similar to *S.aureus.* The drug sensitivity and the severity of disease are different from other CNS pathogens. Therefore, it is often compared with *S. aureus.* The ability of CNS to produce a biofilm on the surfaces is an important factor facilitating to onset of the infections. (15) Biofilms, in general, resist to antimicrobial agents at high levels, and this situation makes the treatment of biological organism infections a difficult and an expensive effort, whereas it will be more complicated if the causal organism is resistant to several medicines.

It was aimed in the study to present the current risk and to examine the biofilm formation ability and the antibiotic sensitivity of *S.lugdunensis* by defining the bacteria that were isolated from the cataract surgeries in order to minimize the risks due to *S.lugdunensis* which is stated among coagulase bacteria as no identification is made in the isolates or which can be confused with *S.aureus*, and in particular to prevent eye infections that might occur in immunosuppressed individuals.

Material And Methods

Identification of Bacteria

The bacteria that had been isolated from previous cataract surgeries and stored at the Microbiology Laboratory of Eskisehir Technical University, Faculty of Science, Department of Biology were used for the study.

Staphylococcus strains were growth on blood agar plates (BioMerieux, Marcy l'Etoile, France) at 37°C in aerobic conditions for 24 h. Isolates that were obtained from plates were identified using conventional and molecular microbiological methods. The isolates were tested with gram stain, catalase, coagulase and oxidase tests. (16)The strains were further identified with ID 32 Staph and VITEK II system (BioMerieux). Thereafter, Automated EcoRIRibotyping was carried out with a RiboPrinter® Microbial Characterization System (Dupont Qualicon) and the standard EcoRI DNA preparation kit according to the manufacturer's operations and analytical guides. The reliability of these systems depends upon the number and diversity of bacteria in the databases.

Biofilm Formation

The isolates were assessed for biofilm production according to a modified microtiter plate method. (17) The isolates of *S. lugdunensis* were developed in 96-well elisa petris containing 2% glucose tryptic soy broth (TSB). The plates were incubated for 24 at 37°C temperature. At the end of that period, the plates were emptied carefully, washed with phosphate buffered saline (PBS), and then dried. The plates were washed with PBS following the staining. The stained plates were evaluated spectrophotometrically at A492 nm.

The criterion described by Chistensen et al. (17) was used to identify whether isolates were strongly biofilm-positive (A492 > 0.12) or non-adherent and biofilm-negative (A492 <0.12). The mean biofilm absorbance values were used after repeating assays six times

Slime production in strains was also determined by cultivation on Congo Red Agar (CRA) plates. (18)

Antibiotic sensitivity test

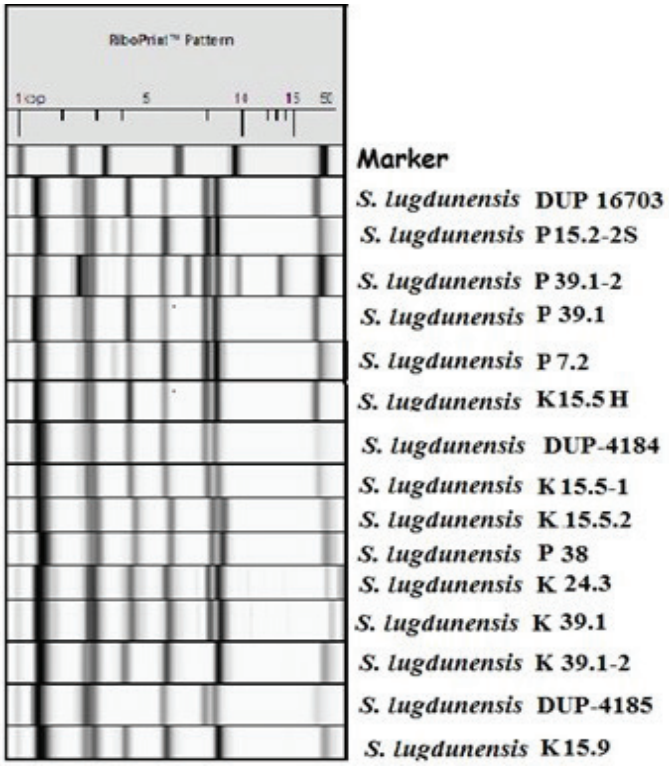
Antibiotic sensitivity profile of the *Staphylococcus spp.* against the most common antibiotics used in ocular infections were assessed by the disc diffusion method according to the Clinical Laboratory Standards Institute guidelines.(19). Disks contained these antibiotics: gatifloxacin (5 µg), (5 µg), cefuroxime (30 µg), ceftazidim (30 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), lomefloxacin (10 µg), moxifloxacin (5 µg). Vancomycin and methicillin were assessed by microdilution method. (19,20)

Results

We identified 12 *S.lugdunensis* isolates from ocular surface of patients who underwent cataract surgery. All of the isolates produced beta hemolytic rough white colonies in the blood agar. In other tests, the catalase was determined as positive, oxidase as negative, tube coagulase as negative and the slide coagulase as positive. While the free coagulase enzyme was negative, the clumping factor (bound coagulase enzyme) was positive in *S.lugdunensis.* The coagulase test comes out negative when analysis is performed in the tube. However, the clumping factor was identified as positive in latex agglu-

tination test and could have be identified as *S.aureus* mistakenly. For this reason, by applying together in both tests, the isolates of which clumping factor was positive and coagulase test was negative were marked. In the test made with Vitek 2 gram-positive card, the pyrrolidonyl arylamidase (PYR), ornithine decarboxylase, urease and mannitol were found positive. These findings supported that the tested isolated could be *S.lugdunensis* . Also in the analysis made with Vitek 2-GP card, the isolates were identified as *S.lugdunensis* .

Figure 1. Riboprinter band profiles of *S.lugdunensis* isolates



In the analysis made with automatic riboprinter, whereas 16S rRNA is predicated upon in this automatic system; the EcoR I enzyme is used automatically to cut, and ran on a gel, and the formed band sizes are compared with marker. Thereafter, the result is found if the similarity to the data stored in the library of the riboprinter is above 85%. All of the tested isolates were identified as *S.lugdunensis* in the test (Figure 1)

Regarding antibiotic sensitivity results, all of the tested isolates were found to be sensitive to vancomycin and levofloxacin. (Table 1).

While 1 isolate produced strong biofilm, 4 isolates produced a moderate biofilm, with CRA method. Six isolates did not produce any biofilm. As for microtitration method, 3 isolates produced strong biofilm, while 5 isolates did not produce any biofilm (Table 2).

Isolates no	Biofilm	
	Microtiter	CRA
P 7.2	++	++
K 15.5.1	++	++
P 15.5.2	-	-
K 15.5H	+++	-
K 15.9H	-	-
K 24.3 H	+	+
P 39.1.2	-	-
P 39.1	-	-
K 39.1H	+++	+++
K 39.1.1	-	-
P 38	++	++
P 15.5.2	+++	++

OD < 0,120 = (-); OD < 0,240 = (+); OD < 0,500 = (++); OD > 0,500 = (+++)

OD: Optical Density

Table 2. The biofilm formation states of isolates.

Isolates no	Antibiogram of the isolates									
	Cefuroxime (30 µg)	Methicillin	Ceftazidim (30 µg)	Ciprofloxacin (5 µg)	Gentamicin (10µg)	Amikacin (30 µg)	Vancomycin*	Gatifloxacin (5 µg)	Levofloxacin (10 µg)	Moxifloxacin (5 µg)
P 7.2	R	R	R	S	R	S	S	S	S	S
K 15.5.1	R	R	R	S	S	S	S	S	S	S
P 15.5.2	R	R	R	S	S	S	S	S	S	S
K 15.5H	R	R	R	S	S	S	S	S	S	S
K 15.9H	S	S	S	S	S	S	S	S	S	S
K 24.3 H	S	S	S	S	S	S	S	S	S	S
P 39.1.2	S	S	S	S	S	S	S	S	S	S
P 39.1	R	R	R	I	I	R	S	S	S	R
K 39.1H	R	R	S	S	R	R	S	R	S	R
K 39.1.1	S	S	S	S	S	S	S	S	S	S
P 38	R	R	R	S	S	S	S	S	S	S
P 15.5.2	R	R	R	I	R	R	S	S	S	R

Table 1. Antibiotic sensitivity results of test isolates.

Discussion

In our study, all the *S.lugdunensis* isolates produced beta hemolytic white colonies in the blood agar. Yazgi and Uyanik (21) also reported similar findings.

Babu and Oropello (1) reported that the coagulase test applied to Staphylococcus isolates could give different results according to the test to be made. Therefore, we marked which clumping factor was positive and which coagulase factor which was negative since the positive clumping factor in latex agglutination test could have been identified as *S.aureus.* Researchers recommended PYR and ornithine decarboxylase tests in addition to coagulase tests as key test for identification of *S.lugdunensis* as we performed. (10,14)

The researchers reported that *S.lugdunensis* is sensitive against antibiotics in general (22), however its resistance to penicillin increased lately. (23, 24) Tan et al. (25) reported that 5% of isolates are resistant to methicillin. Eight (66.7%) of them were resistant to 3 or more antibiotics. Garoon et al. (26) reported that half of the *S.lugdunensis* isolates they isolated from 6 cases were resistant to oxacillin. As we put up in our study, they reported that *S.lugdunensis* isolated from 83.3% of the cases was resistant to ciprofloxacin, and similarly the cultures were resistant to levofloxacin and moxifloxacin. In our study, 3 isolates were found to be resistant to moxifloxacin and 9 isolates were sensitive. Higher MIC limit values of *S.lugdunensis* for methicillin and vancomycin are found compared to those for other coagulase negative Staphylococcus species and they are more similar to MIC values of *S.aureus.* (25) Approximately 25% of clinical isolates of *S.lugdunensis* was reported to produce extracellular agents that play a role in bacterial colonization and intervene in the activities concerning phagocytosis. (27) Biofilms are important in the pathogenesis of *S.lugdunensis* infections, however studies on this subject are still rare. The biofilms corrupt the efficiency of antimicrobial agents, and this is an important factor to determine the clinical progress of the treatment. (28) Bacterial biofilms are responsible for the emergence of resistance to antibiotherapy. (29) In our study, 1 isolate produced strong biofilm, 4 isolates produced a moderate biofilm and 1 isolate produced mild biofilm with CRA method. Six isolates did not produce any biofilm with CRA. As for microtitration method, 3 isolates produced strong biofilm, 3 produced moderate and 1 mild while 5 isolates did not produce any biofilm. As depicted, half of the isolates produced biofilm, which can be clinically significant in ocular infections such as endophthalmitis.

Acute endophthalmitis due to *S.lugdunensis* is generally insidious as it is primarily related to intraocular surgery. The microorganisms are generally resistant to most of the antibiotics including vancomycin; however if they are related to open globe injury they can be resistant to oxacillin, and rarely show resistance to ciprofloxacin.

In parallel with *S.aureus*, *S.lugdunensis* is inclined to infections causing from skin; however, those related to cataract surgery or intravenous injections showed a less resistance to antibiotics, as they come from an infectious origin exogen source, particularly in relation with an open globe injury. While vancomycin resistance was observed for some Staphylococcus species, the vancomycin provided a consistent coverage with no resistance known for *S.lugdunensis*. Vancomycin should be selected as the first line of treatment for acute endophthalmitis caused by coagulase-negative staphylococcus. (30) In our study, two thirds of the *S.lugdunensis* isolates were multi drug resistant, and these isolates were resistant to cefuroxime which is used as intracameral antibiotic.

S.lugdunensis should be kept in mind as an infrequent but agressive agent in post operative endophthalmitis especially in diabetic patients.

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