ORIGINAL PAPER
Polish Journal of Microbiology
2024, Vol. 73, No 1, 21–28
https://doi.org/10.33073/pjm-2024-003

Antimicrobial Resistance and the Prevalence of the Panton-Valentine Leukocidin Gene among Clinical Isolates of *Staphylococcus aureus* in Lithuania

AGNĖ KIRKLIAUSKIENĖ[1]*, JONAS KRIŠČIŪNAS[2], JOLANTA MICIULEVIČIENĖ[3], DAIVA RADZIŠAUSKIENĖ[4], TOMAS KAČERGIUS[1], MAKSIM BRATCHIKOV[1] and LINA KAPLERIENĖ[1]

[1] Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[2] Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[3] Vilnius City Clinical Hospital, Vilnius, Lithuania
[4] Department of Infectious Diseases and Dermatovenerology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

Submitted 29 September 2023, accepted 18 December 2023, published online 8 March 2024

Abstract

This study aimed to determine resistance to antimicrobials of *Staphylococcus aureus* strains isolated from clinical specimens in Lithuanian hospitals and to identify the genes conferring resistance and virulence. The study was carried out from June 2019 to September 2021. *S. aureus* strains were isolated from skin, soft tissues, blood, lower respiratory tract, urine and other specimens. Antibiotic susceptibility testing was performed using the disc diffusion method according to EUCAST guidelines. All isolates were analyzed for detection of the *ermA*, *ermC*, *mecA*, *mecC*, *tetK*, *tetM*, and *lukF-PV* genes by multiplex real-time PCR. The 16S rRNA coding sequence was applied as an internal PCR control. Altogether, 745 *S. aureus* strains were analyzed. Antimicrobial susceptibility testing revealed that all isolates were susceptible to rifampin and vancomycin. Of the 745 strains, 94.8% were susceptible to tetracycline, 94.5% to clindamycin, and 88.3% to erythromycin. The lowest susceptibility rate was found for penicillin (25.8%). Six percent of the tested strains were methicillin-resistant *S. aureus* (MRSA). The majority of methicillin-resistant strains were isolated from skin and soft tissues (73.3%), with a smaller portion isolated from blood (17.8%) and respiratory tract (8.9%). The *ermC* gene was detected in 41.1% of erythromycin-resistant *S. aureus* strains, whereas *ermA* was detected in 32.2% of erythromycin-resistant *S. aureus* strains. 69.2% of tetracycline-resistant *S. aureus* strains had *tetK* gene, and 28.2% had *tetM* gene. 7.3% of *S. aureus* isolates harbored *lukF-PV* gene. The frequency of the *pvl* gene detection was significantly higher in MRSA isolates than in methicillin-susceptible *S. aureus* isolates ($p < 0.0001$).

K e y w o r d s: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, resistance genes, Panton-Valentine leucocidin

Introduction

The Gram-positive bacterium *Staphylococcus aureus* is an asymptomatic colonizer of the anterior nares (Wertheim et al. 2005). It is a common bacterial pathogen responsible for a wide range of human infections, including skin infections, pneumonia, respiratory tract infections, surgical wound infections, prosthetic joint infections, cardiovascular infections, and nosocomial bacteremia (Tong et al. 2015). While various antimicrobial compounds are used to treat staphylococcal infections, the emergence of antimicrobial resistance is an increasing concern (Vestergaard et al. 2019). Unsuccessful treatment due to antimicrobial resistance leads to significant financial and human losses (Rungelrath and DeLeo 2021). There are numerous mechanisms by which antibiotic resistance arises, including changes in drug targets, enzymatic drug inactivation, enhanced efflux of antimicrobial substances, and altered drug accessibility (Boyle-Vavra and Daum 2016). Horizontal acquisition of the *mecA* gene, which encodes PBP2a, an alternative transpeptidase with poor affinity for the majority of β-lactam antibiotics, confers resistance to methicillin as well as almost all other members of the broad class of β-lactam antibiotics (Vestergaard et al. 2019). Resistance to macrolide, lincosamide,

* Corresponding author: Kirkliauskienė, Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania; **e-mail: agne.kirkliauskiene@mf.vu.lt**

streptogramin B (MLSb) compounds (including erythromycin) is encoded by the *erm* genes, primarily *ermA* and *ermC*, which encode proteins responsible for ribosomal-binding site alteration (by mutation or/and methylation of the 23S rRNA gene) (Alekshun and Levy 2007; Toh et al. 2007; Cetin et al. 2008). Active efflux or target-site protection has been linked to clinically relevant tetracycline resistance (Jensen and Lyon 2009). The genes *tetK* and *tetL* encode efflux pumps of the main facilitator superfamily, which facilitate active efflux by exchanging a proton for a tetracycline molecule (Jensen and Lyon 2009), while the *tetM* gene encodes a protein responsible for ribosomal site protection (Burdett 1991).

Furthermore, virulence factors such as the Panton-Valentine leukocidin gene have been identified a a significant concern for public health. Both methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) strains can possess the *lukS-lukF* gene, which is transcribed as a tandem mRNA sequence with two open reading frames and encodes a pore-forming protein called Panton-Valentine leukocidin (PVL) (Prévost et al. 1995; Löffler et al. 2010; Chi et al. 2014). PVL causes these cells to degranulate prematurely, severely damaging the surrounding tissue. As a result, this process allows the bacteria to avoid the local immune system within the tissue, increasing the invasiveness and virulence of *S. aureus* producing PVL (Shallcross et al. 2013; Sakr et al. 2018). In Lithuania, the prevalence of MRSA infections has remained constant, with rates between 5% and 10% (ECDC 2020). While the number of invasive MRSA infections in other EU countries, such as Italy, Slovakia, Greece, Romania, and Portugal, has dropped to 25% (ECDC 2020), MRSA still poses a threat because of the high rates of hospitalization and mortality associated with MRSA infection, as well as its resistance to antimicrobial drugs (Yahav et al. 2016). Therefore, it is critical to continue developing innovative prevention and treatment strategies for MRSA infections. The main aim of this study was to investigate the prevalence of antimicrobial resistance and virulence genes, including the Panton-Valentine leukocidin gene, among clinical isolates of *S. aureus* in Lithuanian hospitals. This study may provide valuable insights into managing and preventing staphylococcal infections, particularly MRSA infections in Lithuania, and contribute to developing more effective infection prevention and treatment strategies.

Experimental

Materials and Methods

Study design. A retrospective study was conducted from June 1, 2019, to September 2, 2021. Two hospitals in Vilnius, Lithuania, took part in the study: Vilnius City Clinical Hospital (a 510-bed regional hospital) and Vilnius University Hospital Santaros Klinikos Center of Infectious Diseases (a 50-bed centre). All consecutive adult patients (≥ 18 years) with *S. aureus* isolated from clinical specimens were included in this study. *S. aureus* strain isolated from the same person repeatedly was excluded from the study. The patients' sociodemographic data were evaluated using a questionnaire.

Phenotypic identification of isolates. Collected specimens were assigned into five groups: skin and soft tissue, blood, respiratory tract, urine, and other specimens (peritoneal cavity, joint cavity, prostheses, and cerebrospinal fluid). All specimens were inoculated onto blood agar with 5% sheep blood (Graso, Poland) and the selective chromogenic media ChromID® (bioMèrieux, France) and ChromID® MRSA SMART (bioMèrieux, France). All plates were aerobically incubated for 24–48 h at 35 ± 2°C. The MRSA and MSSA clinical isolates were identified with the MALDI--TOF VITEK® MS Microbial Identification System (bioMérieux, France).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing and interpretation of the results for the tested *S. aureus* strains were performed using the disk diffusion method on Muller-Hinton agar (Bio-Rad, France) according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2022). The susceptibility of all *S. aureus* isolates to cefoxitin (30 μg), rifampin (5 μg), clindamycin (2 μg), erythromycin (15 μg), norfloxacin (10 μg), fusidic acid (10 μg), benzylpenicillin G (1 U), ciprofloxacin (5 μg), tetracycline (30 μg), gentamicin (10 μg) and trimethoprim-sulfamethoxazole (1.25–23.75 μg) was tested using commercial discs (Liofilchem®, Italy). Inducible or constitutive clindamycin resistance was examined using the double-disk method (D-test). Susceptibility to vancomycin was determined by using the gradient method (Liofilchem®, Italy). For quality control, *Staphylococcus aureus* ATCC® 29213™ was used (EUCAST 2022).

Molecular typing. All *S. aureus* strains were analysed for detection of the *mecA*, *mecC* (methicillin resistance), *ermA*, *ermC* (erythromycin resistance), *tetK*, *tetM* (tetracycline resistance) and *lukF-PV* (virulence) genes by our designed multiplex real-time PCR protocols. The 16S rRNA coding sequence was applied as an internal PCR control. The primer and probe sequences were designed using the Vector NTI Advance™ program (Thermo Fisher Scientific, Inc., USA) for sequence alignment and FastPCR online (http://primerdigital.com/tools/pcr.html) Java applet for primer tests (Table I). The multiplex reactions were divided into the mixture I (targeting *ermA*, *lukF-PV*, *ermC*, and 16S rRNA), mixture II (targeting *tetK*, *tetM*,

mecA, and 16S rRNA), and mixture III (targeting *mecC* and 16S rRNA). The reactions were carried out in a total volume of 15 µl with 1 µl of *S. aureus* lysate DNA. The multiplex real-time PCR mixture composition was 7.5 µl of 2 × SensiMix™ II Probe (Bioline Reagents Ltd., UK), 200 nM of each primer (Biolegio, The Netherlands), and 100 nM of each hydrolysis probe (Biolegio, The Netherlands). The reactions were performed using a Rotor-Gene Q 5plex HRM thermal cycler (QIAGEN, Germany) under the following conditions: initial denaturation at 95°C for 10 min (1 cycle) followed by 40 cycles of denaturation at 95°C for 20 s and primer annealing and extension at 55°C for 1 min.

Statistical analysis. Statistical analyses were performed using SPSS® 24.0 software (IBM® SPSS® Statistics for Windows, Version 24.0, IBM Corp., USA). Standard descriptive analysis was applied for the continuous variables using the mean, standard deviation, and minimal and maximal values. For the categorical data, the p-value was calculated using χ^2 statistics. Fisher's exact iterative test was applied if the expected values were less than 5. Differences were considered statistically significant at $p \leq 0.05$.

Results

The study included 745 patients from whose specimens *S. aureus* was isolated. The mean age of the participants was 57.4 years (median – 60, SD – 20.7). The youngest patient and study participant was 18 years old, and the oldest was 98 years old. Fifty-one percent (n = 380) of patients were female, and 49% (n = 365) were male.

The most significant number of strains was isolated from patients' skin and soft tissues (69.0%, n = 514), with additional strains from blood (14.5%, n = 108), respiratory tract (11.7%, n = 87), urine (2.7%, n = 20), and 2.1% (n = 16) from other specimens (reproductive system, peritoneal cavity, breast milk, joint cavity, prostheses, feces, and cerebrospinal fluid) (Table II).

Susceptibility to antimicrobials. In total, 21.1% (157/745) of *S. aureus* strains were susceptible to all tested antimicrobials. All 745 isolates were susceptible to rifampin and vancomycin. More than 95% of the *S. aureus* strains were susceptible to trimethoprim-sulfamethoxazole, fusidic acid, and gentamicin (99.7%, 96.4%, and 95.3%, respectively). Susceptibility to tetracycline was found in 94.8% (706/745) of isolates, to

Table I
Sequences of primers and detection hydrolysis probes.

Target gene	Detection primers and hydrolysis probes	
ermA	ermA_F	CAATGGTTGATGTCGYTCAAGAAC
	ermA_R	ATCTGCAACGAGCTTTGGG
	ermA_P	FAM/TCAATACAGAGTCTACACTTGGCTTAGGATG/BHQ1
ermC	ermC_F	ATCTTTGAAATYGGCTCAGGAA
	ermC_R	AACAAGTTTATKTTCTGTARTYTTGCA
	ermC_P	ROX/AGTACAG+AGGTGTAATTTCGTAACTGCYA/BHQ2
mecA	mecA_F	CTTTACGATAAAAAGCTCCAACATGA
	mecA_R	CTATTAATGTATGTGCGATTGTATTGC
	mecA_P	ROX/TGGCTATCGTGTCACAATCGTTGACGA/BHQ2
mecC	mecC_F	ACTAATGGTATGGAACGTGTAGT
	mecC_R	CCAACCTATTTGTCTTCCRGTTTC
	mecC_P	FAM/TTTT+TAATTCTGCTGTKCCAGATTTACC/BHQ1
tetK	tetK_F	GATTGCTTTTATTGGTCACAATCAC
	tetK_R	CTTGTAATATTTCTAGCTACAACCACC
	tetK_Pr	FAM/TGAAGGGAATGCAGCAGATCCTACTCCT/BHQ1
tetM	tetM_F	AGAACTAAAAGAGCCTACAGTCA
	tetM_R	TACAGATAAACCAATGGAAGCCC
	tetM_Pr	HEX/TGGCGGCACTTCGATGTGAATGGTA/BHQ1
lukF-PV	Pvl_F	TGGTTGGGATGTTGAAGCACA
	Pvl_R	TTGCAGCGTTTTGTTTTCGAG
	Pvl_P	HEX/TGCCAGTGTTATCCAGAGGTAACT/BHQ1
16S rRNA	Frrs_m	ACAGGATTAGATACCCTGGTAGTCC
	Rrrs_m	CGTTGCGGGACTTAACCCAAC
	Rrrs_Pr	Cy5/TCACRACACGAGCTGACGACAGCCA/BHQ2

Table II
Antimicrobial resistance profile of *Staphylococcus aureus* strains isolated from different clinical specimens.

		Skin and soft tissues (n = 514, 69.0%)	Blood (n = 108, 14.5%)	Respiratory tract (n = 87, 11.7%)	Urine (n = 20, 2.7%)	Other specimens (n = 16, 2.1%)	Total (n = 745)
Susceptible to all antimicrobials		114 (22.2%)	19 (17.6%)	16 (18.4%)	6 (30.0%)	2 (12.5%)	157 (21.1%)
FOX	R	33 (6.4%)	8 (7.4%)	4 (4.6%)	0 (0.0%)	0 (0.0%)	45 (6.0%)
	S	481 (93.6%)	100 (92.6%)	83 (95.4%)	20 (100%)	16 (100%)	700 (94.0%)
CD	R	25 (4.9%)	5 (4.6%)	11 (12.6%)	0 (0.0%)	0 (0.0%)	41 (5.5%)
	S	489 (95.1%)	103 (95.4%)	76 (87.4%)	20 (100%)	16 (100%)	704 (94.5%)
E	R	53 (10.3%)	8 (7.4%)	24 (27.6%)	1 (5.0%)	1 (6.3%)	87 (11.7%)
	S	461 (89.7%)	100 (92.6%)	63 (72.4%)	19 (95.0%)	15 (93.8%)	658 (88.3%)
NOR	R	40 (7.8%)	8 (7.4%)	4 (4.6%)	2 (10.0%)	0 (0.0%)	54 (7.2%)
	S	474 (92.2%)	100 (92.6%)	83 (95.4%)	18 (90.0%)	16 (100%)	691 (92.8%)
FA	R	22 (4.3%)	2 (1.9%)	2 (2.3%)	0 (0.0%)	1 (6.3%)	27 (3.6%)
	S	492 (95.7%)	106 (98.1%)	85 (97.7%)	20 (100%)	15 (93.8%)	718 (96.4%)
P	R	378 (73.5%)	84 (77.8%)	65 (74.7%)	13 (65.0%)	13 (81.3%)	553 (74.2%)
	S	136 (26.5%)	24 (22.2%)	22 (25.3%)	7 (35.0%)	3 (18.8%)	192 (25.8%)
CIP	R	38 (7.4%)	8 (7.4%)	2 (2.3%)	1 (5.0%)	0 (0.0%)	49 (6.6%)
	S	476 (92.6%)	100 (92.6%)	85 (97.7%)	19 (95.0%)	16 (100%)	696 (93.4%)
TE	R	29 (5.6%)	4 (3.7%)	5 (5.7%)	1 (5.0%)	0 (0.0%)	39 (5.2%)
	S	485 (94.4%)	104 (96.3%)	82 (94.3%)	19 (95.0%)	16 (100%)	706 (94.8%)
GN	R	23 (4.5%)	1 (0.9%)	11 (12.6%)	0 (0.0%)	0 (0.0%)	35 (4.7%)
	S	491 (95.5%)	107 (99.1%)	76 (87.4%)	20 (100%)	16 (100%)	710 (95.3%)
SXT	R	1 (0.2%)	1 (0.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.3%)
	S	513 (99.8%)	107 (99.1%)	87 (100%)	20 (100%)	16 (100%)	743 (99.7%)
RD	R	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	S	514 (100%)	108 (100%)	87 (100%)	20 (100%)	16 (100%)	745 (100%)
VA	R	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	S	514 (100%)	108 (100%)	87 (100%)	20 (100%)	16 (100%)	745 (100%)

R – resistant, S – susceptible, FOX – cefoxitin, CD – clindamycin, E – erythromycin, NOR – norfloxacin, FA – fusidic acid, P – penicillin, CIP – ciprofloxacin, TE – tetracycline, GN – gentamicin, SXT – trimethoprim-sulfamethoxazole, RD – rifampin, VA – vancomycin

clindamycin in 94.5% (704/745), and to erythromycin in 88.3% (658/745). The lowest susceptibility rate was to penicillin (25.8%, 192/745). Most *S. aureus* strains resistant to clindamycin showed inducible resistance (65.9%, 27/41) to this lincosamide. MRSA comprised 6% (45/745) of the tested strains. Methicillin-resistant strains were isolated from skin and soft tissues (73.3%, 33/45), blood (17.8%, 8/45), and the respiratory tract (8.9%, 4/45). The antimicrobial resistance profiles of the clinical *S. aureus* isolates are shown in Table II.

All isolated MRSA strains were resistant to penicillin. MRSA strains showed significantly lower susceptibility to clindamycin, erythromycin, norfloxacin, ciprofloxacin, tetracycline, and gentamicin than MSSA isolates ($p < 0.05$). A comparison of MRSA and MSSA susceptibility patterns is shown in Table III.

Antimicrobial resistance and virulence genes. Eighty-seven erythromycin-resistant *S. aureus* isolates were found, and the presence of the *ermA* and *ermC* genes in these isolates was determined. The most prevalent *erm* gene was *ermC* (41.4%, 36/87), followed by *ermA* (32.2%, 28/87). Neither the *ermA* nor the *ermC* gene was detected in 26.4% (23/87) of the tested erythromycin-resistant *S. aureus* strains (Table IV). The frequency of the *ermC* gene was higher in MSSA strains than in MRSA strains (49.2%, 29/59 vs. 25.0%, 7/28) ($\chi^2 = 5.033$, $p = 0.037$). The erythromycin-resistant MRSA isolates exhibited higher frequencies of *ermA* than the erythromycin-resistant MSSA isolates (60.7%, 17/28 vs. 18.6%, 11/59) ($\chi^2 = 14.323$, $p < 0.0001$). A total of 0.5% (3/658) of *S. aureus* isolates susceptible to erythromycin had the *ermC* gene.

A total of 97.4% (38/39) of tetracycline-resistant isolates carried the tetracycline resistance gene *tet* (Table IV). The prevalence of *tetK* and *tetM* were 69.2% (27/39) and 28.2% (11/39), respectively. The most

Table III
Comparison of the antimicrobial susceptibility of MSSA and MRSA strains.

Antimicrobial	MSSA (n = 700)	MRSA (n = 45)	*p*-value	OR (95% CI)
Clindamycin	670 (95.7%)	34 (75.6%)	0.000*	7.23 (2.99–16.34)
Erythromycin	641 (91.6%)	17 (37.8%)	0.000*	17.89 (8.81–36.76)
Norfloxacin	683 (97.6%)	8 (17.8%)	0.000*	185.82 (70.11–518.18)
Fusidic acid	674 (96.3%)	44 (97.8%)	0.820	0.59 (0.01–3.77)
Penicillin	192 (27.4%)	0 (0.0%)	NA	NA
Ciprofloxacin	686 (98.0%)	10 (22.2%)	0.000*	171.50 (65.93–457.45)
Tetracycline	677 (95.3%)	39 (86.7%)	0.025	3.11 (1.00–8.12)
Gentamicin	673 (96.1%)	37 (82.2%)	0.001	5.39 (1.97–13.24)
Trimethoprim-sulfamethoxazole	699 (99.9%)	44 (97.8%)	NA	NA

*$p < 0.0001$, NA – not applicable, OR – odds ratio, CI – confidence interval

prevalent gene in the tetracycline-resistant MSSA isolates was *tetK* (72.7%, 24/33), followed by *tetM* (24.2%, 8/33). One MSSA strain had inducible lincosamide resistance and the *ermA* gene, and it was sensitive to tetracycline but positive for *tetM*. The *tetM* and *tetK* genes were equally found in tetracycline-resistant MRSA strains (3/6 and 3/6, respectively).

All 745 *S. aureus* strains were tested for the *mecA* and *mecC* genes. The *mec* genes were found in 6.4% (48/745) of the tested isolates. Forty-five *S. aureus* strains were cefoxitin resistant, and the analysis showed that 95.6% (43/45) of them harboured the *mecA* gene, whereas 4.4% (2/45) harboured *mecC*.

An analysis of the *S. aureus* virulence gene *lukF-PV*, which encodes the PVL protein, was performed in all 745 strains. A total of 7.3% (54/745) of the isolates had the *pvl* gene. The frequency of *pvl* was significantly higher in the MRSA isolates than in the MSSA isolates (42.2%, 19/45 vs. 5.0%, 37/700; $p < 0.0001$). The highest proportion of *pvl*-positive strains was among those isolated from skin and soft tissue specimens – 72.2% (39/54).

Table IV
The prevalence of erythromycin and tetracycline resistance and virulence genes in MSSA and MRSA strains.

Gene	MSSA	MRSA	In total
ermA	11 (18.6%)	17 (60.7%)	28 (32.2%)
ermC	29 (49.2%)	7 (25.0%)	36 (41.4%)
ND *erm* genes	19 (32.2%)	4 (14.3%)	23 (26.4%)
tetM	8 (24.2%)	3 (50.0%)	11 (28.2%)
tetK	24 (72.7%)	3 (50.0%)	27 (69.2%)
ND *tet* genes	1 (3.0%)	0 (0.0%)	1 (2.6%)
pvl	35/700 (5.0%)	19/45 (42.2%)	54/745 (7.3%)

ND – not detected

Discussion

This study shows that the skin and soft tissues were the most common sites for infection, probably because *S. aureus* colonizes typically the skin. Antimicrobial resistance testing revealed that the highest resistance rate was to penicillin, as has been previously demonstrated by others (from 69 to 78.9%) (Resman et al. 2016; Davido et al. 2018). On the other hand, the rate of *S. aureus* resistance to penicillin is decreasing, and there are suggestions to use penicillin to treat penicillin-susceptible *S. aureus* infections (Cheng et al. 2016; Davido et al. 2018). Differences in tetracycline and erythromycin resistance vary between various countries. In our study, tetracycline resistance differed from that reported in Poland (5.2% and 10.7%, respectively) (Kot et al. 2020). The rate of resistance to erythromycin in this study was lower than that reported by Romaniszyn et al. (2015) in Poland (32.6%) and Karki et al. (2001) in Estonia (25.3%). According to our study data, the prevalence of MRSA is comparable with that reported by other countries that have sampled patients in hospitals (ECDC 2020). Similar MRSA prevalence rates were reported in Latvia, Germany, Austria, Slovenia, Belgium, and Luxemburg for invasive strains, where 5–10% of the isolated *S. aureus* strains were reportedly MRSA (ECDC 2020). All *S. aureus* isolates were found to be susceptible to rifampin and vancomycin. Based on data from a recent systematic review and meta-analysis, the prevalence of vancomycin-resistance *S. aureus* (VRSA) was 1% in Europe, but a higher prevalence rate of vancomycin resistance was reported in developing countries. In Egypt, 5.5–8.8% of *S. aureus* isolates were VRSA (Amr and Gammal 2017; ElSayed et al. 2018). The determination of *S. aureus* resistance is crucial for

optimal antimicrobial therapy, as well as for epidemiological knowledge and effective infection control strategies (Prestinaci et al. 2015; Coia et al. 2021). Since 2008, Lithuania has applied a national prevention program to control the spread of antimicrobial resistance (Ministry of Health of The Republic of Lithuania 2007). The differences in the resistance of isolated *S. aureus* strains to antimicrobials in our study as compared to the data presented by other scientists (Gianino et al. 2018) may be linked not only to the consumption of antibiotics in the country but also to the internal policy of antibiotics consumption in the hospitals under study. In Lithuania, first- and second-generation cephalosporins and broad-spectrum penicillins are the most common antimicrobials in the treatment of nosocomial infections (Ašembergienė et al. 2009). Also, according to the data of the Institute of Hygiene, penicillins with inhibitors are the most often prescribed group of antimicrobials (39.2% of all bacterial diseases in Intensive care units). Other often-prescribed groups of antibiotics are first and second-generation cephalosporins (16.1% of all bacterial diseases in Intensive care units) (Institute of Hygiene 2020). In our study, we observe *S. aureus* resistance to the most used group of antibiotics. Only two groups of antibiotics are prescribed for treating MRSA: glycopeptide (vancomycin) and oxazolidinone (linezolid). Antimicrobials of other groups (cyclic lipopeptides, streptogramins, glycyclins) are not registered in our country (Institute of Hygiene 2020). Considering the existing antibiotic prescribing practice in primary healthcare in Lithuania, we assume that it may have influenced our findings that the susceptibility to some antimicrobials is the same or higher as in other European countries. Further research is needed to confirm the findings.

Our results indicated that erythromycin and tetracycline resistance in *S. aureus* strains from Vilnius hospitals was dependent on the presence of corresponding resistance genes. This study's predominant erythromycin resistance gene was *ermC* (41.4%), while the less predominant gene was *ermA* (32.2%).

These findings differed from those of earlier research, which indicated that most erythromycin-resistant bacteria possessed *ermA* (Lim et al. 2012). In France, the prevalence of *ermA* and *ermC* in erythromycin-resistant strains was 63.2% and 25.0%, respectively. Among MRSA strains, the prevalence of *ermA* was higher than that of the *ermC* gene (60.7% and 25.0%, respectively), consistent with previous studies (Lina et al. 1999). Interestingly, a significant proportion (26.4%) of *S. aureus* bacteria resistant to erythromycin tested negative for both the *ermC* and *ermA* genes. This result can occur because other genes, such as *msr*, *lun*, and others in the *erm* gene cluster, are responsible for bacterial resistance to erythromycin (Khodabandeh et al. 2019). Regarding tetracycline resistance, our study showed that the predominant gene among tetracycline-resistant *S. aureus* from Vilnius hospitals was *tetK*, with a prevalence of 69.2%. This differed from the results of other studies, which showed that the tetracycline gene *tetM* was more prevalent (Trzcinski et al. 2000; Schmitz et al. 2001). These results suggest that *S. aureus* strains from Vilnius contain characteristic resistance genes for tetracycline resistance. The *mecA* gene was found in 6.4% of all 745 tested *S. aureus* strains. In 95.6% of MRSA strains, the presence of *mecA* was detected. The prevalence of *mecA* was consistent with that reported in North American and European hospitals, where *mecA* prevalence was also found to be 95% (Wielders et al. 2002). Even though MRSA bacteria are resistant to β-lactam antibiotics due to the presence of the *mecA* gene, the level of resistance can vary significantly among MRSA strains. Some MRSA strains exhibit resistance that is barely above that of susceptible isolates (methicillin minimum inhibitory concentrations (MICs) < 3 μg/ml). In contrast, other strains show high resistance (methicillin MICs up to 1,600 μg/ml) (Parvez et al. 2008). The reasons for these differences in resistance are not fully understood. However, some research suggests that increased expression or duplication of the *mecA* gene or enhanced transcription of this gene may contribute to higher resistance levels (Gallagher et al. 2017). Finally, the virulence factor PVL plays a vital role during infections caused by *S. aureus* (Shallcross et al. 2013; Sakr et al. 2018). All *S. aureus* strains were genotypically tested for the prevalence of the *lukF-PV* gene. A total of 7.3% of strains were found to carry the *pvl* gene. The gene was found in both MRSA and MSSA strains. However, out of all strains carrying the *pvl* gene, there were more bacteria resistant to methicillin (42.2%) than strains susceptible to methicillin (5.0%). These results are consistent with data from other studies (Dufour et al. 2002; Wannet et al. 2005; Diederen and Kluytmans 2006).

Conclusions

All tested strains were susceptible to rifampicin and vancomycin. MRSA consisted of 6% of the strains. The lowest susceptibility rate was found to penicillin. The *tetK* gene was predominant among *S. aureus* strains resistant to tetracycline, and the *ermC* gene was predominant among erythromycin-resistant strains. Seven percent of *S. aureus* isolates harbored the *pvl* gene, which was more prevalent in methicillin-resistant strains. *S. aureus* resistance to tested antimicrobials is lower than in other European countries due to the low antibiotic consumption in primary health care and rational antibiotic use strategy in Lithuania.

ORCID
Agnė Kirkliauskienė https://orcid.org/0000-0001-5701-5594

Availability of data and material

All data used to support the findings of this study are available from the corresponding author upon request.

Ethical statement

Ethical approval for the study was obtained from the Vilnius regional biomedical ethics review board (approval No. 158200-17-969-481).

Acknowledgments

The authors would like to thank Vilnius University, Faculty of Medicine Microbiology, laboratory technicians Olga Purakevic and Halina Gluchoviene for technical help with microbiology work.

Author contributions

AK, LK, JM, DR, JK, TK, and MB contributed to the conception and design of the study. LK and JK wrote the first draft of the manuscript. AK, LK, JK, TK, and MB were responsible for data management, analyses and interpretation. JM, DR, and LK participated in the collection and processing of the samples and identification of microorganisms. AK and LK performed the antimicrobial susceptibility testing. AK, MB, and JK carried out the molecular genetics studies. AK, TK, DR, JM, and MB participated in the revision and provided significant assistance towards the drafting of the manuscript. All authors read and approved the final manuscript.

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.

Literature

Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell. 2007 Mar;128(6):1037–1050. https://doi.org/10.1016/j.cell.2007.03.004

Amr GE, Gammal SA. Emergence of vancomycin resistant *Staphylococcus aureus* isolated from patients in ICUs of Zagazig University Hospitals. Egypt J Med Microbiol. 2017 Apr;26(2):53–59.

Ašembergienė J, Gurskis V, Kėvalas R, Valintėlienė R. Nosocomial infections in the pediatric intensive care units in Lithuania. Medicina. 2009;45(1):29. https://doi.org/10.3390/medicina45010005

Boyle-Vavra S, Daum RS. Molecular strategies of *Staphylococcus aureus* for resisting antibiotics, In: Somerville GA, editor. *Staphylococcus*: Genetics and physiology. Norfolk (UK): Caister Academic Press; 2016. p 249–300. https://doi.org/10.21775/9781910190494.11

Burdett V. Purification and characterization of Tet(M), a protein that renders ribosomes resistant to tetracycline. J Biol Chem. 1991 Feb;266(5):2872–2877. https://doi.org/10.1016/S0021-9258(18)49928-0

Cetin ES, Gunes H, Kaya S, Aridogan BC, Demirci M. Macrolide-lincosamide-streptogramin B resistance phenotypes in clinical staphylococcal isolates. Int J Antimicrob Agents. 2008 Apr;31(4):364–368. https://doi.org/10.1016/j.ijantimicag.2007.11.014

Cheng MP, René P, Cheng AP, Lee TC. Back to the future: Penicillin-susceptible *Staphylococcus aureus*. Am J Med. 2016 Dec;129(12):1331–1333. https://doi.org/10.1016/j.amjmed.2016.01.048

Chi CY, Lin CC, Liao IC, Yao YC, Shen FC, Liu CC, Lin CF. Panton-Valentine leukocidin facilitates the escape of *Staphylococcus aureus* from human keratinocyte endosomes and induces apoptosis. J Infect Dis. 2014 Jan;209(2):224–235. https://doi.org/10.1093/infdis/jit445

Coia JE, Wilson JA, Bak A, Marsden GL, Shimonovich M, Loveday HP, Humphreys H, Wigglesworth N, Demirjian A, Brooks J, et al. Joint Healthcare Infection Society (HIS) and Infection Prevention Society (IPS) guidelines for the prevention and control of meticillin-resistant *Staphylococcus aureus* (MRSA) in healthcare facilities. J Hosp Infect. 2021 Dec;118S:S1–S39. https://doi.org/10.1016/j.jhin.2021.09.022

Davido B, Lawrence C, Dinh A, Bouchand F. Back to the future with the use of penicillin in penicillin-susceptible *Staphylococcus aureus* (PSSA) bacteremia. Am J Med. 2018 Apr;131(4):e155. https://doi.org/10.1016/j.amjmed.2017.10.032

Diederen BM, Kluytmans JA. The emergence of infections with community-associated methicillin resistant *Staphylococcus aureus*. J Infect. 2006 Mar;52(3):157–168. https://doi.org/10.1016/j.jinf.2005.09.001

Dufour P, Gillet Y, Bes M, Lina G, Vandenesch F, Floret D, Etienne J, Richet H. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: Emergence of a single clone that produces Panton-Valentine leukocidin. Clin Infect Dis. 2002 Oct;35(7):819–824. https://doi.org/10.1086/342576

ElSayed N, Ashour M, Amine AEK. Vancomycin resistance among *Staphylococcus aureus* isolates in a rural setting, Egypt. Germs. 2018 Sep;8(3):134–139. https://doi.org/10.18683/germs.2018.1140

ECDC. Antimicrobial resistance in the EU/EEA (EARS-Net) – Annual Epidemiological Report 2019. Stockholm (Sweden): European Centre for Disease Prevention and Control; 2020 [cited 2022 Apr 26]. Available from https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2019

Gallagher LA, Coughlan S, Black NS, Lalor P, Waters EM, Wee B, Watson M, Downing T, Fitzgerald JR, Fleming GTA, et al. Tandem amplification of the staphylococcal cassette chromosome *mec* element can drive high-level methicillin resistance in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2017 Aug;61(9):e00869-17. https://doi.org/10.1128/AAC.00869-17

Gianino MM, Lenzi J, Bonaudo M, Fantini MP, Ricciardi W, Damiani G. Predictors and trajectories of antibiotic consumption in 22 EU countries: Findings from a time series analysis (2000–2014). PLoS One. 2018 Jun;13(6):e0199436. https://doi.org/10.1371/journal.pone.0199436

Institute of Hygiene. [Epidemiological monitoring of nosocomial infections in resuscitation and intensive care units in 2020 reports] (in Lithuanian). Vilnius (Lithuania): Health Information Center of the Institute of Hygiene. Institute of Hygiene; 2020 [cited 2022 Apr 26]. Available from https://sena.hi.lt/lt/hospitaliniu-infekciju-epidemiologines-priezi-uros-reanimacijos-ir-intensyvios-terapijos-skyriuose-ataskaitos.html

Jensen SO, Lyon BR. Genetics of antimicrobial resistance in *Staphylococcus aureus*. Future Microbiol. 2009 Jun;4(5):565–582. https://doi.org/10.2217/fmb.09.30

Karki T, Truusalu K, Vainumäe I, Mikelsaar M. Antibiotic susceptibility patterns of community- and hospital-acquired *Staphylococcus aureus* and *Escherichia coli* in Estonia. Scand J Infect Dis. 2001;33(5):333–338. https://doi.org/10.1080/003655401750173904

Khodabandeh M, Mohammadi M, Abdolsalehi MR, Alvandimanesh A, Gholami M, Bibalan MH, Pournajaf A, Kafshgari R, Rajabnia R. Analysis of resistance to macrolide-lincosamide-streptogramin b among *mecA*-positive *Staphylococcus Aureus* isolates. Osong Public Health Res Perspect. 2019 Feb;10(1):25–31. https://doi.org/10.24171/j.phrp.2019.10.1.06

Kot B, Wierzchowska K, Piechota M, Grużewska A. Antimicrobial resistance patterns in methicillin-resistant *Staphylococcus aureus* from patients hospitalized during 2015–2017 in hospitals in Poland. Med Princ Pract. 2020;29(1):61–68. https://doi.org/10.1159/000501788

Lim KT, Hanifah YA, Yusof M, Thong KL. *ermA*, *ermC*, *tetM* and *tetK* are essential for erythromycin and tetracycline resistance among methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary hospital in Malaysia. Indian J Med Microbiol. 2012 Apr–Jun;30(2):203–207. https://doi.org/10.4103/0255-0857.96693

Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother. 1999 May;43(5):1062–1066. https://doi.org/10.1128/aac.43.5.1062

Löffler B, Hussain M, Grundmeier M, Brück M, Holzinger D, Varga G, Roth J, Kahl BC, Proctor RA, Peters G. *Staphylococcus aureus* Panton-Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog. 2010 Jan;6(1):e1000715. https://doi.org/10.1371/journal.ppat.1000715

Ministry of Health of The Republic of Lithuania. [Order of the minister of health protection of the republic of Lithuania on the approval of the program for the prevention of the spread of antimicrobial drug resistant microorganisms for 2008–2014] (in Lithuanian). Vilnius (Lithuania): Ministry of Health of The Republic of Lithuania; 2007 [cited 2022 Apr 26]. Available from https://e-seimas.lrs.lt/rs/legalact/TAD/TAIS.309573

Parvez MA, Shibata H, Nakano T, Niimi S, Fujii N, Arakaki N, Higuti T. No relationship exists between PBP 2a amounts expressed in different MRSA strains obtained clinically and their β-lactam MIC values. J Med Invest. 2008 Aug;55(3–4):246–253. https://doi.org/10.2152/jmi.55.246

Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. Pathog Glob Health. 2015; 109(7): 309–318. https://doi.org/10.1179/2047773215Y.0000000030

Prévost G, Cribier B, Couppié P, Petiau P, Supersac G, Finck-Barbançon V, Monteil H, Piemont Y. Panton-Valentine leucocidin and gamma-hemolysin from *Staphylococcus aureus* ATCC 49775 are encoded by distinct genetic loci and have different biological activities. Infect Immun. 1995 Oct;63(10):4121–4129. https://doi.org/10.1128/iai.63.10.4121-4129.1995

Resman F, Thegerström J, Månsson F, Ahl J, Tham J, Riesbeck K. The prevalence, population structure and screening test specificity of penicillin-susceptible *Staphylococcus aureus* bacteremia isolates in Malmö, Sweden. J Infect. 2016 Aug;73(2):129–135. https://doi.org/10.1016/j.jinf.2016.05.011

Romaniszyn D, Różańska A, Wójkowska-Mach J, Chmielarczyk A, Pobiega M, Adamski P, Helwich E, Lauterbach R, Borszewska-Kornacka M, Gulczyńska E, et al. Epidemiology, antibiotic consumption and molecular characterisation of *Staphylococcus aureus* infections – data from the Polish Neonatology Surveillance Network, 2009–2012. BMC Infect Dis. 2015 Apr;15:169. https://doi.org/10.1186/s12879-015-0890-3

Rungelrath V, DeLeo FR. *Staphylococcus aureus*, antibiotic resistance, and the interaction with human neutrophils. Antioxid Redox Signal. 2021 Feb;34(6):452–470. https://doi.org/10.1089/ars.2020.8127

Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O. *Staphylococcus aureus* nasal colonization: An update on mechanisms, epidemiology, risk factors, and subsequent infections. Front Microbiol. 2018 Oct; 9:2419. https://doi.org/10.3389/fmicb.2018.02419

Schmitz FJ, Krey A, Sadurski R, Verhoef J, Milatovic D, Fluit AC; European SENTRY Participants. Resistance to tetracycline and distribution of tetracycline resistance genes in European *Staphylococcus aureus* isolates. J Antimicrob Chemother. 2001 Feb;47(2):239–240. https://doi.org/10.1093/jac/47.2.239

Shallcross LJ, Fragaszy E, Johnson AM, Hayward AC. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: A systematic review and meta-analysis. Lancet Infect Dis. 2013 Jan; 13(1):43–54. https://doi.org/10.1016/S1473-3099(12)70238-4

EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0. Basel (Switzerland): The European Committee on Antimicrobial Susceptibility Testing; 2022.

Toh SM, Xiong L, Arias CA, Villegas MV, Lolans K, Quinn J, Mankin AS. Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant *Staphylococcus aureus* resistant to the synthetic antibiotic linezolid. Mol Microbiol. 2007 Jun;64(6): 1506–1514. https://doi.org/10.1111/j.1365-2958.2007.05744.x

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015 Jul;28(3):603–661. https://doi.org/10.1128/CMR.00134-14

Trzcinski K, Cooper BS, Hryniewicz W, Dowson CG. Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother. 2000 Jun;45(6):763–770. https://doi.org/10.1093/jac/45.6.763

Vestergaard M, Frees D, Ingmer H. Antibiotic resistance and the MRSA problem. Microbiol Spectr. 2019 Mar;7(2). https://doi.org/10.1128/microbiolspec.GPP3-0057-2018

Wannet WJ, Spalburg E, Heck ME, Pluister GN, Tiemersma E, Willems RJ, Huijsdens XW, de Neeling AJ, Etienne J. Emergence of virulent methicillin-resistant *Staphylococcus aureus* strains carrying Panton-Valentine leucocidin genes in The Netherlands. J Clin Microbiol. 2005 Jul;43(7):3341–3345. https://doi.org/10.1128/JCM.43.7.3341-3345.2005

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005 Dec;5(12):751–762. https://doi.org/10.1016/S1473-3099(05)70295-4

Wielders CL, Fluit AC, Brisse S, Verhoef J, Schmitz FJ. *mecA* gene is widely disseminated in *Staphylococcus aureus* population. J Clin Microbiol. 2002 Nov;40(11):3970–3975. https://doi.org/10.1128/JCM.40.11.3970-3975.2002

Yahav D, Yassin S, Shaked H, Goldberg E, Bishara J, Paul M, Leibovici L. Risk factors for long-term mortality of *Staphylococcus aureus* bacteremia. Eur J Clin Microbiol Infect Dis. 2016 May; 35(5):785–790. https://doi.org/10.1007/s10096-016-2598-8