Epidemiology, Drug Resistance, and Virulence of *Staphylococcus aureus* Isolated from Ocular Infections in Polish Patients

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Abstract

Analysis of the epidemiology of *Staphylococcus aureus* (SA) ocular infections and virulence factors of the isolates with a special emphasis on their drug resistance, and the ability of biofilm formation. In a period from 2009 to 2013, 83 isolates of SA were prospectively collected and preserved in a multicenter laboratory-based study carried out in southern Poland. Epidemiological, phenotypic, and genotypic analyses were performed. The resistance and virulence genes were analyzed. Screening for the biofilm formation was provided. Among the materials derived from ocular infections from 456 patients, SA was found in 18.2% (n = 83) of cases (one SA isolate per one patient). Most infections were identified in the age group of over 65 years (OR 8.4 95%CI; 1.03-68.49). The majority of patients (73.4%) were hospitalized. Among the virulence and resistance genes, the most frequently detected were the *luk*E (72.2%, n = 60) and *erm*A (15.6%, n = 13) genes. A positive result of the CRA test (the ability of biofilm formation) was found in 66.2% (n = 55) of isolates. Among the strains under study, 6.0% (n = 5) had the methicillin-resistant *Staphylococcus aureus* phenotype, and 26.5% (n = 22) had the macrolide-lincosamide-streptogramin B phenotype. In 48 (57.8%) isolates the neomycin resistance was revealed. All isolates under study were sensitive to vancomycin. The population most susceptible to ocular SA infections consists of hospitalized patients aged 65 and more. The SA strains under study showed the increased ability to biofilm formation. In the strains tested, high susceptibility to chloramphenicol and fluoroquinolones was demonstrated. However, the high level of drug resistance to neomycin detected in this study among SA isolates and the blood-ocular barrier makes it difficult to treat ocular infections.

K e y w o r d s: *Staphylococcus aureus*, ocular infections, virulence factors, epidemiology, surgical interventions, soft contact lenses

Introduction

Bacteria are considered as the main contributor to ocular infections all over the world (Teweldemedhin et al. 2017). In the study by Long et al. conducted between 1990–2009, the most frequently isolated bacteria from ocular infections were Gram-positive cocci (41.9%) (Long et al. 2014). Analysis of databases proved that *Staphylococcus aureus* (SA) is predominant regardless of the geographical area or population examined (Teweldemedhin et al. 2017). The results of the research regarding the prevalence of SA isolates from ocular infections showed their distribution in the range from 13% in India to 28.1% in Ethiopia; the average prevalence was 20.1% (Teweldemedhin et al. 2017).

The most common ocular infection is conjunctivitis, which constitutes 50–70% of infectious conjunctivitis (Bertino 2009; Galvis et al. 2014; Teweldemedhin et al. 2017). Moreover, one should also point out the frequent incidents of bacterial keratitis and endophthalmitis (West et al. 2005; Bertino 2009; Pozzi et al. 2012; Teweldemedhin et al. 2017). Untreated ocular infections may cause injuries in the ocular structure and lead to visual impairments and blindness (Bertino 2009; Teweldemedhin et al. 2017). Researchers indicate a strong relationship between ocular trauma, contact lenses, and bacterial keratitis lesions in the anatomical ocular surface that may lead to the development of staphylococcal infection (Bourcier et al. 2003; Ly et al. 2006; Teweldemedhin et al. 2017). Moreover, a patient's

immunity to ocular infections can be reduced by underlying diseases, operative procedures, the use of corticosteroids, hospitalization, and the use of medical devices (Teweldemedhin et al. 2017).

One of the main SA virulence factors that contribute to ocular infections is its ability to the formation of biofilms on the surface of biomedical implants or contact lenses (Cramton et al. 1999). Through this process, the bacteria become more resistant to various physicochemical stresses, e.g. antibiotics (Mathur et al. 2018). Cramton and coworkers reported that SA was more frequently isolated from corneal infections related to the contact lenses wearing (Cramton et al. 1999). The extended wear of contact lenses and lack of eye hygiene increase the risk of keratitis. The morbidity of ocular infections is associated with the increasing number of cataract surgery and lens replacement (Astley et al. 2019). The ability of SA strains to aggregate and form biofilm is related to their capacity of producing slime – an extracellular mucoid substance whose main components are glycosaminoglycans. The well-established phenotypic methods, such as the Congo Red Agar (CRA) test, are still used for the identification of the virulent biofilm-forming bacteria confirming phenotypically their ability to develop a biofilm. It has been shown that the results of this method coincide with the presence of the *ica*A and *ica*D genes in staphylococci (Arciola et al. 2002).

There is little information on human SA ocular infections in databases such as PubMed, a fact that makes it impossible to work out and implement effective and plausible measures to prevent infections. Concerning Polish patients, there is no epidemiological data at all. We sought to describe the epidemiology and various types of treatment for SA ocular infections with a special emphasis on cataract postoperative complications or the consequences of soft contact lenses wearing.

Experimental

Materials and Methods

SA isolates. Isolates from this multicenter laboratory-based study were obtained by the Department of Microbiology of the Jagiellonian University Medical College and were collected in collaboration with KORLAB from 1 January to 31 December 2013. Non-repetitive samples from ocular infection were collected from hospitalized patients (62) or outpatients (21) throughout the south of Poland. In total, clinical materials from 456 patients with symptoms of infection were examined and 83 isolates of SA were found, including 47 strains from the vitreous and corneas. The remaining clinical materials were conjunctival swabs.

The relevant patient information including age, sex, and type of care (ambulatory/hospitalization) was collected. The identification of microorganisms was performed using the MALDI-TOF Biotyper (Bruker Corporation, the Netherlands) according to standard methods.

Susceptibility testing. Antimicrobial susceptibility testing of all SA isolates was performed according to the current guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, http://www.eucast.org/clinical_breakpoints/; accessed December 2017) by disc diffusion or the E-test method on Müeller-Hinton agar plates. The antimicrobial discs (Oxoid Ltd., UK) contained gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), neomycin (10 µg), ciprofloxacin (5 µg), moxifloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), clindamycin (2 µg), erythromycin (15 µg), chloramphenicol (30 µg), and tetracycline (30 µg). Antimicrobial susceptibility to neomycin was interpreted according to the standards of the British Society for Antimicrobial Chemotherapy Version 14.0, 05.01.2015 (BSAC, http://bsac.org.uk/wp-content/uploads/2012/02/BSAC-Susceptibility-testing-version-14.pdf). For vancomycin, the minimal inhibitory concentration (MIC) was determined by E-test (bioMérieux, France).

The methicillin-resistant *Staphylococcus aureus* (MRSA) phenotype was detected using a cefoxitin disc (30 µg). The macrolide-lincosamide-streptogramin B (MLS$_B$) phenotype was determined according to a previously published protocol (Leclercq 2002).

The categories of antimicrobial resistance. Strains were divided into six categories based on their resistance to several antimicrobial agent categories (aminoglycosides, fluoroquinolones, folate pathway inhibitors, lincosamides, macrolides, phenicols, and tetracyclines). The susceptibility of the isolate to all antimicrobial agents from all categories examined denoted "0"; "5 or more" meant resistance to five or more categories.

DNA isolation. The bacterial strains were grown overnight at 37°C in tryptic soy broth medium and total DNA was isolated with the Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions.

Polymerase Chain Reaction (PCR)-based detection of resistance and virulence genes. PCR amplification was used to detect the *mec*A gene using previously described primers (Pereira et al. 2010). As controls, SA ATCC 33591 (*mec*A+) and SA ATCC 25923 (*mec*A−) were employed. PCR was also used to detect the presence of a gene resistant to mupirocin (*mup*A gene) (Anthony et al. 1999). SA ATCC BAA-1708 (*mup*A+) was employed as a control. The erythromycin resistance genes (*erm*A, *erm*B, *erm*C, and *msr*) were detected by multiplex PCR (Sutcliffe et al. 1996). The bands were visualized with the UVP GelDocIT Imaging System

Table I
Staphylococcus aureus strains isolated from ocular infections in different patient age groups with consideration
of gender, hospitalization, and the results of the CRA test.

Characteristics of the study group	Hospitalization (n; %)			OR (95% CI)	p-value
	Yes, n = 61 (73.4%)	No, n = 22 (26.5%)	Total, N = 83		
Age (years) by categories [n; %]					
<= 18 years	15 (24.5%)	1 (4.5%)	16 (19.2%)	2.5 (0.20–32.99)	
19–64 years	12 (19.6%)	2 (9.0%)	14 (16.8%)	1.00 (ref.)	0.027
>= 65 years	34 (55.7%)	19 (86.3%)	53 (63.8%)	8.4 (1.03–68.49)	
Gender [n; %]					
Female	26 (42.6%)	12 (54.5%)	38 (45.7%)	0.6 (0.23–1.65)	0.454
Male	35 (57.3%)	10 (45.4%)	45 (54.2%)		
The positive CRA (Congo Red Agar) biofilm test result (n; %)					
yes	45 (73.7%)	10 (45.4%)	55 (66.2%)	3.3 (1.22–9.31)	0.016
no	16 (26.2%)	12 (54.5%)	28 (33.7%)		

OR (95%CI) – 95% confidence intervals of the odds ratio

(UVP, Upland, Canada) after 1.5%-TBE-agarose electrophoresis (70 min, 90 mV) and staining with ethidium bromide (Bio-Rad, Warsaw, Poland). A DNA-ladder of 100–1000 bp (Thermo Scientific, Waltham, MA, USA) was used as a size marker.

SA isolates were verified for the presence of the following virulence genes: *luk*E (LukDE leukocidin), *pvl* (Panton-Valentine leukocidin, PVL), *tsst-1* (toxic shock syndrome toxin-1, TSST-1), *et*A, and *et*B (exfoliative toxin A or B; EtA, EtB) using PCR and the previously described primers (Johnson et al. 1991; Lina et al. 1999). The strains used as controls were kindly provided by Prof. Marek Gniadkowski, National Medicines Institute, Warsaw, Poland.

To determine the *spa* type of the polymorphic X-region of the SA protein A, the *spa* gene was amplified by PCR and sequenced. Chromatograms obtained from sequencing were analyzed using DNAGear Spa Typing Software (Al-Tam et al. 2012).

Biofilm formation. Screening for the ability of SA isolates to develop a biofilm was carried out according to the method described by Arciola et al. with the CRA test (Arciola et al. 2002). 0.8 g of Congo Red and 36 g of saccharose (Sigma, St. Louis, MO, USA) were added to 1 L of brain heart infusion agar (Oxoid, Basingstoke, Hampshire, England) to prepare CRA plates. The plates were incubated at 37°C for 24 h and then overnight at room temperature. On CRA plates, black colonies were formed by slime-producing strains and red ones by non-producing strains. A six-color scale was used to accurately assess all the possible chromatic variations exhibited by the growing colonies. The scale ranged from very black (vb), thorough black (b), and almost black (ab) to burgundy (brd), red (r), and very red (vr). Very black, black, and almost black colonies were classified as the slime producer strains, while very red, red, and burgundy-colored colonies were classified as the strains unable to produce slime.

Ethics. All SA isolates under the study were collected as part of routine clinical care. No medical records or identifying information about the patients were accessed as part of this study. The isolates and any relevant information about the cases were obtained and analyzed in a fully anonymized and de-identified form. All data analyzed during this study were blinded before analysis. The utilization of this data for analysis without patients' agreement was consistent with Polish law and approved by the Bioethics Committee of the Jagiellonian University Medical College (No. BET/227/B/2012).

Results

Among the 456 cases of ocular infections examined, 83 (18.2%) SA strains were isolated (one strain from one patient). Slightly more than half of SA strains (54.2%) came from men. The majority of patients, i.e. 73.4% (42.6% of women and 57.3% of men, respectively) constituted the hospitalized cases (Table I). The results showed a large difference in SA-ocular infection prevalence between hospitalized and ambulatory patients. The most infection cases were observed in the group of people over 65 years (63.8%); the least in the biggest group of age in the range between 19 and 64 years (16.8%). The infections in the oldest patients were treated five times more often in an outpatient setting (OR 95%CI 8.4; 1.03-68.49; $p = 0.027$, Table I).

One of the virulence characteristics, which is biofilm formation, was evaluated with the CRA test. A positive result of the CRA test was found in 66.2% of all cases (Table I). It was demonstrated that 66.2% of the strains showed biofilm formation capacity, with 22% of them

Table II
The presence of various genes encoding for the resistance and virulence factors of
Staphylococcus aureus strains isolated from ocular infections.

Studied genes	Hospitalization (n;%)		Total n = 83
	Yes, n = 61 (73.4%)	No, n = 22 (26.5%)	
*mec*A (n = 6; 7%)	4 (6.5%)	2 (9.0%)	6 (7.2%)
*erm*A (n = 13; 16%)	8 (13.1%)	5 (22.7%)	13 (15.6%)
mup (n = 4; 5%)	4 (6,5%)	0	4 (4.8%)
*luk*E (n = 60; 72%)	45 (73.7%)	15 (68.1%)	60 (72.2%)
tsst-1 (n = 10; 12%)	10 (16.3%)	0	10 (12.0%)
*et*A (n = 3; 4%)	2 (3.2%)	1 (4.5%)	3 (3.6%)
*et*B (n = 2; 2%)	2 (3.2%)	N0	2 (2.4%)

*et*A/B – exfoliative toxin A and/or B; *luk*E – lukDE leukocidins; N/A – not applicable;
OR (95%CI) – 95% confidence intervals of odds ratio

being strong biofilm formers (very black and black colors), and 44% being weaker (almost black color). Among the biofilm-forming strains, the hospital strains dominated (73.4%), whereas among the ambulatory strains the ratio between biofilm-forming strains and non-producing ones was more even (45.4% vs. 54.5%; OR 95%CI 3.3; 1.22–9.31; $p = 0.016$, Table I).

The most frequent virulence and resistance genes were *luk*E and *erm*A (Table II). The presence of other virulence genes oscillated within the range of 1.2–12.0% of cases. The *pvl* gene was found in one strain. Strains from hospitalized patients were the main source of virulence genes. In the isolates from ambulatory patients, the *mup*, *tsst*-1, *pvl* or *et*B genes were not found. Interestingly, all cases of the *pvl*, *tsst* and *et*B genes detected in this study as well as two out of three the *et*A genes and 63% of the *luk*E genes were found among the strains positive in the CRA test.

Among the SA strains, most were resistant to neomycin and comprised 57.8% (n = 48). The level of erythromycin resistance amounted to 25.3%; 13.2% of isolates were resistant to ciprofloxacin, and 7.2% to moxifloxacin (Table III). Resistance to fluoroquinolones was five times more often found in ambulatory patients. Additionally, resistance to tobramycin was recorded for 14 strains (16.8%), to gentamicin for five strains (6.0%), and to chloramphenicol also for five strains (6.0%). All the isolates under study were sensitive to vancomycin, and the MIC value was equal to 1 µg/ml. Out of the isolates under study, 73.4% belonged to the category of fully susceptible to antimicrobial agents. The highest percentage of strains resistant to at least one antimicrobial was identified in hospitalized patients (40.9% for one category) and in outpatients (27.2% for two categories) (Table III). On the other hand, the strains isolated from hospitalized patients were four times more likely to show full susceptibility (they belonged to the "fully susceptible" category, Table III) than strains from non-hospitalized patients.

Among the strains under study, five isolates (6.0%) had the MRSA phenotype and 22 had the MLS$_B$ phenotype (26.5%), including 17 strains that had the inducible (iMLS$_B$) and five strains that had the constitutive (cMLS$_B$) phenotypes (Table III). Four strains manifested both mechanisms at the same time. Each of the five MRSA strains had the *mec*A gene. Additionally, one strain had the *mec*A gene without the MRSA phenotype. Thirteen strains contained the *erm*A gene, including all those with the mechanism of cMLS$_B$ resistance and seven with that of iMLS$_B$. One strain with the iMLS$_B$ mechanism had the *msr*A/B gene, and in eight strains none of the genes of resistance under study was found.

Spa typing of five MRSA isolates showed the presence of three different *spa* types – three strains belonged to t003, one to t015, and one to t1192.

Discussion

In the studied population, the contribution of SA strains to ocular infections was slightly higher than in the American population as it has been shown by Gentile and coworkers, and where the most prevalent pathogens were coagulase-negative staphylococci (39.4%), followed by *Streptococcus viridans* (12.1%), and SA (11.1%) (Gentile et al. 2014). Similar findings came from Canada and Europe (Asencio et al. 2014; Assaad et al. 2015). A Chinese analysis of corneal samples have provided that Gram-positive cocci (69.88%) are the most commonly isolated; nevertheless, a decreasing trend was observed over the nine years of the study (Lin et al. 2019).

However, despite its non-dominant role in ocular infections, SA is an important etiologic agent of ocular infections. Callegan and coworkers have reported that ocular SA infections were more difficult to treat and the sharpness of vision was restored only in 30%

Table III
Drug resistance of *Staphylococcus aureus* strains isolated from ocular infections.

Antimicrobial category	Antimicrobial agent	Hospitalization n (%)		Total, N = 83
		Yes, n = 61 (73.4%)	No, n = 22 (26.5%)	
Aminoglycosides	Gentamicin	4 (6.5%)	1 (4.5%)	5 (6.0%)
	Amikacin	5 (8.1%)	3 (13.6%)	8 (9.6%)
	Tobramycin	9 (14.7%)	5 (22.7%)	14 (16.8%)
	Neomycin	37 (60.6%)	11 (50.0%)	48 (57.8%)
Fluoroquinolones	Ciprofloxacin	4 (6.5%)	7 (31.8%)	11 (13.2%)
	Moxifloxacin	2 (3.2%)	4 (18.1%)	6 (7.2%)
Folate pathway inhibitors	Trimethoprim/sulfamethoxazole	3 (4.9%)	2 (9.0%)	5 (6.0%)
Lincosamides	Clindamycin	13 (21.3%)	8 (36.3%)	21 (25.3%)
Macrolides	Erythromycin	13 (21.3%)	8 (36.3%)	21 (25.3%)
Phenicols	Chloramphenicol	4 (6.5%)	1 (4.5%)	5 (6.0%)
Tetracyclines	Tetracycline	11 (18.0%)	3 (13.6%)	14 (16.8%)
Non-susceptible to antimicrobial agents in (above) categories				
	fully susceptible (0 categories)	37 (60.6%)	6 (27.2%)	61 (73.4%)
	one category	25 (40.9%)	4 (18.1%)	29 (34.9%)
	2 categories	12 (19.6%)	6 (27.2%)	18 (21.6%)
	3 categories	5 (8.1%)	1 (4.5%)	6 (7.2%)
	4 categories	2 (3.2%)	1 (4.5%)	3 (3.6%)
	5 categories or more	2 (3.2%)	4 (18.1%)	5 (6.0%)
MRSA, yes		3 (4.9%)	2 (9.0%)	5 (6.0%)
MLS$_B$, yes		14 (22.9%)	8 (36.3%)	22 (26.5%)

MLS$_B$ – macrolide/lincosamide/streptogramin B resistant *Staphylococcus aureus*; MRSA – methicillin-resistant *Staphylococcus aureus*; OR (95% CI) – 95% confidence intervals of odds ratio

of the patients (Callegan et al. 2007). The research conducted by West and coworkers from 1994 to 2001 in the American population has indicated an increase in endophthalmitis incidence as a complication of cataract surgery, a fact that is challenging because this was the most common surgery in the USA (West et al. 2005; Astley et al. 2019). The reports by West and coworkers were confirmed by the results of Callegan and coworkers, which showed that postoperative endophthalmitis was a result of almost every ocular surgery, mainly cataract surgery (Callegan et al. 2007). Astley and coworkers also pointed to an increase in injection-related complications following intravitreal injections (Astley et al. 2019). One of the important elements that interfere with proper postoperative healing, and is the cause of therapeutic failures can be the virulence of pathogens. In any operation with the use of implants, such as cataract surgery, SA can present its capacity to form a biofilm. This problem was discussed by Ammendolia and coworkers who demonstrated the presence of a very high proportion of biofilm-forming strains (88.9%) higher than in the population investigated here (66.2%) (Ammendolia et al. 1999). At the same time, Ammendolia and coworkers has initially claimed that slime production was never considered as a virulence

factor, but their studies generally dealt with various types of hospital infections, not only ocular infections (Ammendolia et al. 1999). The studies considering the problem of biofilm-forming strains in ocular infections, however, have not been conducted so far. Atshan and coworkers have indicated the biofilm formation to varied extent and diverse adherence capacities of MRSA strains depending on their *spa* type (Atshan et al. 2012).

The results from the Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) group have shown that of MRSA amounts to 39% of ocular infections and there is also an increase in the resistance to fluoroquinolones among the ophthalmic strains in the United States (Haas et al. 2011; Vola et al. 2013). This was confirmed by a study by Morrissey and coworkers conducted in European countries, where MRSA was shown to be an etiologic agent of 22% of all ocular SA infections (Morrissey et al. 2004; Vola et al. 2013). Fortunately, according to the data analyzed and presented here, the problem of MRSA does not concern southern Poland since the prevalence of MRSA is lower. The authors' previous experience regarding other clinical forms of both hospital and outpatient infections in southern Poland indicated a high prevalence of MRSA in bloodstream infections (20.4%), and pneumonia

(32.7%) (Pomorska-Wesołowska et al. 2017). The general hospital prevalence of MRSA is 15.1%, and it is three times higher than it was established in the recent ocular infection study (Chmielarczyk et al. 2016). As reported previously, and also in this study, the *spa* typing confirmed that *spa* type t003 was the most predominant among MRSA strains (Chmielarczyk et al. 2016; Pomorska-Wesołowska et al. 2017).

Between the above-mentioned studies and ours, there was no difference in SA resistance to MLS_B, which was observed at a similar level (less than 30% in the studied patients' population with ocular infections) as well as in other populations of patients in southern Poland (Chmielarczyk et al. 2016; Pomorska-Wesołowska et al. 2017). Unfortunately, there are no known reports on MLS_B resistance in ocular infections coming from other parts of the world.

The most common antibiotics administered in ocular infections are fluoroquinolones, chloramphenicol, and aminoglycosides (Brown 2007). Unluckily, both Polish data and evidence from other centers, including those from Europe, indicate a low sensitivity of SA to aminoglycosides and some fluoroquinolones (Galvis et al. 2014; Gentile et al. 2014). Nevertheless, in the latest ARMOR surveillance studies from the USA, there was no difference in the level of resistance to older- (ciprofloxacin) and newer-generation fluoroquinolones (moxifloxacin), and it was 35.8% vs 33.6%, respectively. In our study, resistance was lower to moxifloxacin (7.2%) than to ciprofloxacin (13.2%), so the newer generation of fluoroquinolones can be more effective in therapy (Thomas et al. 2019).

Given the rising resistance of 4th generation fluoroquinolones that have been observed in recent years, researches were conducted on the effectiveness of aminoglycosides (Galvis et al. 2014). Chinese research on corneal infections caused by SA confirmed the lowest resistance of the strains to neomycin (Wang et al. 2016). The possibility of treatment with the aminoglycoside group was confirmed independently by studies by Blanco and coworkers and Lin and coworkers, which showed high susceptibility of those strains to chloramphenicol (Blanco et al. 2013; Lin et al. 2019). Our results also confirm the high susceptibility of the SA isolates to fluoroquinolones and chloramphenicol. This is important information because the results of systematic review and meta-analysis suggested that fluoroquinolones might be the first choice for empirical treatment of most cases of the suspected bacterial keratitis (Hanet et al. 2012; Austin et al. 2017).

Unfortunately, the findings of this study have indicated that in Poland a serious problem, rarely described by other authors, occurs i.e. the resistance of SA to neomycin in almost 60% of strains. It appears that this is quite a rare situation because the reports of Wang and coworkers from China have recently determined neomycin resistance in 7.8% of strains, i.e. at a considerably lower level than that established for the isolates from Polish patients (Wang et al. 2016). Therefore, this situation is surprising as neomycin is not frequently or routinely used systemically in the treatment of more common infections as opposed to ocular infections. All pharmaceutical preparations with neomycin associated with ocular treatment are available in Poland on prescription and none of them is a combined preparation. For the topical dermatological treatment, there are available over-the-counter medicines containing neomycin in combination with e.g. bacitracin, which could lead to such high neomycin resistance but the lack of Polish historical data or data from other countries makes it difficult to interpret the phenomenon observed.

Ocular antibiotics are usually administered locally, in the form of solution or suspension, to obtain a high concentration of antibacterial in the place of infection. Since the 1980s, the antibiotics can be administered in the form of injections directly into the vitreous, with the visual outcome of patients not changed considerably (Callegan et al. 2007). In ocular infections, therapeutic success depends on quick and accurate diagnosis and also on the administration of antibiotics (Callegan et al. 2007). This is due to the bacterial toxins and enzymes that may damage the integrity of the ocular tissues (Bertino 2009). Astley and coworkers reported some of those, including α-toxin (a role in the pathogenesis of SA keratitis and endophthalmitis) and PVL (cytotoxin) (Astley et al. 2019). The key anatomic barriers, such as the delicate nature of the interior of the eye and the blood-ocular barrier are factors to be considered during treatment (Callegan et al. 2007). Drug administration and contact lenses consist of a problem.

Study limitations

There are some limitations associated with this laboratory-based study. First, the demographic information on the study population is limited. For example, previous hospitalization and/or surgery and antimicrobial usage, co-morbidity, disability, and patient outcome data were not available because of the retrospective nature of the study. Additionally, these results may not be generalizable to the other parts of Poland.

Conclusions

In conclusion, the most common microorganisms in ocular infections were Gram-positive cocci, especially SA strains. The main virulence factor was the biofilm formation capacity of isolates and a high percentage of

strains with the *luk*E gene was also observed. Although high resistance to neomycin was noted, our research indicates a high efficacy of treatment with chloramphenicol and fluoroquinolones, as well as the need to implement new solutions due to the aforementioned bacteria's high resistance to neomycin and anatomic barrier difficulties.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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