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ANALYSIS OF PHENOTYPIC AND GENOTYPIC ANTIBIOTIC RESISTANCE OF FOOD ISOLATES OF *STAPHYLOCOCCUS* SPP.

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ABSTRACT

Background. *Staphylococcus* bacteria are potentially pathogenic species that are more and more often isolated from the natural environment. They are often responsible for the spoilage of food products and for food poisoning in consumers. The aim of this study was to analyse the phenotypic and genotypic antibiotic resistance of food isolates of the genus *Staphylococcus*.
Materials and methods. The biological material consisted of 5 coagulase-negative strains of the genus *Staphylococcus* isolated from food (radish sprouts, fresh milk and a dietary supplement) and a reference strain, *Staphylococcus epidermidis* DSMZ 3270. Phenotypic antibiotic resistance was examined using the disc method, while genotypic antibiotic resistance assessment was examined using Real-Time PCR.
Results. All of the tested isolates turned out to be resistant to 6 antibiotics: erythromycin, gentamycin, rifampicin, linezolid, tigecycline and methicillin. On this basis, the isolates were classified as multi-drug-resistant strains. For all 5 isolates and *Staphylococcus epidermidis* DSMZ 3270, only 2 common genes present in the DNA (plasmid and/or genomic) were identified: *tet*(L) and *int*(Tn916/Tn1545). However, none of them had the *mec*(A) gene.
Conclusion. On this basis, the isolates were classified as multi-drug-resistant strains. The study confirms the increasing prevalence of multi-drug-resistant bacteria of the genus *Staphylococcus* in the environment.

Keywords: *Staphylococcus* spp., coagulase-negative, antibiotic resistance, genotyping, phenotyping, food

INTRODUCTION

Staphylococcus bacteria are gram-positive cocci with a diameter of up to 1.5 µm. To date, over 40 species of staphylococci have been identified and described (Canovas et al., 2016). As of 2014, 47 species (with 23 subspecies) belong to the genus *Staphylococcus*, 38 of which are coagulase-negative staphylococci (Becker et al., 2014).

Due to the ability of some *Staphylococcus* spp. to synthesise coagulase, since 1903, this genus has been divided into coagulase-positive species (CoPS) and coagulase-negative species (CoNS) (Wolny-Kołladka, 2018). Until recently, the former were considered more virulent (with the best-known member of this group being *Staphylococcus aureus*). Recently, it has been observed that newly isolated strains of the genus *Staphylococcus* are not able to synthesise coagulase (coagulase-negative strains prevail among environmental isolates). These isolates are often identified as representatives of pathogenic species. The best-known coagulase-negative species are *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. Traditionally, coagulase-negative staphylococci

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include *Staphylococcus capitis*, *Staphylococcus hominis*, *Staphylococcus simulans* and *Staphylococcus warneri* (Becker et al., 2014). Coagulase-negative staphylococci are a group of potentially pathogenic bacteria that are common food contaminants (Fowoyo and Ogunbanwo, 2017). The main challenge in combating coagulase-negative staphylococci isolated from food is the difficulty in limiting their growth. This is because isolates from food show resistance to many antimicrobial agents. Chemotherapeutic agents used to treat animals and feed additives (to improve the growth of food animals) are a primary factor in the rising drug resistance of *Staphylococcus*. Staphylococci often contaminate milk, meat and fermented foods made from contaminated raw material (Olsen et al., 2006). Among products of animal origin, staphylococci are most often isolated from raw meat and meat products, poultry and egg products or milk and dairy products. Salads and bread (especially cakes with custard and whipped cream) can also be a source of staphylococci infection (Cho et al., 2019; Mekonnen et al., 2018; Pondit et al., 2018; Saifullah et al., 2018; Wu et al., 2018). *Staphylococcus* bacteria have an ability to grow in environments with high salt concentration (even up to 20% [w/w]) and low water activity (0.86). Therefore, they may be present in salty products such as ham, canned food and − less frequently − smoked fish (Argudin et al., 2010).

For strains that are difficult to control, those antibiotics which are most commonly used have proven unable to fight them (Aarestrup et al., 2008). The coagulase-negative staphylococci which have been described in the literature to date are known to have acquired resistance to a variety of chemotherapeutic agents used in human, veterinary and agricultural applications. This is a serious problem in the modern world (Simeoni et al., 2008). The ease with which antibiotic resistance is transmitted between coagulase-negative *Staphylococcus* strains is mainly due to the presence of mobile genetic elements in cells (e.g., plasmids). In this way, genes are easily and frequently transferred by horizontal transfer between bacteria in the same environment (Hanssen et al., 2004). An unfortunate consequence of this phenomenon is the increasingly frequent isolation of methicillin-resistant coagulase-negative staphylococci (MRCoNS) from food samples (in comparison with the previously isolated *Staphylococcus aureus*; Bania et al., 2014; Resch et al., 2008; Urdez-Hernandez et al., 1999).

It is believed that coagulase-negative staphylococci can acquire antibiotic resistance genes from strains with high pathogenic potential, such as *Staphylococcus aureus*. This is because they often inhabit the same ecological niche (e.g., human nostrils). Unfortunately, among coagulase-negative staphylococci there are also strains resistant to the 'antibiotics of last resort,' i.e., linezolid and daptomycin. The antibiotic resistance of *Staphylococcus* strains depends primarily on the environment from which they were isolated and the latitude where the food product originated (Marincola et al., 2021). Although infections caused by coagulase-negative staphylococci are less serious than *Staphylococcus aureus* infections, it should be remembered that it is very often more complicated to treat them. The rising resistance to common antibiotics (penicillin, oxacillin, methicillin, gentamicin, clindamycin, ciprofloxacin and erythromycin) makes staph infections troublesome to control. In addition, coagulase-negative staphylococci are characterised by the rapid acquisition, possession and modification of the gene pool responsible for antibiotic resistance. It is worth remembering that this characteristic further promotes the transfer of these genes to other bacteria species as well (not only within the genus *Staphylococcus*) (Allen et al., 2010; Davies and Davies, 2010; O'Neill, 2016).

At least 40 different genes that determine tetracycline resistance have been characterized for tetracycline. Tetracycline resistance is regulated by *tet* genes. These take part in active removal of the drug, protection of ribosomes and enzymatic modification of the drug. The *tet*(A), *tet*(B), *tet*(D), *tet*(E) and *tet*(G) genes are most commonly observed in gram-negative bacteria. In contrast, for gram-positive bacteria, the *tet*(K), tet(L), *tet*(M), tet(O) and *tet*(S) genes are observed (Hedayatianfard et al., 2014). Methicillin resistance, on the other hand, depends on a penicillin-binding protein that is encoded by a mobile genetic element called the methicillin resistance gene, *mec*(A). In addition, up to three different mechanisms of resistance to macrolide, lincosamide and streptogramin B-type antibiotics have been found in bacteria of the genus *Staphylococcus*. One mechanism is based on methylation of the ribosomal site of antibiotics mediated

by the presence of erythromycin-resistant methylase genes – *erm*. The primary function of the methylase is to reduce the binding of antibiotics to the target site on the 50S subunit of the ribosome. The most common genes observed in *Staphylococcus* bacteria mediating the above-described mechanism are *erm*(A) and *erm*(C). In addition, *erm*(A) genes are often spread in methicillin-resistant isolates and are generated by transposons. In contrast, *erm*(C) genes are mainly responsible for erythromycin resistance among methicillin-susceptible isolates and are carried by plasmids (Hosseini et al., 2016). Ribosome site modification is also encoded by the *erm*(B), *erm*(Y) and *erm*(F) genes. Currently, there are as many as 21 different classes of modified *erm* genes. An active effect on inhibiting protein synthesis is also attributed to the presence of the *msr*(A/B) gene. The *msr*(A), *msr* (B) and the newly discovered *msr*(C) genes encode an ATP-dependent efflux pump that mediates resistance to macrolides and B-type streptogramin in *Staphylococcus* bacteria (Mišić et al., 2017).

The aim of this study was to characterise the phenotypic and genotypic antibiotic resistance of 5 coagulase-negative isolates from food.

MATERIALS AND METHODS

Staphyloccoci isolation from food

The biological material in this study consisted of 5 strains of bacteria from the genus *Staphylococcus*. One strain – *Staphylococcus epidermidis* DSMZ 3270 – was used as a reference strain. The other 5 strains were food-derived isolates.

In order to isolate bacteria of the genus *Staphylococcus*, a series of 10-fold dilutions of the tested samples was prepared (vegetable salad, cottage cheese with chives, powdered milk, fresh unpasteurised milk, radish sprouts, broccoli sprouts, a dietary supplement – powdered acai berry extract), and then the suspensions were surface plated onto Chapman agar (Merck, Germany). The plates were incubated for 24 hours at 37°C. After incubation, single white-cream colonies grown on Chapman's medium were collected using sterile loops (except from the samples of cottage cheese with chives, vegetable salad and powdered milk, where no presence of staphylococci was found) and streaked (to obtain single colonies) on Nutrient Agar (Merck, Germany). The samples were incubated for 24 hours at 37°C. From the colonies that were grown on Nutrient Agar, microscopic slides were made and stained using the Gram staining technique, according to the method described by Moneta and Piątkiewicz (2007). Of the obtained isolates (40), five were selected for further research (Table 1).

The species affiliation of these isolates was confirmed by API®Staph testing and molecular methods based on sequence analysis of the 16S ribosomal RNA gene. The sequences of the strains are stored in the GenBank biotechnology database (US National Center for Biotechnology Information, Bethesda, MD, USA). The species affiliation and the sequence access numbers of the tested strains are presented in Table 1.

During identification, all the food isolates were found to lack the ability to synthesise free coagulase according to the methodology described by the American Society of Microbiology and bound coagulase according to the methodology described by Berke and Tilton (1986). Thus, they were classified as coagulase-negative strains. All strains used in the study were stored in the MAST CRYOBANK (both isolates and reference strain). The culture medium used for growth and storage of the *Staphylococcus* bacteria contained: glucose 1 g/L, peptone 15 g/L, sodium chloride 6 g/L and yeast extract 3 g/L. The inoculated medium was incubated at 37°C for 24 h.

Isolation of genomic and plasmid DNA

Genomic DNA was extracted from colonies selected for the API®Staph test using a Genomic Mini Kit (A&A BIOTECHNOLOGY, Poland), while plasmid DNA was isolated using a Plasmid Miniprep DNA Purification Kit (EURx, Poland).

To isolate the genomic DNA, 100 µL of bacterial culture (24-hour culture, 37°C, Nutrient Broth medium, Merck, Germany) was transferred to an eppendorf-type tube. The bacterial cells were treated with lysozyme (10 mg/mL) for 20 minutes at 37°C. Then 200 µL of LT lysis solution and 20 µL of Proteinase K were added to the tubes. The whole mixture was mixed by inverting the tube and then it was incubated again for 20 minutes at 37°C. After incubation, the samples were kept for 5 minutes at 70°C (water bath) and then intensively vortexed for 20 seconds. After being vortexed, the samples were centrifuged 3 minutes

Table 1. Bacterial isolates selected for further phenotypic and genotypic characterisation

	Designation	Source	Accession number in GenBank, NCBI	API®Staph match result	16S rRNA match result
1	A5	food supplement	MW 040699	91.2% *Staphylococcus epidermidis* 5.9% *Staphylococcus capitis*	99.3% *Staphylococcus epidermidis*
2	M5	fresh cow milk	MW 776359	99.7% *Staphylococcus xylosus* 0.1% *Staphylococcus saprophyticus*	99.7% *Staphylococcus xylosus*
3	M6	fresh cow milk	MW 776358	53.2% *Staphylococcus saprophyticus* 31.4% *Staphylococcus haemolyticus*	99.4% *Staphylococcus haemolyticus*
4	KR6	radish sprouts	MW 776357	99.4% *Staphylococcus capitis* 0.4% *Staphylococcus caprae*	99.9% *Staphylococcus capitis*
5	KR2A	radish sprouts	MW 776360	96.5% *Staphylococcus warneri* 1.8% *Staphylococcus hominis*	98.5% *Staphylococcus warneri*

at 12,000 × g. The supernatant was applied to minicolumns and centrifuged for 1 minute at 10,000 × g. After centrifugation, 500 µL of A1 wash solution was added to the minicolumns and centrifuged again for 1 minute at 10,000 × g. The minicolumns were then transferred to new tubes and 400 µL of A1 wash solution was added. They were centrifuged for 2 minutes at 10,000 × g. The dried minicolumns were transferred to new tubes. 200 µL of Tris buffer (previously heated in a water bath to 70°C) was applied to the deposits at the bottom of the minicolumns. The samples were incubated for 2 minutes on the laboratory counter (at 25 ±2°C). After incubation, the samples were centrifuged for 1 minute at 10,000 × g. The minicolumns were removed, and the tubes with the purified DNA were stored in a freezer (−20°C) until subsequent analysis.

To isolate the plasmid DNA, 30 µL of Buffer PL activation buffer (containing RNAase – 100 µg/mL; complete membrane saturation promotes maximum DNA binding efficiency) was added to the minicolumns and not vortexed. The minicolumns were left at room temperature until the lysate was applied. To obtain the lysate, about 6ml of overnight culture (12 hours, 37°C, Nutrient Broth medium, Merck, Germany) was centrifuged (in 1 mL portions) at 12,000 × g for 5 minutes. The bacterial pellet was additionally resuspended in 50 µL of lysozyme (concentration 20 µg/mL) and incubated for 30 minutes at 37°C. Then 250 µL of Cell R buffer was added to the samples and mixed thoroughly. 250 µL of Lysis Blue lysis buffer was added to the suspension. The contents of the Eppendorf-type tube were slowly mixed by inverting several times until a uniform blue suspension was obtained. Once a uniform color was obtained, 350 µL of Neutral B buffer was added to the tube and the contents of the tube were mixed thoroughly and slowly (by inverting several times) until the color was neutralized. The minicolumn was centrifuged for 7 minutes at 12,000 × g. A clear supernatant was transferred to the binding column (in a portion of 700 µL maximum), and then it was centrifuged for 1 minute at 11,000 × g. The supernatant was poured off and the minicolumn was placed back in the collection tube. 500 µL of wash buffer (Wash PLX1) was added to the minicolumn and centrifuged for 1 minute at 11,000 × g. The minicolumn was removed, the filtrate was discarded and then the minicolumn was placed back in the tube 650 µL of a second wash buffer (Wash PLX2) was added and the tube was centrifuged for 1 minute at 11,000 × g. After pouring off the filtrate to remove any residual wash buffer, the minicolumn in the tube was centrifuged again (2 minutes, 11,000 × g). The minicolumn was placed in a new Eppendorf-type tube, and 80 µL of Elution buffer (previously warmed to 70°C) was added. The minicolumn was allowed to stand at room temperature for 2 minutes and then centrifuged for 1 minute at 11,000 × g. The minicolumns were removed, and the tubes with the purified

plasmid DNA were stored in a freezer (−20°C) until subsequent analysis.

The genomic and plasmid DNA were analysed by electrophoresis in 1% [w/v] agarose gel in 0.5× TBE buffer (Sigma Aldrich, USA) with Simply Safe dye (EURx, Poland). The gel was visualised under UV light (UVP, BioDoc-It™ Imaging System, USA). The purified genomic and plasmid DNA were stored at −20°C until subsequent analysis.

Genetic identification of isolates

For the food isolates, a fragment of the 16S rRNA gene was sequenced. The universal primers 27F and 1492 R were used to amplify the 1400-bp gene fragment. The reaction mixture was prepared in a volume of 25 µL, containing 40 pmol of each primer, 1.5 U Red-Taq ReadyMix DNA polymerase and 20 ng genomic DNA as a template. The PCR reaction was performed in an MJ Mini Gradient Thermal Cycler (Bio-Rad, Hercules, CA, USA) and consisted of 35 cycles: initial denaturation at 94°C for 2 min, denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min and a final extension at 72°C for 2 min. The resulting PCR products were analysed by electrophoresis in 1% [w/v] agarose gel in 0.5× TBE buffer (Sigma Aldrich, USA) and then purified. Sequencing was performed by Genomed (Warsaw, Poland) using an Applied Biosystems model 3730 Genetic Analyzer sequencer. The nucleotide sequences of the 16S rRNA gene were assembled and compared with the sequences available in GenBank using the basic local alignment search tool, BLAST +2.12.0 (Papamanoli et al., 2003) and later deposited in the GenBank database (Table 1).

Phenotypic antibiotic resistance

Phenotypic resistance to antibiotics was investigated by slightly modifying the method described by Chajecka-Wierzchowska et al. (2015). For this purpose, a 24-hour *Staphylococcus* bacterial culture was prepared using pour plating on a Mueller-Hinton agar medium (Merck Germany). Phenotypic antibiotic resistance was tested using the disc diffusion method with the following antibiotics (Oxoid, UK) in the corresponding amounts: erythromycin (E; 15 µg), clindamycin (DA; 2 µg), gentamicin (CN; 10 µg), cefoxitin (FOX; 30 µg), norfloxacin (NOR; 10 µg), ciprofloxacin (CIP; 5 µg), tetracycline (TE; 30 µg), rifampicin (RD; 5 µg), nitrofurantoin (F; 100 µg), linezolid (LZD; 10 µg), chloramphenicol (C; 30 µg), trimethoprim (W; 5 µg), trimethoprim/sulfamethoxazole (SXT; 25 µg) and tigecycline (TGC; 15 µg). *Staphylococcus epidermidis* DSMZ 3270 was selected as a control. The plates were then incubated for 24 h at 37°C. After incubation, the zones of growth inhibition were measured and the susceptibility of staphylococci to individual antibiotics was determined, according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), 'Breakpoint tables for interpretation of MICs and zone diameters, 2015'.

According to the methodology described by French et al. (1987), the sensitivity to methicillin was also assessed using methicillin sodium salt (Sigma Aldrich, USA) at a concentration of 10 µg/mL.

Genotypic antibiotic resistance

Real-time PCR analysis (BIO-RAD, CFX96™ Real-Time System/1000™ Thermal Cycler) was performed for all food isolates in order to detect the genes responsible for antibiotic resistance. The template in the real-time PCR reaction consisted of both genomic and plasmid DNA. The primers used in the reaction are shown in Table 2.

The reaction mixture consisted of 10 µL of Eva Green SSoFast™ polymerase (BIO-RAD, USA), 8 µL of dH_2O (EURx, Poland), 0.5 µL of each primer (Genomed, Poland) and 1 µL of template (plasmid or genomic DNA).

The control samples were the DNA of the following strains: *Enterococcus faecalis* ATCC 29212 (*tet*(M)), *Enterococcus faecalis* ATCC 51299 (*erm*(B)), *Staphylococcus epidermidis* ATCC 35984 (*mec*(A) and *erm*(A)), *Staphylococcus epidermidis* ATCC 12228 (*tet*(K)) and *Staphylococcus aureus* ATCC 43300 (*mec*(A)). Additionally, the products of real-time PCR reactions were analysed by electrophoresis, as described in section 2.2.

Statistical analysis

The results of the phenotypic and genotypic antibiotic resistance tests were subjected to principal component analysis (PCA) using Statistica 12 software (StatStoft, USA).

Table 2. Oligonucleotides used in real-time PCR

Antibiotic drug / Group of antibiotic drugs	Gene	Primers	References
Metacycline	*mec*(A)	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGGCACTACCGGATTTGC	Murakami et al. (1991)
Tetracycline	*tet*(L)	F: TGGTGGAATGATAGCCCATT R: CAGGAATGACAGCACGCTAA	Malhotra-Kumar et al. (2005)
	tet(M)	F: GTGGACAAAGGTACAACGAG R: CGGTAAAGTTCGTCACACAC	Warsa et al. (1996)
	tet(K)	F: TTATGGTGGTTGTAGCTAGAAA R: AAAGGGTTAGAAACTCTTGAAA	Aminov et al. (2001)
Erythromycin	*erm*(A)	F: TCTAAAAAGCATGTAAAAGAA R: TGATTATTATTTGATAGCTTC	Sutcliffe et al. (1996)
	erm(B)	F: TGGTATTCCAAATGCGTAATG R: CTGTGGTATGGCGGGTAAGT	Malhotra-Kumar et al. (2005)
	erm(C)	F: TCAAAACATAATATAGATAAA R: TAACTGCTAAATTTGTTATAATCG	Rizzotti et al. (2005)
Mobile genetic elements involved in the spread of antibiotic resistance	*int* (Tn916/Tn1545)	F: GCGTGATTGTATCTCACT R: GACGCTCCTGTTGCTTCT	Brenciani et al. (2007)
Macrolides	*msr*(A/B)	F: GCAAATGGTGTAGGTAAGACAACT R: TAAAACAAATGTAGTGTACTA	Singh et al. (2001)

RESULTS

Phenotypic antibiotic resistance

The diameters of the zones of growth inhibition of the tested *Staphylococcus* bacteria with each antibiotic are presented in Table 3. The largest zones of growth inhibition in the case of almost all strains were observed for cefoxitin, for which the largest zones ranged from 27 to 37 mm. The exception was *Staphylococcus epidermidis* A5, for which the largest zone of growth inhibition was observed for ciprofloxacin (31.5 mm). A high sensitivity was also observed in all of the staphylococci with the antibiotics trimethoprim/sulfamethoxazole and nitrofurantoin.

The smallest inhibition zones for *Staphylococcus* were observed for erythromycin, clindamycin, linezolid and methicillin. In most cases, no zone of growth inhibition was observed with these antibiotics, or it was no larger than 13 mm. Interestingly, only in the case of *Staphylococcus xylosus* M5 did clindamycin create a zone of growth inhibition of more than 22 mm (23.3 mm). According to data provided by EUCAST, a zone of growth inhibition for *Staphylococcus* bacteria larger than 22 mm indicates clindamycin sensitivity.

For the other antibiotics, the zone of inhibition ranged from 11 to 24 mm. For most of the cases tested, the smallest zones of growth inhibition (the highest antibiotic resistance) were observed for the reference strain *Staphylococcus epidermidis* DSMZ 3270, while the largest zones of growth inhibition were observed for the isolate *Staphylococcus warneri* KR2A.

The results of the antibiotic resistance tests of the *Staphylococcus* samples isolated from food are presented in Table 4. The criterion for assessing susceptibility or resistance to antibiotics were the sizes of the growth inhibition zones (shown in Table 3). Based on the data on antibiotic resistance provided by EUCAST, the results were interpreted, and the antibiotic resistance profile of the tested strains was established.

All of the tested strains (including the control strain, *Staphylococcus epidermidis* DSMZ 3270) showed sensitivity to 6 or more antibiotics. All the

Table 3. Zones of growth inhibition of *Staphylococcus* spp. bacteria

Antibiotic	Zone of growth inhibition, mm					
	DSMZ 3270	A5	M5	M6	KR6	KR2A
E	13.0 ±0.0	9.0 ±0.0	10.0 ±0.0	10.0 ±0.0	11.5 ±0.6	0.0 ±0.0
DA	0.0 ±0.0	0.0 ±0.0	23.3 ±1.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
CN	18.3 ±1.0	15.8 ±0.5	18.3 ±1.0	14.3 ±1.0	16.0 ±0.8	16.5 ±0.6
FOX	30.5 ±1.3	27.8 ±1.0	28.3 ±1.5	30.5 ±1.3	28.0 ±0.0	37.0 ±0.0
NOR	24.0 ±2.3	29.5 ±1.3	22.0 ±0.0	28.3 ±2.1	27.5 ±0.6	22.5 ±1.3
CIP	24.5 ±0.6	31.5 ±1.3	25.5 ±0.6	28.8 ±2.1	26.5 ±1.7	31.3 ±1.0
SXT	20.0 ±1.8	21.5 ±0.6	21.0 ±0.8	22.0 ±0.8	23.5 ±0.6	32.0 ±1.8
TGC	15.8 ±0.5	13.3 ±1.0	16.0 ±0.0	14.8 ±0.5	16.3 ±0.5	16.8 ±0.5
TE	23.8 ±1.0	23.5 ±0.8	11.0 ±0.0	22.0 ±0.0	22.0 ±0.0	23.0 ±0.0
RD	11.0 ±0.0	13.0 ±0.0	21.8 ±1.0	11.0 ±0.0	11.8 ±0.5	12.3 ±0.5
F	17.8 ±0.5	19.0 ±0.0	20.0 ±0.0	19.5 ±0.6	18.0 ±0.0	19.0 ±0.0
LZD	7.0 ±0.0	8.3 ±0.5	0.0 ±0.0	8.0 ±0.0	0.0 ±0.0	8.5 ±1.7
C	16.8 ±0.8	26.0 ±0.8	20.3 ±1.0	23.0 ±1.6	21.0 ±0.8	21.8 ±0.5
W	19.5 ±0.6	21.3 ±1.0	21.5 ±1.0	13.0 ±0.8	18.5 ±0.6	22.3 ±1.0
Met	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	10.3 ±1.0	0.0 ±0.0	10.5 ±0.6

Antibiotics: E – erythromycin, DA – clindamycin, CN – gentamicin, FOX – cefoxitin, NOR – norfloxacin, CIP – ciprofloxacin, SXT – trimethoprim / sulfamethoxazole, TGC – tigecycline, TE – tetracycline, RD – rifampicin, F – nitrofurantoin, LZD – linezolid, C – chloramphenicol, W – trimethoprim, Met – methicillin.

Staphylococcus strains: *Staphylococcus epidermidis* DSMZ 3270, *Staphylococcus epidermidis* A5, *Staphylococcus xylosus* M5, *Staphylococcus haemolyticus* M6, *Staphylococcus capitis* KR6, *Staphylococcus warneri* KR2A.

food isolates and the reference strain showed sensitivity to cefoxitin, norfloxacin, trimethoprim/sulfamethoxazole and nitrofurantoin.

In contrast, all of the strains were resistant to at least 7 antibiotics. Of all the strains tested, only the *Staphylococcus xylosus* M5 isolate was sensitive to clindamycin. All strains were resistant to erythromycin, gentamycin, rifampicin, linezolid, tigecycline and methicillin. Only *Staphylococcus xylosus* M5 was resistant to tetracycline, while *Staphylococcus haemolyticus* M6 was resistant to trimethoprim. It is worth mentioning that the milk isolates (M5 and M6), being strains from the natural environment, showed resistance to antibiotics that are not characteristic of other isolates (in terms of individual antibiotic resistance profiles, i.e., strain and/or environmental dependence).

In addition, the results for resistance to ciprofloxacin for all strains were marked as ‘susceptible, increased exposure’. According to EUCAST guidelines, strains for which this designation is used have a high probability of successful antibiotic therapy due to increased exposure to the chemical. Exposure refers to the relationship between the mode of the antibiotic’s administration, dosage, dose intervals and infusion time, as well as the distribution and excretion of the antimicrobial at the site of infection.

Principal component analysis (PCA) was prepared based on data (Table 3 and 4) on the phenotypic antibiotic resistance of the tested isolates. The main objective of the PCA was to determine whether the original dataset on antibiotic resistance and susceptibility concealed any unobserved relationships. The correlation

Table 4. Phenotypic antibiotic resistance profile of the tested strains of *Staphylococcus* spp.

Strain	Profile of phenotypic antibiotic resistance		
	Susceptible	Resistant	Susceptible, increased exposure
Staphylococcus epidermidis DSMZ 3270	FOX, NOR, SXT, TE, F, W	E, DA, CN, TGC, RD, LZD, C, Met	CIP
Staphylococcus epidermidis A5	FOX, NOR, SXT, TE, F, C, W	E, DA, CN, TGC, RD, LZD, Met	CIP
Staphylococcus xylosus M5	FOX, DA, NOR, SXT, F, C, W	E, CN, TGC, TE, RD, LZD, Met	CIP
Staphylococcus haemolyticus M6	FOX, NOR, SXT, TE, F, C	E, DA, CN, TGC, RD, LZD, W, Met	CIP
Staphylococcus capitis KR6	FOX, NOR, SXT, TE, F, C, W	E, DA, CN, TGC, RD, LZD, Met	CIP
Staphylococcus warneri KR2A	FOX, NOR, SXT, TE, F, C, W	E, DA, CN, TGC, RD, LZD, Met	CIP

Antibiotics: E – erythromycin, DA – clindamycin, CN – gentamicin, FOX – cefoxitin, NOR – norfloxacin, CIP – ciprofloxacin, SXT − trimethoprim / sulfamethoxazole, TGC – tigecycline, TE – tetracycline, RD – rifampicin, F – nitrofurantoin, LZD – linezolid, C – chloramphenicol, W – trimethoprim, Met – methicillin.

coefficients of the PCA analysis of phenotypic antibiotic resistance of *Staphylococcus* isolates were in the range of −1 to 1. They were located within what is known as the unit circle (correlation circle). The further a given point is from the centre of the circle, the higher the correlation is between a given variable and a given ordinal axis (principal component). Angles between vectors depicting given variables indicate the correlation of these variables. Acute angles indicate a positive correlation, while obtuse angles indicate a negative correlation. A right angle between two vectors shows that there is no correlation.

The first principal component (factor 1) in the PCA of phenotypic antibiotic resistance carried 85% of the information about the antibiotic resistance of the tested strains. The above analyses demonstrate the correlation of low values of the following strains: *Staphylococcus epidermidis* A5, *Staphylococcus xylosus* M5, *Staphylococcus haemolyticus* M6, *Staphylococcus capitis* KR6, *Staphylococcus warneri* KR2A and *Staphylococcus epidermidis* DSMZ 3270. The 2 groups of antibiotics, one of which contains 6 compounds to which all tested strains were resistant, are presented in Figure 1B. The core phenotypic antibiotic resistance of the tested biological material was to 6 antibiotics: CN-LZD-RD-E-TGC-Met. In contrast, FOX-SXT-F-NOR formed a group of compounds to which the tested isolates showed sensitivity. The vector bundle is clearly visible in Figure 1A. This bundle includes all tested strains (isolates and reference strain). The data are further correlated within each group (sensitive and resistance). Negative correlations occurred between pairs of variables from different groups. Based on Figure 1A, strongly positively correlated strains can be distinguished. The strains *Staphylococcus epidermidis* A5, *Staphylococcus capitis* KR6 and *Staphylococcus warneri* KR2A were strongly correlated with the reference strain and *Staphylococcus haemolyticus* M6, while they were less strongly correlated with *Staphylococcus xylosus* M5. No negative correlation was observed.

Genotypic antibiotic resistance

The results of the antibiotic resistance tests for the food-derived *Staphylococcus* spp. isolates are presented in Table 5. Both the plasmid and genomic DNA were analysed for the presence of genes encoding tetracycline resistance (*tet*(L), *tet*(M) and *tet*(K)). For

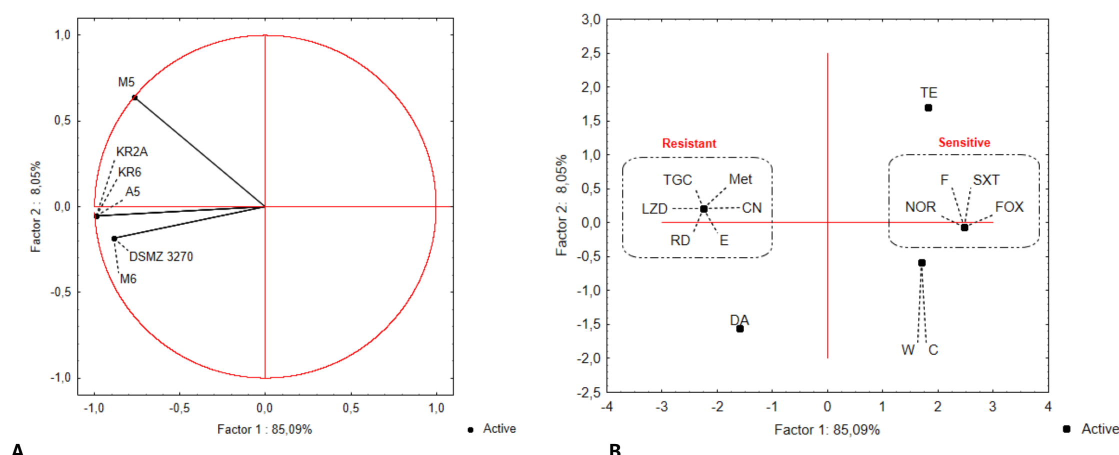

Fig. 1. Principal component analysis of the phenotypic antibiotic resistance of selected strains of the genus *Staphylococcus*: A – a variable factor load scatter plot (correlation circle), showing the correlations between the primary and principal component variables; B – an antibiotic resistance scatterplot in space from the first major components

Table 5. Genotypic antibiotic resistance of *Staphylococcus* strains isolated from food

Strain		Genotype
Staphylococcus epidermidis DSMZ 3270	Plasmid DNA	*tet*(L), *erm*(B), *int*(Tn916/Tn1545)
	Genomic DNA	*tet*(L), *int*(Tn916/Tn1545)
Staphylococcus epidermidis A5	Plasmid DNA	*tet*(K), *tet*(M), *erm*(B), *msr*(A/B), *int*(Tn916/Tn1545)
	Genomic DNA	*tet*(K), *tet*(M), *tet*(L), *erm*(C), *msr*(A/B)
Staphylococcus xylosus M5	Plasmid DNA	*tet*(K), *tet*(L), *erm*(C), *msr*(A/B), *int*(Tn916/Tn1545)
	Genomic DNA	*tet*(K), *tet*(L), *erm*(C), *int*(Tn916/Tn1545)
Staphylococcus haemolyticus M6	Plasmid DNA	*tet*(L), *msr*(A/B)
	Genomic DNA	*tet*(L), *int* (Tn916/Tn1545)
Staphylococcus capitis KR6	Plasmid DNA	*tet*(K), *tet*(M), *tet*(L), *erm*(A), *erm*(B), *int*(Tn916/Tn1545)
	Genomic DNA	*tet*(M), *tet*(L)
Staphylococcus warneri KR2A	Plasmid DNA	*tet*(K), *tet*(L), *tet*(M),*erm*(A)
	Genomic DNA	*tet*(L), *tet*(M), *int*(Tn916/Tn1545)

Staphylococcus xylosus M5 (which phenotypically showed tetracycline resistance), the presence of the *tet*(K) and *tet*(L) genes was determined in reactions with both plasmid and genomic DNA (Table 5). None of the other isolates had exactly this pair of genes marked. *Staphylococcus epidermidis* DSMZ 3270 and *Staphylococcus haemolyticus* M6 had only the *tet*(L) gene (in both plasmid and genomic DNA). In the case of *Staphylococcus epidermidis* A5 and *Staphylococcus capitis* KR6, the *tet*(K) and *tet*(M) genes were identified on the plasmid DNA. *Staphylococcus epidermidis* A5 had the same genes encoded on the genomic DNA,

while *Staphylococcus capitis* KR6 had only the *tet*(M) gene. In the case of *Staphylococcus warneri* KR2A, all 3 genes encoding tetracycline resistance were identified on the plasmid DNA, while in the case of genomic DNA only the *tet*(M) gene was found.

Based on the data in Tables 4 and 5, it can be concluded that there was no clear correlation between the presence of genes encoding genomic DNA resistance to erythromycin and the phenotypic response of the strains. Only *Staphylococcus epidermidis* A5 and *Staphylococcus xylosus* M5 had the *erm*(C) gene on their genomic DNA. The remaining strains did not have the genes *erm*(A), *erm*(B) or *erm*(C) on their genomic DNA. Apart from *Staphylococcus haemolyticus* M6, all the tested isolates had at least 1 erm gene encoded on the plasmid DNA. It is interesting that *Staphylococcus haemolyticus* M6 showed phenotypic resistance to erythromycin, despite the lack of genes encoding antibiotic resistance to it.

In nearly half of the studied strains, the *msr*(A/B) gene was detected on the plasmid DNA (*Staphylococcus epidermidis* A5, *Staphylococcus xylosus* M5 and *Staphylococcus haemolyticus* M6). In one of them, this gene was also present on the genomic DNA (*Staphylococcus epidermidis* A5).

The presence of the transposon *int*(Tn916/Tn1545) was also found in all strains. In the case of *Staphylococcus epidermidis* DSMZ 3270 and *Staphylococcus xylosus* M5, it was present on both the plasmid and genomic DNA. For *Staphylococcus epidermidis* A5 and *Staphylococcus capitis* KR6, it was found only on the plasmid DNA, while for *Staphylococcus haemolyticus* M6 and *Staphylococcus warneri* KR2A it was only identified on the genomic DNA.

PCA was based on the genotypic antibiotic resistance data of the tested isolates. The main objective of the PCA was to determine whether the original data set regarding the presence/absence of genes associated with antibiotic resistance was hiding an initially unobserved relationship. The correlation coefficients of the PCA analysis of the genotypic antibiotic resistance of *Staphylococcus* isolates were in the range of −1 to 1.

Using the data in Table 5, PCA analysis was performed to illustrate the frequency dependence of the genes responsible for antibiotic resistance in staphylococci isolated from food. For all 5 isolates and the reference strain, only 2 genes (highlighted in the boxed image) were present in the DNA (plasmid and/or genomic). The *tet*(L) and *int*(Tn916/Tn1545) genes were detected in all the tested microorganisms, while the *mec*(A) gene was not found in any of them. The presence of the *tet*(K) gene, which was found in 4 of the 6 strains, may have had a significant impact on the degree of similarity between the staphylococci isolated from food.

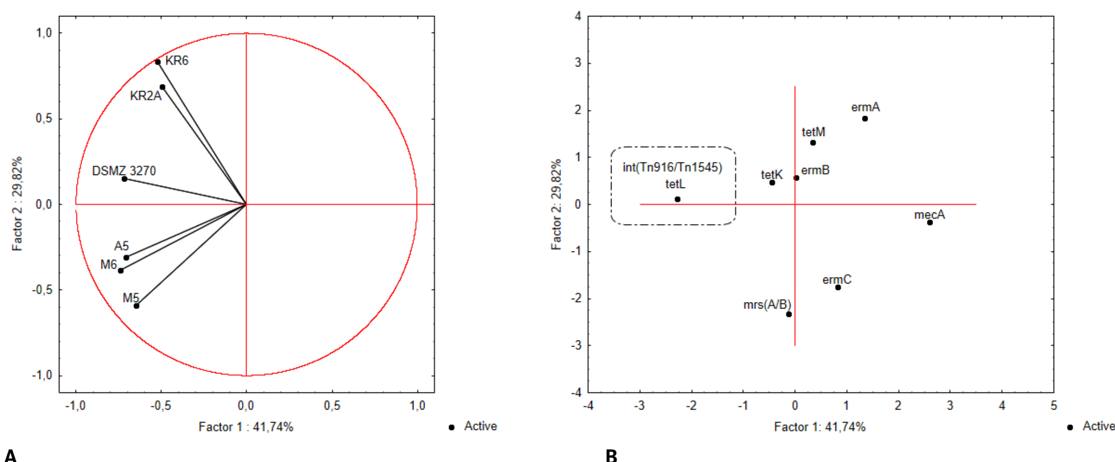

Fig. 2. Principal component analysis of the phenotypic antibiotic resistance of selected strains of the genus *Staphylococcus*: A – a variable factor load scatter plot (correlation circle) showing the correlations between the primary and principal component variables; B – an antibiotic resistance scatterplot in space from the first major components

The correlations between the primary and principal component variables are presented in Figure 2. This figure is based on the presence/absence of genes associated with antibiotic resistance in *Staphylococcus* bacteria. The first principal component (factor 1) in the PCA of genotypic antibiotic resistance carried nearly 42% of the information about the antibiotic resistance of the tested strains. Based on Figure 2A, we can distinguish pairs of strains with a strong positive correlation as well as uncorrelated pairs. There was a strong correlation between *Staphylococcus epidermidis* A5 and *Staphylococcus haemolyticus* M6, and between *Staphylococcus capitis* KR6 and *Staphylococcus warneri* KR2A. A moderate positive correlation was found between the following pairs of strains: *Staphylococcus epidermidis* DSMZ 3270 and *Staphylococcus epidermidis* A5, *Staphylococcus epidermidis* DSMZ 3270 and *Staphylococcus haemolyticus* M6, *Staphylococcus epidermidis* DSMZ 3270 and *Staphylococcus capitis* KR6, *Staphylococcus epidermidis* A5 and *Staphylococcus xylosus* M5, and *Staphylococcus xylosus* M5 and *Staphylococcus haemolyticus* M6. No correlation was observed between *Staphylococcus capitis* KR6 or *Staphylococcus warneri* KR2A and *Staphylococcus epidermidis* A5, *Staphylococcus xylosus* M5 and *Staphylococcus haemolyticus* M6.

Genotypic antibiotic resistance – genomic DNA. Using the data in Table 5, PCA was performed to illustrate the frequency dependence of genes on the genomic DNA responsible for antibiotic resistance in staphylococci isolated from food. Only 1 common gene was identified on the genomic DNA of all 5 isolates and the reference strain. The *tet*(L) gene was detected in all of the tested microorganisms. In contrast, none of them had as many as 3 genes present on the genomic DNA: *erm*(A), *erm*(B) and *mec*(A).

The similarity of the different staphylococci to each other (based on antibiotic resistance genes encoded on genomic DNA) are presented in Figure 3. The first principal component (factor 1) in the PCA of genotypic antibiotic resistance (on genomic DNA) carried more than 56% of the information about the antibiotic resistance of the tested strains. Based on Figure 3A, we can distinguish pairs of strains with a strong positive correlation as well as uncorrelated pairs. There was a strong correlation of both *Staphylococcus epidermidis* DSMZ 3270 and *Staphylococcus haemolyticus* M6 with *Staphylococcus xylosus* M5. There was a moderate positive correlation between *Staphylococcus xylosus* M5 and *Staphylococcus warneri* KR2A. *Staphylococcus capitis* KR6 was positively correlated with *Staphylococcus epidermidis* A5 and positively (but less strongly) correlated with *Staphylococcus warneri* KR2A. A much weaker positive correlation

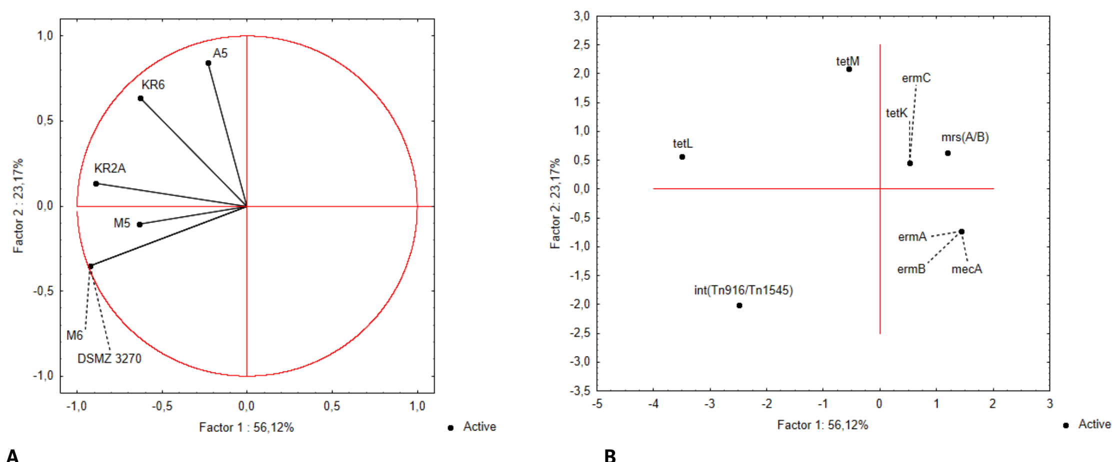

Fig. 3. Principal component analysis of the phenotypic antibiotic resistance of selected strains of the genus *Staphylococcus*: A – a variable factor load scatter plot (correlation circle) showing the correlations between the primary and principal component variables; B – an antibiotic resistance scatterplot in space from the first major components

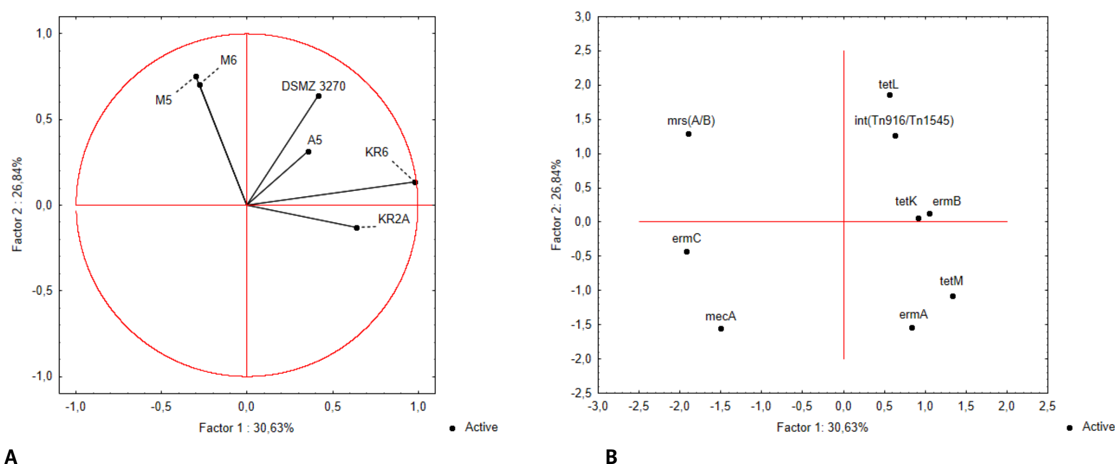

Fig. 4. Principal component analysis of the phenotypic antibiotic resistance of selected strains of the genus *Staphylococcus*: A – a variable factor load scatter plot (correlation circle) showing the correlations between the primary and principal component variables; B – an antibiotic resistance scatterplot in space from the first major components

was observed between *Staphylococcus xylosus* M5 and *Staphylococcus capitis* KR6. In contrast, the weakest positive correlation was observed for *Staphylococcus epidermidis* A5 and *Staphylococcus xylosus* M5. No correlation was observed between either *Staphylococcus epidermidis* DSMZ 3270 or *Staphylococcus haemolyticus* M6 and *Staphylococcus epidermidis* A5.

Genotypic antibiotic resistance – plasmid DNA. Using the data in Table 5, PCA was performed to illustrate the frequency dependence of genes on the plasmid DNA that are responsible for antibiotic resistance in staphylococci isolated from food. No common gene was found on the plasmid DNA of all 5 isolates and the reference strain, *Staphylococcus epidermidis* DSMZ 3270.

The similarity (based on genes for antibiotic resistance encoded on plasmid DNA) of the individual staphylococci to each other are presented in Figure 4. The first principal component (factor 1) in the PCA of genotypic antibiotic resistance (on plasmid DNA) carried nearly 31% of the information about the antibiotic resistance of the tested strains. Based on Figure 4A, we can distinguish pairs of strains with a positive correlation as well as uncorrelated pairs. The strongest positive correlation was observed between *Staphylococcus epidermidis* DSMZ 3270 and *Staphylococcus epidermidis* A5. The radish sprout isolates of *Staphylococcus capitis* KR6 and *Staphylococcus warneri* KR2A also correlated strongly. *Staphylococcus epidermidis* DSMZ 3270 was positively correlated with the other isolates (most weakly with *Staphylococcus warneri* KR2A). *Staphylococcus epidermidis* A5 was also positively correlated with the other isolates (most weakly with the strains isolated from milk: *Staphylococcus xylosus* M5 and *Staphylococcus capitis* M6). However, no correlation was observed between the strains isolated from milk (*Staphylococcus xylosus* M5 and *Staphylococcus haemolyticus* M6) or the isolates from sprouts (*Staphylococcus capitis* KR6 and *Staphylococcus warneri* KR2A).

DISCUSSION

The ever-increasing resistance of microorganisms to antimicrobials poses a serious threat to public health. The currently available pool of compounds with bactericidal potential is narrowing because some known antibiotics have lost much of their effectiveness. The number of strains showing resistance to more than one antibiotic at the same time is increasing in the environment. Thus, it is important to standardise the naming system of multi-drug-resistant (MDR) strains. Epidemiological surveillance defines MDR strains as

organisms showing *in vitro* resistance to more than one antimicrobial agent (regardless of the source of strain isolation; Magiorakos et al., 2012). According to Lowy (1998), multi-drug resistance in strains belonging to the genus *Staphylococcus* is defined as antibiotic resistance to at least 3 antibiotics of different classes (regardless of the source of strain isolation). All the strains isolated during the analyses showed resistance to at least 7 antibiotics, each of them representing a completely different class of compounds (macrolides, aminoglycosides, oxazolidinones, betalactams and semi-synthetic ansamycin antibiotics). Thus, coagulase-negative environmental isolates can be classified as MDR strains. According to a study by Osman et al. (2017), coagulase-negative isolates of the genus *Staphylococcus* (isolated from beef) showed resistance to at least one of the following antibiotics: ampicillin, methicillin, erythromycin, chloramphenicol, ciprofloxacin, vancomycin and tetracycline. The basis of the phenotypic multidrug resistance of the studied environmental isolates is resistance to erythromycin, gentamycin, rifampicin, linezolid, tigecycline and methicillin. The results are consistent with the literature on the subject. In a study conducted by Fowoyo and Ogunbanwo (2017), most coagulase-negative staphylococci (strains isolated from traditional Nigerian fermented foods) showed phenotypic resistance to ampicillin, amoxicillin, sulfomethoxazole/trimethoprim and oxacillin. A large proportion of the microorganisms tested were also resistant to ciprofloxacin, erythromycin, gentamicin, cefotaxime and ofloxacin. On the other hand, according to a study by Moawad et al. (2019), environmental coagulase-negative isolates (isolated from healthy turkeys) were also classified as MDR microorganisms. Strains tested by Moawad et al. (2019) showed resistance to penicillin, ampicillin, tetracycline and trimethoprim/sulfomethoxazole. Nearly 98% of the isolates were resistant to erythromycin, 95% to chloramphenicol and 93% to oxacillin. In addition, 90% of the isolates were resistant to tigecycline and 80% were also resistant to ciprofloxacin and linezolid. Resistance to ciprofloxacin was also associated with resistance to gentamicin and oxacillin, while oxacillin resistance was associated with chloramphenicol and tetracycline resistance. According to a study by Manandhar et al. (2021), phenotypic antibiotic resistance in *Staphylococcus* bacteria (clinical isolates) may be related to species affiliation. Chloramphenicol resistance was observed in *Staphylococcus epidermidis* (92.7% of isolates), *Staphylococcus saprophyticus* (87.5% of isolates) and *Staphylococcus haemolyticus* (95.8% of isolates). Tetracycline resistance was common in *Staphylococcus capitis* (100% of isolates) and *Staphylococcus hominis* (93.3% of isolates). It was also found that all the above-mentioned species were resistant to methicillin. It can also be assumed that resistance to one antibiotic also determines resistance to others. Among the isolates tested, only *Staphylococcus xylosus* M5, which was resistant to erythromycin, did not show sensitivity to clindamycin. Similar results were reported by Manandhar et al. (2021). An association between erythromycin and clindamycin resistance was shown for nearly 30% of the isolates.

All the isolates analysed in the phenotypic test were resistant to methicillin. According to many studies, MRCoNS strains are usually phenotypically resistant to 5 or more classes of antibiotics (Hassanzadeh et al., 2015; Navidinia et al., 2015; Poorabbas et al., 2015). However, the presence of the *mec*(A) gene is not sufficient to demonstrate methicillin resistance (strains isolated from pigs) (Nwaogaraku and Smith, 2019). According to a study by Parvin et al. (2021), methicillin-sensitive *Staphylococcus aureus* strains (isolated from frozen chicken meat) containing the *mec*(A) gene were isolated. Therefore, it cannot be conclusively stated that the *mec*(A) gene is the only gene responsible for antibiotic resistance. The *mec*(A) gene can been isolated from strains that are phenotypically resistant to methicillin, as well as those that are not. Staphylococci (isolated from clinical material samples) developed other mechanisms of resistance to methicillin, including the presence of *mec*(B) and *mec*(C) genes and the overproduction of β-lactamases (Asante et al., 2021), though a significant increase in methicillin resistance was observed in coagulase-negative staphylococci (nearly 80% of isolates). On the other hand, over 95% of the strains isolated by Asante et al. (2021) were resistant to the newest antibiotic on the medical market: linezolid. Unfortunately, it is now more common for coagulase-negative staphylococci (isolated from clinical samples from intensive care units) to acquire linezolid resistance than *Staphylococcus aureus* (Balandin et al., 2016; Baos et al., 2013).

Fowoyo and Ogunbanwo (2017) also found a discrepancy between phenotypic methicillin resistance and the presence of the *mec*(A) gene. Coagulase-negative *Staphylococcus* strains (isolated from fermented foods) did not have the *mec*(A) gene, though they showed resistance to cefoxitin and oxacillin in the phenotypic resistance test. Methicillin resistance in *Staphylococcus* bacteria is due to the expression of the protein PBP2a (penicillin-binding), encoded by the *mec*(A) gene, among other things. There are studies in the literature which confirm the possibility of a horizontal transfer of the SCC cassette (staphylococcal cassette chromosome) between different *Staphylococcus* species. Thus, CoNS may represent a reservoir for the spread of resistance genes in the environment. Coagulase-negative species (e.g., isolated from cattle) can transfer antibiotic resistance genes not only to other CoNS strains, but also to CoPS species—including *Staphylococcus aureus*. Thus, there is a risk that bacteria inhabiting the same ecological niches may exchange the same genes, for example, *mec*(A) (Hanssen et al., 2004; Olsen et al., 2006). The *mec*(A) gene has also been linked to methicillin/oxacillin resistance in combination with resistance to ciprofloxacin, gentamicin and vancomycin (in strains isolated from cattle, pigs and chickens) (Lee, 2003). All of the strains we tested showed resistance to methicillin and gentamicin, despite the *mec*(A) gene being absent on both the genomic and plasmid DNA.

Among the genes responsible for the induction of resistance to macrolides and lincosamides are *erm*(A), *erm*(B), *erm*(C) and *msr*(A/B) (Chaieb et al., 2007; Fasihi et al., 2017; Navidinia et al., 2015).

Fasihi et al. (2017) reported that the macrolide resistance genes (*erm*(A, B, C) and *msr*(A/B)) were associated with the *mec*(A) gene and MRSA isolates (methicillin-resistant S*taphylococcus aureus* (isolated from clinical samples)). In our study, we observed that the *mec*(A) gene was not present in the plasmid or genomic DNA of the food isolates, though the strains were phenotypically resistant to methicillin. In a study by Fasihi et al. (2017), 11% of the isolates resistant to erythromycin and clindamycin had the *erm*(A) gene, while 3.5% had the *erm*(B) gene, 20.5% had the *erm*(C) gene, and 10.5% of the isolates had the *msr*(A/B) gene. Among the food isolates tested in this study, all were phenotypically resistant to erythromycin; only 1 of them was not. Zmantar et al. (2011) also found that 44% of the strains (clinical material isolates) that showed resistance to erythromycin and clindamycin lacked the *erm* genes. Among all the genes coding for erythromycin resistance, the *erm*(B) and *erm*(C) genes were most often isolated, followed by *erm*(A). This result confirms a trend that was observed by Chaieb et al. (2007) that the *erm*(A) and *erm*(B) genes were most often isolated in strains showing resistance to erythromycin and clindamycin (clinical material isolates). The variable erythromycin resistance and the different frequency of genes encoding resistance to this antibiotic may be due to the heterogeneous nature of food isolates. In addition, there may be a loss of small plasmids, on which the *erm* and *msr* genes are often located (Fluit et al., 2001; Zmantar et al., 2008; Zmantar et al., 2011).

In turn, resistance to tetracycline is mainly due to the presence of the *tet*(K), *tet*(L) and *tet*(M) genes. According to research conducted by Chajecka-Wierzchowska et al. (2015), the *tet*(M) gene was the one most often isolated in strains demonstrating tetracycline resistance (strains isolated from ready-to-eat food). Each of the isolates obtained for this study had the *tet*(L) gene. In the samples from the dietary supplement, milk and radish sprouts where the *tet*(K) gene was identified, this was the only gene determining resistance to tetracycline to be found (the gene was always paired with *tet*(L) and/or *tet*(M)). Although most strains had at least 1 *tet* gene, only 1 of the isolates was phenotyped for tetracycline resistance. This may be related to the fact that other genes − such as *tet*(O) and *tet*(S), which were not marked in this study − are also responsible for full resistance to tetracycline (Leroy et al., 2019). The incidence of the *tet*(L) gene compared to other genes responsible for antibiotic resistance may have resulted from the fact that it is mainly encoded on plasmids, which may contribute to the spread of these conditions (Chajęcka-Wierzchowska et al., 2015). Although most of the isolates were phenotypically sensitive to tetracycline, they had at least one tetracycline resistance gene. In 4 cases, a similar situation was observed by Chajęcka-Wierzchowska et al. (2015).

Tn916/Tn1545 is a family of conjugation transposons that are integrative conjugation elements. Tn916 contains as many as 24 open reading frames (arranged

in functional modules) that favour transfers and transposition (excision and insertion) and can regulate transcription. They also often play an auxiliary role in promoting the transmission of resistance to antibiotics and antiseptics. The transfer of Tn916 begins with the excision of an element from the original replicon (from the donor cell) to form a round intermediate molecule. Tn916/Tn1545 are often able to make changes at different places in the host genome. The presence of Tn916/Tn1545 in the cell is often associated with the presence of the *tet*(M) gene, which is associated with moving elements (regardless of the source of strain isolation; Lunde et al., 2019). Research on the structure and function of Tn916/Tn1545 transposons has shown that elements belonging to this family are constantly evolving and interacting with other transposons, plasmids, introns and insertion sequences. Many elements similar to Tn916/Tn1545 also contain the *erm*(B) gene and other genes that confer resistance to macrolide and lincosamide antibiotics. It is especially dangerous that the Tn916/Tn1545 family of transposons is not only diverse but also ubiquitous. It is found in many types of bacteria, most of which are resistant to tetracycline or macrolide antibiotics. Therefore, it cannot be denied that this family of transposons plays a very important role in the evolution of the bacterial genome. They are responsible for the induction of changes at the genotypic and phenotypic levels (regardless of the source of strain isolation; Ciric et al., 2013). Inconsistency between phenotypic and genotypic antibiotic resistance is commonly observed. Differences in antibiotic resistance in strains of the genus *Staphylococcus* can be influenced by many factors. The expression of genes associated with antibiotic resistance can be disrupted or stimulated by the expression of other genes (not necessarily associated with antibiotic resistance). Additionally, multi-drug resistance efflux pumps also have a large impact on antibiotic resistance in *Staphylococcus* bacteria (e.g., isolates derived from fresh milk, meat, frozen fruits and vegetables, and clinical isolates; Kosmidis et al., 2012; Xu et al., 2014).

CONCLUSIONS

The tested biological material was classified as multi-drug-resistant and coagulase-negative strains. Therefore, in further research, these strains will serve as test strains for research on antibiotic resistance and the possibility of modelling it. In order to understand the mechanisms which lower antibiotic resistance through chemical and environmental factors, it is necessary to study the genotype and phenotype of the tested bacteria.

The characterisation of 5 new strains classified as coagulase-negative *Staphylococcus* spp. carried out as part of this study will facilitate effective research into food safety and how to limit the spread of coagulase-negative *Staphylococcus* spp. through food.

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