water

MDPI

Article

First Report of Antibiotic-Resistant Coagulase-Negative *Staphylococcus* Strains Isolated from Technical Snow on Ski Slopes in Mountain Areas

Klaudia Stankiewicz and Anna Lenart-Boroń *

Department of Microbiology and Biomonitoring, University of Agriculture in Cracow, Mickiewicza Ave. 24/28, 30-059 Cracow, Poland; klaudia.kulik@student.urk.edu.pl
* Correspondence: anna.lenart-boron@urk.edu.pl

Abstract: Coagulase-negative staphylococci form a heterogeneous group defined solely by the lack of coagulase. Initially considered non-pathogenic, they are now known to be opportunistic pathogens of increasing importance. This study was conducted to examine the prevalence of *Staphylococcus* spp., their taxonomic diversity, antibiotic resistance patterns and genetic determinants of antibiotic resistance in the water resources used within the technical snow production process. The types of samples included (1) river water at intakes where water is drawn for snowmaking, (2) water stored in technical reservoirs, from which it is pumped into the snowmaking systems, (3) and technical snowmelt water. The study was conducted in the catchments of five rivers: Białka, Biały Dunajec, Raba and Wisła in Poland, and Studený Potok in Slovakia. *Staphylococcus* spp. was detected in all types of samples: in 17% of river water, 25% of reservoir-stored water and in 60% of technical snowmelt water. All staphylococci were coagulase-negative (CoNS) and belonged to 10 species, with *S. epidermidis* being the most prevalent in river water, *S. warneri* and *S. pasteuri* in reservoir-stored water and *S. haemolyticus* in snowmelt water. The highest resistance rates to erythromycin and macrolide/lincosamid/streptogramin b (MLSb) types of resistance were detected in all types of samples, accompanied by the erythromycin efflux pump-determining *msrA* gene as the most frequent genetic determinant of antibiotic resistance. This study is the first report of the presence of antibiotic-resistant, including multidrug-resistant, CoNS carrying more than one gene determining antibiotic resistance in technical snow in the mountain areas of the Central European countries.

Keywords: antibiotic resistance; coagulase-negative staphylococci; mountain areas; *Staphylococcus lugdunensis*; *Staphylococcus warneri*; technical snow

Academic Editor: Christos S. Akratos

Received: 15 December 2024
Revised: 3 January 2025
Accepted: 8 January 2025
Published: 10 January 2025

Citation: Stankiewicz, K.; Lenart-Boroń, A. First Report of Antibiotic-Resistant Coagulase-Negative *Staphylococcus* Strains Isolated from Technical Snow on Ski Slopes in Mountain Areas. *Water* **2025**, *17*, 185. https://doi.org/10.3390/w17020185

1. Introduction

Coagulase-negative staphylococci (CoNS) form a large group of Gram-positive cocci commonly characterized by the lack of one *S. aureus*-associated virulence factor, i.e., coagulase [1]. They represent a very heterogeneous group within the genus *Staphylococcus* and are defined only by the diagnostic procedure-based classification for delimitation from coagulase-positive staphylococci [2]. Initially, they were considered non-pathogenic, but they still can produce their own species- or strain-dependent virulence factors, which allow many of them to act as opportunistic pathogens. Importantly, CoNS-associated opportunistic infections are most frequently regarded as being of environmental origin, but the distribution and antibiotic resistance patterns of coagulase-negative staphylococci in the environment are still poorly characterized [3].

Water is one of the most important vehicles of bacterial spread and dissemination. Microbial populations inhabiting aquatic ecosystems connected with water circulation in the environment play an important role in the dissemination of human-associated microorganisms and pathogens, antibiotic-resistant bacteria and antibiotic resistance genes. Human activities have established a type of water cycle within which wastewater treatment and discharge, along with water uptake, treatment and distribution, represent key stages [3]. If antibiotic-resistant bacteria enter or occur within this cycle, it is possible that they may never be eliminated. The presence of antibiotic-resistant opportunistic pathogens, as well as non-pathogenic species, in the environment can contribute to the interspecies exchange of genetic determinants of antibiotic resistance, which is already a very severe global problem [4]. Additionally, the major challenge in the treatment of CoNS-associated infections is the difficulty in therapy due to their escalating antibiotic resistance rates, which is becoming a factor of increasing morbidity and mortality, as well as the evolution of new pathogens [5].

Even though *Staphylococci* are ubiquitous bacteria, and their presence has been reported in a variety of aquatic environments, no studies to date have been conducted on their presence in the technical snow production processes. Technical snowmaking has become an indispensable requirement for snow-based winter tourism, which faces drastic problems due to climate variability and warming [6]. To provide appropriate amounts of snow on the slopes during the ski season, large amounts of water are required. The formation of a snow cover of approx. 30 cm on a one-hectare slope requires approx. 1000 m^3 of water. Vanham et al. [7] estimated a water demand of 2.3 million m^3 for snowmaking in one of the Austrian regions, which exceeded 50% of municipal water consumption needs during the winter season. The important issue of water management in mountain areas and its consumption due to snowmaking is the fact that the aquatic environment in many of the world's mountain regions is significantly contaminated by the discharge of insufficiently treated wastewater [8]. This is due to the fact that these highly tourism-burdened places are also characterized by large fluctuations in wastewater production as a result of seasonally variable numbers of visitors, which result in local treatment plants being overwhelmed and unable to treat theincoming wastewater [9]. For this reason, given that wastewater is one of the most important pathways of pathogen and resistance distribution, wastewater-contaminated water resources can further act as vectors of these pollutants.

With the above in mind, the question that we tried to answer in this study was whether water resources used for technical snow production and technical snow itself can become sources of bacteria that may pose a threat to human health and to the environment. To answer this question, we conducted the study in order to assess the prevalence of *Staphylococcus* spp., their taxonomic diversity, antibiotic resistance patterns and genetic determinants of antibiotic resistance in the water resources that play a part in technical snow production. The types of samples included (1) river water at intakes where water is drawn for the snowmaking systems, (2) water stored in technical reservoirs, from which it is then pumped into the snowmaking systems, and (3) technical snowmelt water.

2. Materials and Methods

2.1. Sample Collection and Analysis

The samples were collected from 15 ski stations located in the vicinity of five watercourses: Białka, Biały Dunajec, Raba and Wisła in Poland, and Studený Potok in Slovakia (Western Tatras). The samples included three stages of technical snow production: (1) river water at intakes where water is drawn for the snowmaking systems; (2) water stored in technical reservoirs, from which it is then pumped into the snowmaking systems; and (3) melt water from freshly produced technical snow collected directly from underneath

snow cannons. The precise location of the examined ski stations cannot be revealed due to confidentiality agreements with the ski station companies. The sampling campaigns were conducted during two winter seasons, when technical snow production takes place, i.e., from late November to early January. The majority of samples were collected between late November and mid-December (Supplementary Table S1) due to the weather conditions that allowed for efficient technical snow production (i.e., temperature below −4 °C). In total, 63 samples were examined: river water at intakes for technical snow production (n = 25), water stored in technical reservoirs (n = 8) and freshly produced technical snow (n = 30).

In all cases, the samples were collected in three instantaneous replications that formed the final mixed sample. Water was collected into sets of 1000 mL sterile polypropylene bottles, while snow was collected by first scratching the superficial layer, followed by the collection of snow with a snow corer (a 1.0 m-long, 10 cm-wide tube) and transferring the snow into sterile plastic string bags, where it melted. Then, snowmelt water was transferred into sets of 1000 mL sterile polypropylene bottles and further analyzed. Due to the fact that the examined bacteria do not form any survival forms, such as cysts or spores, the samples of water and snow were transported to the laboratory (in coolers at 4 °C) shortly after collection, where they were immediately examined.

2.2. Isolation and Identification of Staphylococci

The enumeration and isolation of staphylococci in river water, reservoir water and snowmelt water were carried out using the membrane filtration method. A volume of 100 mL of water was transferred through a nitrocellulose membrane filter (0.22 μm pore size, 47 mm ø, Sarstedt, Nümbrecht, Germany) and placed in a Petri dish containing Baird-Parker agar (Biomaxima, Lublin, Poland). The Petri plates were incubated at 36 ± 1 °C for 48 h, after which typical grey to black colonies were counted. The bacterial counts were expressed as the number of colony-forming units per 100 mL of water (CFU/100 mL). The typical colonies were then subcultured on Baird-Parker agar and preliminary identification was conducted based on microscopic observations of Gram-stained preparations.

Species identification of colonies that were initially identified as *Staphylococcus* spp. was conducted using the Bruker MALDI Biotyper (Bruker, Billerica, MA, USA) by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) [10]. In order to ensure a high confidence of identification, the MALDI Biotyper was calibrated using the proprietary bacterial test standard (BTS), consisting of a mixture of peptides and proteins derived from the DH5-Alpha *Escherichia coli* strain, and two additional proteins, RNase A and myoglobin, with molecular weights of 12,683.2 Da and 16,952.3 Da. The identification itself relies on the automated process of ionizing bacterial proteins with laser light in vacuum conditions, which separates ionized proteins and peptides into fractions of different molecular weight and charge. These create a protein profile—in the form of characteristic peaks—characteristic of a given microbial genera and species. These profiles are compared with the Analyzer's library (here an MBT IVD reference library; version 2023). Based on this comparison, the analyzer determines the species or genus of the tested isolate and provides the numerical value of compliance for the tested microbial profile with the reference. The higher the value, the greater the compliance of both profiles and—consequently—the reliability of the identification. Each bacterial isolate is subjected to this process in two replicates and only the high score values of identification are taken into account (i.e., 2.30–3.00, which indicates a reliable identification to the species level).

2.3. Culture-Based Antibiotic Resistance Determination

A total of 75 staphylococcal isolates, identified as various species, were subjected to antibiotic resistance testing using the Kirby–Bauer [11] disc diffusion method and following

the recommendation of The European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12]. Antimicrobial disc cartridges were obtained from Oxoid (Basingstoke, UK). Suspensions of bacterial isolates with a density of 0.5 MacFarland were streaked onto Mueller-Hinton II agar (Biomaxima, Lublin, Poland). Eight antimicrobial agents of seven classes were tested: cefoxitin (FOX 30 µg; β-lactam, cephalosporin), ciprofloxacin (CIP 5 µg; fluoroquinolones), erythromycin (E 15 µg, macrolides), gentamycin (CN 10 µg; aminoglycosides), clindamycin (DA 2 µg; lincosamids), tetracycline (TE 30 µg; tetracyclins), tobramycin (TOB 10 µg; aminoglycosids) and trimethoprim/sulfamethoxazole (SXT 1.25/23.75 µg; sulphonamide with dihydrofolate reductase inhibitor). The growth inhibition zone diameters were measured after incubation for 18–24 h at 36 ± 1 °C, and the results were compared with the breakpoint values provided by the European Committee on Antimicrobial Susceptibility Testing [12]. Isolates that were non-susceptible to at least one agent in a minimum of three antibiotic classes were considered multidrug-resistant (MDR) [13]. The type of resistance to macrolides, lincosamids and streptogramins b (MLSb) was assessed according to Fiebelkorn [14]. The methicillin resistance assessment of staphylococci was based on their resistance to cefoxitin (FOX 30 µg), following the results' interpretation according to [12]. The quality control for the disk diffusion method was conducted each time according to the EUCAST recommendations on quality control [15]. Methicillin-resistant, *mecA*-positive *S. aureus* NCTC 12493 was used as a positive control for methicillin resistance testing (see the strain description at https://www.microbiologics.com/01065L (accessed on 2 January 2025)), while all-susceptible *S. aureus* ATCC 29213 was used as a negative control (see the strain description at https://www.microbiologics.com/0365L (accessed on 2 January 2025)) with the relevant inhibition zone diameter validation. The media were prepared fresh on the days of use, and new batches of disks were employed.

2.4. Detection of Antibiotic Resistance Genes

For DNA extraction, loopfuls of fresh colonies were suspended in 100 µL of Tris. Then, the DNA extraction procedure was conducted using the Genomic Mini DNA extraction kit (A&A Biotechnology, Gdańsk, Poland) according to the manufacturer's instructions.

The genes *mecA*, *msrA*, *ereA*, *mphA*, *lnuA* and *vga* were screened using primer pairs and PCR reaction conditions, as described in Table 1. The PCR reaction mixtures were prepared in volumes of 25 µL, containing ~50 ng of DNA template, 12.5 pM of each primer, 2.0 mM of dNTP, 1 × PCR buffer and 2.4 U Taq DNA polymerase (PCR Mix Plus Green, A&A Biotechnology, Gdańsk, Poland). The reactions were performed in a T100 thermal cycler (BioRad, Hercules, CA, USA) using the following temperature profile: 3 min at 94 °C and 34 cycles of amplification consisting of 30 s at 94 °C, an annealing temperature suitable for each primer (Table 1), and 1 min at 72 °C with 10 min at 72 °C for the final extension. The PCR products were visualized and examined in 1% agarose gels (in 1 × TBE buffer) stained with SimplySafe (EurX, Gdańsk, Poland), with a DNA 3 size marker (A&A Biotechnology, Gdańsk, Poland) allowing for assessment of the product length. *S. aureus* ATCC 29213 was used as a negative control (please see strain description at https://www.microbiologics.com/0365L (accessed on 2 January 2025)) and *S. aureus* NCTC 12493 was used as a positive control for *mecA* testing (please see strain description at https://www.microbiologics.com/01065L (accessed on 2 January 2025)).

Table 1. PCR primers used in the study and antibiotic resistance gene characterization.

Gene	Mode of Action	Primer Sequence (5′-3′)	Amplicon Size (bp)	Annealing Temperature (°C)	Reference
mecA	alternative penicillin-binding protein, PBP 2a	F: GTAGAAAATGACTGAACGTCCGATAA R: CCAATTCCACATTGTTTCGGTCTAA	310	55	[16]
msrA	macrolide efflux protein	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	50	[17]
ereA	macrolide lactone esterase	F: AACACCCTGAACCCAAGGGACG R: CTTCACATCCGGATTCGCTCGA	420	57	[18]
mphA	macrolide-active phosphotransferase	F: AACTGTACGCACTTGC R: GGTACTCTTCGTTACC	837	50	[18]
lnuA	lincosamide nucleotidyltransferase	F: GGTGGCTGGGGGGTAGATGTATTAACTGG R: GCTTCTTTTGAAATACATGGTATTTTTCGATC	323	57	[17]
vga	ABC-F subfamily protein conferring resistance to streptogramin A	F: CCAGAACTGCTATTAGCAGATGAA R: AAGTTCGTTTCTCTTTTCGACG	470	54	[17]

2.5. Statistical Analysis

The statistical significance of differences in the numbers of bacteria, the prevalence of CoNS strains resistant to the examined antimicrobials, and the prevalence of genetic determinants of antibiotic resistance in different types of water samples, as well as those between the catchment areas, was assessed using a simple ANOVA, followed by post-hoc tests (e.g., least significant differences between each site; LSD). The correlations between all parameters examined in this study were assessed based on Pearson's correlation coefficient values. In all tests, p-values less than 0.05 were considered statistically significant. The statistical analyses were conducted using Statistica v. 13 software (TIBCO Software, Palo Alto, CA, USA).

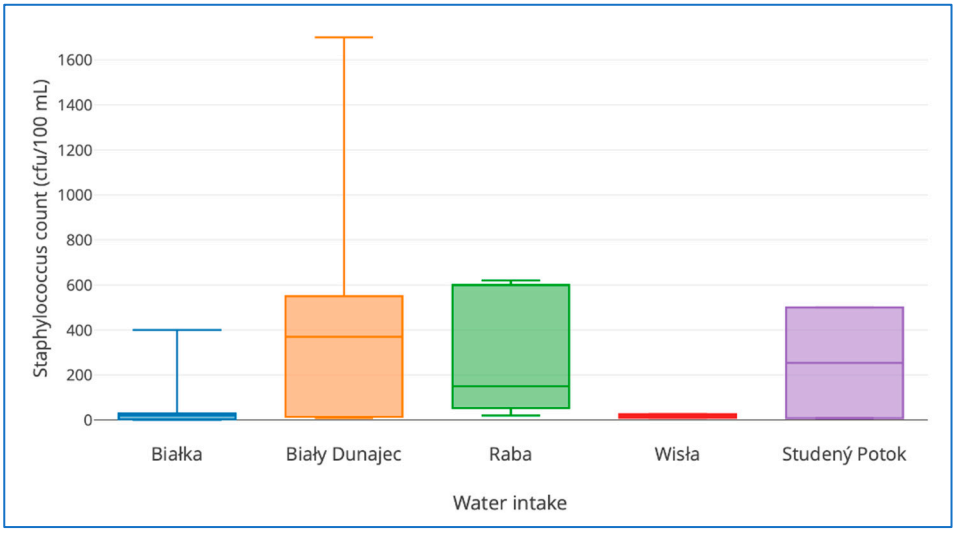
3. Results and Discussion

3.1. Staphylococcal Counts

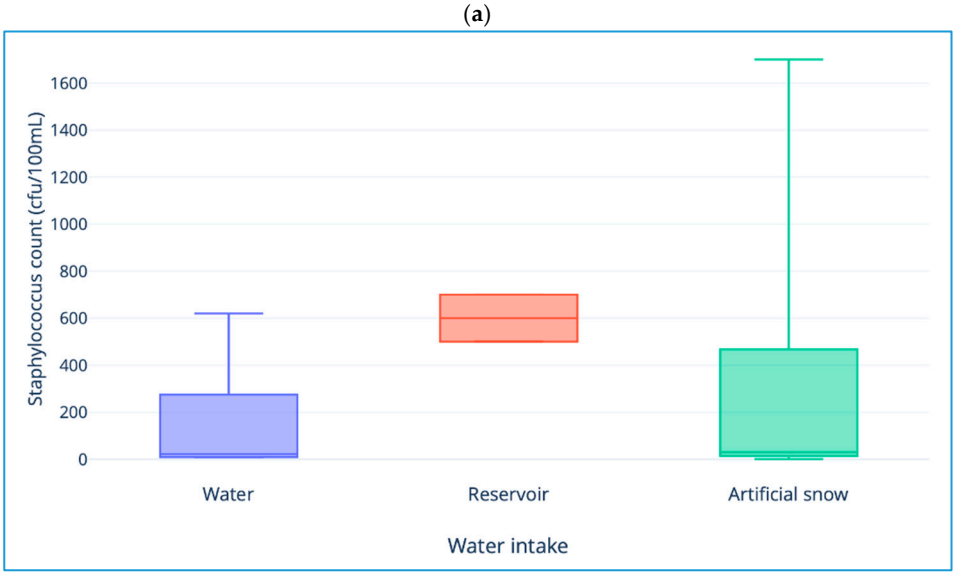
Out of the total number of 63 samples, collected from 15 ski stations, 38 scored positive for the presence of *Staphylococcus* spp., including 17% (n = 18) of river water, 25% (n = 2) of water stored in technical reservoirs and 60% (n = 18) of technical snowmelt water. The staphylococcal counts on Baird-Parker agar ranged from 1 CFU/100 mL (two sites: technical snow, Białka river catchment and river water, Biały Dunajec catchment) to as many as 50,000 CFU/100 mL (technical snow, Western Tatras, Slovakia; Figure 1a,b; Supplementary Table S1; statistically significant differences between types of samples: $F = 4.45$; $p = 0.015$; statistically significant differences between river catchments: $F = 6.66$, $p = 0.00013$). Based on our previous study [19], it can be expected that the presence of staphylococci in river water at intakes for technical snow production, which then results in their presence in reservoir-stored water and technical snow, results from river water contamination by leaking septic tanks, illegal discharges of wastewater from households, and the improper functioning and insufficient effectiveness of wastewater treatment plants and water resource recovery facilities [20].

In some cases (i.e., Western Tatras, Slovakia or in one of the ski stations situated in the Biały Dunajec catchment), the staphylococcal counts in the technical snowmelt water exceeded those detected in the river water or reservoir-stored water, or the staphylococcal counts in the reservoir exceeded those in river water (Figure 1b). One of the possible explanation for the high bacterial counts in snowmelt water might be the differences between the ski stations in the frequency of cleaning and exchange of the snow cannon

filters (unpublished data, information provided verbally by the ski station owners). High bacterial loads in reservoir water may result from the fact that the reservoir collects water over a period of around a month, which allows for the microorganisms to accumulate over time. This—in the case of too infrequent maintenance procedures—might result in the biofilm formation by the staphylococci-containing water microbiota, followed by its detachment [3,21].



(**a**)



(**b**)

Figure 1. Boxplots of total staphylococcal counts (CFU/100 mL) showing the differences between (**a**) river catchments and (**b**) types of water samples.

3.2. *Staphylococcus* spp. *Species Prevalence*

A total of 75 isolates, including 25 from river water, eight from technical reservoirs and 42 from technical snowmelt water were obtained in the first stage of research. These were subjected to MALDI-TOF analysis for species identification. All 75 *Staphylococcus* spp. isolates were coagulase-negative and belonged to ten species (Figure 2). The most prevalent species varied between different types of samples. When considering all the samples in total, *S. warneri*, *S. haemolyticus* and *S. equorum* were the three most prevalent, *S. epidermidis*, *S. warneri* and *S. equorum* were the three most frequently detected in river water, *S. pasteuri*, *S. warneri* and *S. equorum* were the only three species identified in the reservoir-stored water and finally *S. haemolyticus*, *S. warneri* and the group of unidentified to the species

level (grouped as *Staphylococcus* spp.) were the three most prevalent in technical snow (Figure 2, Supplementary Table S1). Importantly, two strains of *S. lugdunensis* have been isolated from the technical snow. This species has already been recognized as a CoNS "intermediate" between *S. aureus* and *S. epidermidis* groups and is characterized by some clinical features that it shares with *S. aureus* [2,22]. It is also known that CoNS are responsible for laryngological infections and *S. lugdunensis* has been reported as an etiological agent of necrotizing sinusitis in hospitalized patients [23]. This species has been isolated from maxillary sinuses of laryngological patients and several virulence factors carried by strains of this species have been demonstrated [23]. According to [22], six CoNS species are of higher clinical significance—*S. epidermidis*, *S. saprophyticus*, *S. haemolyticus*, *S. capitis*, *S. hominis* and *S. lugdunensis*—and five of them were identified in our study (all of them in technical snow). *Staphylococcus warneri*, which was the most numerous species isolated in our study (and the second most numerous in technical snow), has been recently recognized as a new emerging pathogen, leading to severe invasive infections in immunocompromised patients and a variety of other types of infections even in healthy individuals [24].

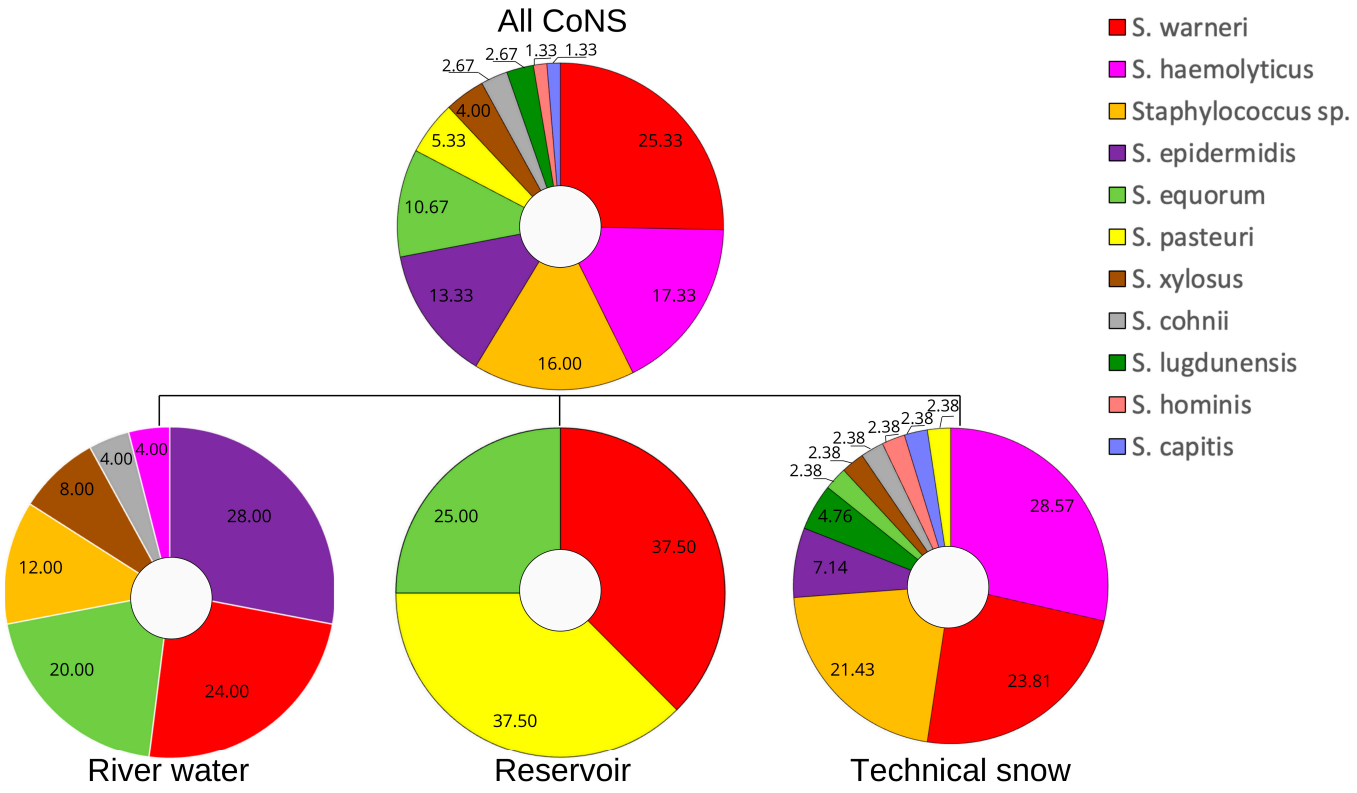


Figure 2. Species distribution of coagulase-negative staphylococci (CoNS) identified in the study.

3.3. Antibiotic Resistance of Isolated CoNS

Among the most significant problems associated with the prevalence of CoNS is their increasing resistance to antimicrobial agents. Firstly, the increasing rates of resistant and multi-resistant infections caused by CoNS limit therapeutic options and aggravate treatment strategies [2,22,25]. Secondly, CoNS have been regarded as important reservoirs of resistance genes, which are often located on mobile genetic elements; therefore, they could easily be transferred (via horizontal gene transfer) to other species, including more pathogenic ones, such as *S. aureus* [5,25,26]. Faria et al. [3] suggest that CoNS may represent a relevant antibiotic resistance reservoir, particularly in habitats with restrictive conditions, i.e., technical snow. Out of the 75 CoNS isolates examined in this study, 25 (33.33%) were susceptible to all tested antimicrobial agents, while none were resistant to all antimicrobials. Three isolates were

multidrug-resistant (i.e., non-susceptible to at least one agent in a minimum of three antibiotic classes) [13] and these included two technical snow-derived isolates (*S. lugdunensis*, Biały Dunajec catchment and *S. haemolyticus*, Raba river catchment) and one river water-derived isolate (*S. haemolyticus*, Raba river catchment). Four isolates were MRS-positive (three MDR isolates and one snow-derived *Staphylococcus* spp., Białka river catchment; Table 2). As many as 41 (54.67%) showed one of the macrolide/lincosamid/streptogramin b (MLSb) resistance types. Within these, 27 (36%) presented MSb resistance, 11 presented (14.67%) inducible MLSb and three presented (4%) constitutive MLSb types of resistance (Table 2).

Figure 3 presents the resistance rates of CoNS isolates to all antibiotics tested along with their MLSb and MRS profiles and compares them between the examined types of samples. Resistance to erythromycin (macrolide) was most frequently detected and was the only one observed in all types of samples. Out of the specific types of resistance, MLSb was similarly very frequent. Very high or the highest rates of resistance to erythromycin have been reported in many studies worldwide [3,27,28]. Such high rates of erythromycin resistance, as observed in this and other studies, may be due to the fact that erythromycin is the first-discovered 14-membered macrolide and has been widely used since its clinical introduction in 1952 [29]. This fact might influence the co-occurrence, as observed in our study, of the frequent MLSb type of resistance (i.e., 32% in river water, 50% in reservoir-stored water and 69% in snowmelt water).

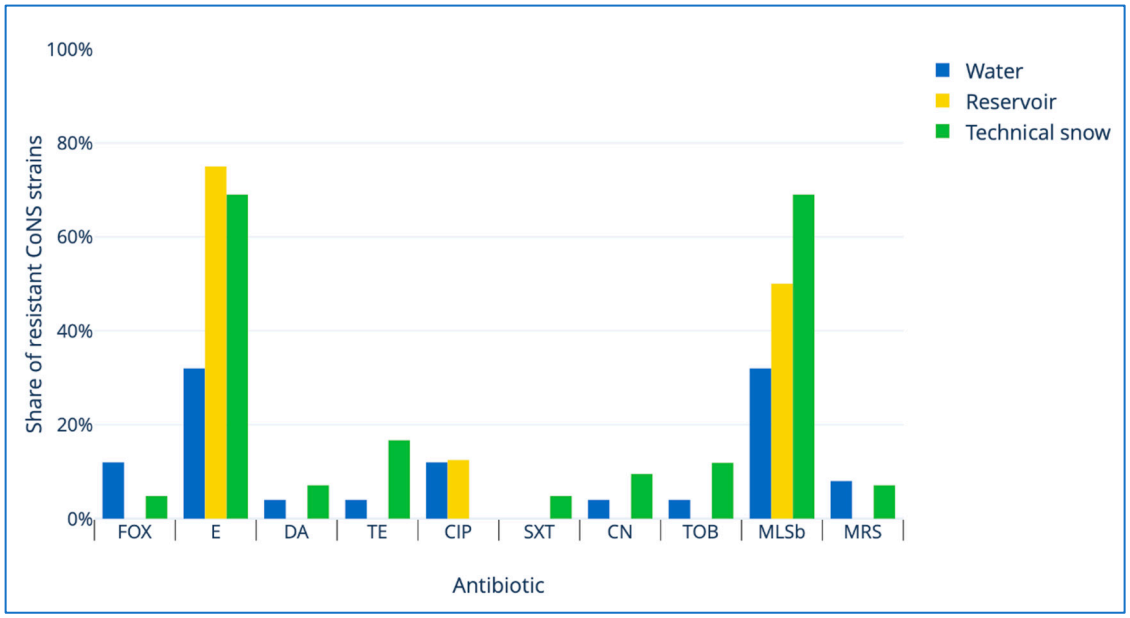


Figure 3. Resistance rates of CoNS strains divided by the types of samples examined in the study. FOX—cefoxitin; E—erythromycin; DA—clindamycin; TE—tetracycline; CIP—ciprofloxacin; SXT—trimethoprim/sulfamethoxazole; CN—gentamycin; TOB—tobramycin; MLSb—macrolide/lincosamid/streptogramin b type of resistance; MRS—methicillin resistance.

Table 2. Summary of antibiotic resistance and the resistance-determining genes in the coagulase-negative staphylococci analyzed in this study. Numbers in brackets represent the count of strains within a given species detected in various river catchments, types of samples, phenotypic resistance and antibiotic resistance-carrying genes.

No.	Species	River Catchment *	Type of Sample **	Antibiotic Resistance Phenotype ***	Antibiotic Resistance Genes
1	*S. epidermidis* $n = 10$	B (4), BD (0) R (0), W (0) S (6)	W (7) R (0) S (3)	FOX (1), E (8), DA (0), TE (0), CIP (2), SXT (1), CN (0), TOB (0), MS_B (8), $cMLS_B$ (0), $iMLS_B$ (0), MDR (0)	*mecA* (7), *msrA* (8), *ereA* (0), *mphA* (0), *lnuA* (0), *vga* (0)
2	*S. haemolyticus* $n = 13$	B (1), BD (3) R (8), W (1) S (0)	W (1) R (0) S (12)	FOX (2), E (11), DA (1), TE (7), CIP (1), SXT (0), CN (4), TOB (3), MS_B (1), $cMLS_B$ (1), $iMLS_B$ (9), MDR (2)	*mecA* (1), *msrA* (6), *ereA* (0), *mphA* (0), *lnuA* (5), *vga* (3)
3	*S. lugdunensis* $n = 2$	B (0), BD (1) R (1), W (0) S (0)	W (0) R (0) S (2)	FOX (0), E (1), DA (1), TE (0), CIP (0), SXT (0), CN (1), TOB (1), MS_B (0), $cMLS_B$ (1), $iMLS_B$ (0), MDR (1)	*mecA* (0), *msrA* (1), *ereA* (0), *mphA* (0), *lnuA* (1), *vga* (1)
4	*S. warneri* $n = 19$	B (2), BD (6) R (7), W (0) S (4)	W (6) R (3) S (10)	FOX (0), E (9), DA (1), TE (0), CIP (1), SXT (1), CN (0), TOB (1), MS_B (7), $cMLS_B$ (0), $iMLS_B$ (0), MDR (0)	*mecA* (0), *msrA* (12), *ereA* (0), *mphA* (0), *lnuA* (0), *vga* (2)
5	*S. pasteuri* $n = 4$	B (0), BD (3) R (0), W (1) S (0)	W (0) R (3) S (1)	FOX (0), E (3), DA (0), TE (1), CIP (0), SXT (0), CN (0), TOB (0), MS_B (3), $cMLS_B$ (0), $iMLS_B$ (0), MDR (0)	*mecA* (0), *msrA* (1), *ereA* (0), *mphA* (0), *lnuA* (0), *vga* (0)
6	*S. equorum* $n= 8$	B (0), BD (7) R (1), W (0) S (0)	W (5) R (2) S (1)	FOX (1), E (1), DA (0), TE (0), CIP (0), SXT (0), CN (0), TOB (0), MS_B (1), $cMLS_B$ (0), $iMLS_B$ (0), MDR (0)	*mecA* (0), *msrA* (3), *ereA* (0), *mphA* (1), *lnuA* (0), *vga* (0)
7	*S. xylosus* $n = 3$	B (0), BD (2) R (1), W (0) S (0)	W (2) R (0) S (1)	FOX (0), E (1), DA (0), TE (0), CIP (0), SXT (0), CN (0), TOB (0), MS_B (0), $cMLS_B$ (0), $iMLS_B$ (1), MDR (0)	*mecA* (0), *msrA* (0), *ereA* (0), *mphA* (0), *lnuA* (0), *vga* (0)
8	*S. hominis* $n = 1$	B (0), BD (1) R (0), W (0) S (0)	W (0) R (0) S (1)	FOX (0), E (1), DA (0), TE (0), CIP (0), SXT (0), CN (0), TOB (0), MS_B (0), $cMLS_B$ (0), $iMLS_B$ (1), MDR (0)	*mecA* (0), *msrA* (0), *ereA* (0), *mphA* (0), *lnuA* (0), *vga* (0)
9	*S. cohnii* $n = 2$	B (0), BD (0) R (1), W (0) S (1)	W (1) R (0) S (1)	FOX (0), E (1), DA (1), TE (0), CIP (0), SXT (0), CN (0), TOB (0), MS_B (0), $cMLS_B$ (1), $iMLS_B$ (0), MDR (0)	*mecA* (1), *msrA* (2), *ereA* (0), *mphA* (0), *lnuA* (0), *vga* (1)
10	*S. capitis* $n = 1$	B (0), BD (0) R (0), W (1) S (0)	W (0) R (0) S (1)	FOX (0), E (1), DA (0), TE (0), CIP (0), SXT (0), CN (0), TOB (0), MS_B (1), $cMLS_B$ (0), $iMLS_B$ (0), MDR (0)	*mecA* (0), *msrA* (1), *ereA* (0), *mphA* (0), *lnuA* (0), *vga* (0)
11	*Staphylococcus* sp. $n = 12$	B (8), BD (1) R (1), W (2) S (0)	W (3) R (0) S (9)	FOX (1), E (6), DA (0), TE (0), CIP (0), SXT (0), CN (0), TOB (1), MS_B (6), $cMLS_B$ (0), $iMLS_B$ (0), MDR (0)	*mecA* (0), *msrA* (10), *ereA* (0), *mphA* (0), *lnuA* (1), *vga* (3)

* River catchment: B—Białka; BD—Biały Dunajec; R—Raba; W—Wisła; S—Studený Potok. ** Type of sample: W—river water; R—reservoir; S—technical snow, *** Antibiotic resistance: FOX—cefoxitin; E—erythromycin; DA—clindamycin; TE—tetracycline; CIP—ciprofloxacin; SXT—trimethoprim/sulfamethoxazole; CN—gentamicin; TOB—tobramycin; MS_B—resistance to macrolide and streptogramin B; $cMLS_B$—constitutive resistance to macrolide, lincosamide and streptogramin Bl $iMLS_B$—inducible resistance to macrolide, lincosamide and streptogramin B; MDR—multidrug resistance.

3.4. Genetic Determinants of Staphylococcal Resistance to MLSb Antibiotics

Given the high prevalence of erythromycin resistance along with the MLSb phenotype among the isolated CoNS, we examined the genes determining resistance to macrolides (*msrA* encoding efflux protein [17], *ereA* encoding macrolide lactone ring esterase, and *mphA* encoding macrolide-active phosphotransferase [18]), lincosamids (*lnuA* encoding lincosamide nucleotidyltransferase [17]) and streptogramins b (*vga* encoding ATP-binding cassette protein [17]). Due to the fact that CoNS may act as reservoirs of genes determining resistance to, e.g., *S. aureus*, we also screened the isolates for the presence of the *mecA* gene, encoding the alternative penicillin-binding protein, PBP 2a [16]. Out of the three macrolide resistance determining genes, *msrA* was most frequently detected (i.e., in 44 isolates, 58.67%). Among all examined genes, *vga* was the second most frequent (in 10 isolates, 13.33%), followed by the *mecA* gene (in 9 isolates, 12%). In eight out of the nine *mecA*-positive isolates, this gene co-occurred with *msrA* (and *msrA* only). Two isolates

(*S. haemolyticus*, technical snow-derived, Białka river catchment and *S. lugdunensis*, technical snow-derived, Biały Dunajec catchment) were characterized by the presence of three of the examined genes, i.e., *msrA*, *lnuA* and *vga* (a set encoding resistance to macrolides, lincosamids and streptogramins b) (Table 2).

3.5. Resistance Rates Through the Technical Snow Production Cycle

To further examine the spread of resistance throughout the cycle of technical snow production, we created a heatmap (Figure 4) presenting the percentage share of resistance phenotypes, MRS and MLSb types of resistance and their genetic determinants. It shows a clear relationship between the highest prevalence of erythromycin resistance, combined with the MLSb type of resistance, and the genetic determinant thereof, i.e., the *msrA* gene. Worryingly, the technical snow was characterized by the highest percentage of MLSb-positive and *msrA*-positive isolates (Figures 2 and 4, Supplementary Table S1). What can also be noticed in both Figures 2 and 4, is that the rates of phenotypic resistance and genetic determinants of resistance to MLSb antibiotics vary between the types of samples and that the highest rates do not follow a clear pattern. The highest resistance rate in river intake water was observed only in the case of cefoxitin (and accordingly in the MRS type of resistance). The highest resistance rate in reservoir water was observed in the case of erythromycin and ciprofloxacin, while the resistance to remaining antibiotics was highest in technical snowmelt water. There may be two reasons for such a situation. The first would be—as in the case of higher staphylococcal counts in technical snow than in the water used for its production—the ability of bacteria to form biofilms on biotic and abiotic surfaces, which allows them to resist and survive environmental stresses, allowing their further spread [25]. The second reason might be the co-occurrence of antibiotic resistance and cold stress resistance in certain strains of CoNS. In the case of the highest resistance rates in reservoir water, the reason might be the timespan of water collection in reservoirs (of c.a. a month), which may contribute to the accumulation of certain bacterial strains. However, verification of the above hypotheses requires further studies.

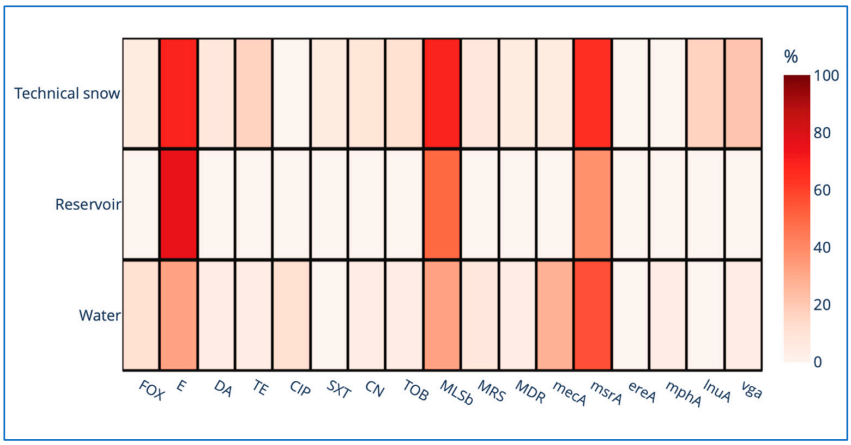


Figure 4. Heatmap showing the antibiotic resistance phenotypes, MLSb and MRS types of resistance and the genetic determinants of antibiotic resistance in river water used for the technical snow production (Water), water stored in reservoirs prior to technical snow production (Reservoir) and in technical snowmelt water (Technical snow). FOX—cefoxitin; E—erythromycin; DA—clindamycin; TE—tetracycline; CIP—ciprofloxacin; SXT—trimethoprim/sulfamethoxazole; CN—gentamycin; TOB—tobramycin; MLSb—macrolide/lincosamid/streptogramin b type of resistance; MRS—methicillin resistance; MDR – multidrug resistance. The differences in resistance to erythromycin and ciprofloxacin and in the detection rates of *mecA* and *lnuA* were statistically significant (F = 5.50, 5.23, 5.06 and 3.17, respectively; p = 0.006, 0.008, 0.009 and 0.048, respectively).

3.6. Resistance Rates in Various River Catchments

A similar relationship (i.e., co-occurrence of erythromycin resistance phenotype with MLSb type of resistance and/*msrA* gene) was observed when various river catchments were examined (Figure 5). The highest or nearly the highest resistance phenotypes and gene prevalence were observed in Studený Potok in Slovakia (e.g., the highest percentage of *mecA*-positive isolates). Yeamans et al. [30] mention the irrational use of antibiotics as the most common cause of antibiotic resistance; at the same time, they explored the prevalence of self-medication in the populations of different European countries. Poland and Slovakia are both characterized by high self-medication rates, i.e., 51% in Slovakia and 46% in Poland. The European Centre for Disease Prevention and Control (ECDC) has raised the alarm on the continuously increasing threat of antibiotic resistance, estimating that over 35,000 people die every year due to antibiotic-resistant infections across the EU/EEA countries [31], and lists overall antibiotic consumption as the major AMR contributor. The ECDC reports on antibiotic consumption by country list Poland and Slovakia high (23.2 and 20.1 DDD/1000 inhabitants/day in Poland and Slovakia, respectively) in the consumption of antibacterials for systemic use [32]. Furthermore, the MRSA detection rate is the same in Poland and Slovakia (i.e., 15.2% according to the report by [33]). However, what needs to be mentioned here, is the fact that the phenotypic resistance and genetic determinant detection rates observed locally can vary significantly by regions (as seen in, e.g., Figure 5—part referring to Polish watercourses). Moreover, when considering resistance rates and the prevalence of genetic determinants in individual types of samples (i.e., river water, reservoir water and snowmelt water) from the examined catchments, the highest share of strains resistant to erythromycin coupled with the MLSb type of resistance was observed in snowmelt water in Polish catchments and in river water in Slovakia (Supplementary Table S1). However, in the case of the remaining parameters, there is no regularity in terms of the highest prevalence of resistance rates and genetic determinants of antibiotic resistance (e.g., in the Białka river catchment, the highest resistance rate to cefoxitin, erythromycin, trimethoprim/sulfamethoxazole and gentamycin was observed in snowmelt water, but for ciprofloxacin, it was the highest in river water; in Raba the highest resistance rate to cefoxitin, clindamycin, gentamycin and tobramycin was detected in rover water, while in snowmelt water, there was the highest resistance rate to erythromycin, tetracycline and ciprofloxacin, and so on; Supplementary Table S1).

The Pearson's correlation coefficient between the antibiotic presence, concentration [34], the resistance phenotypes and their genetic determinants (Supplementary Table S1) shows no correlation between the concentrations or the presence of various antimicrobial agents and the corresponding resistances or genetic determinants thereof. This is very likely, as the major sources of antibiotic-resistant bacteria and resistance genes in the environment include wastewater treatment plants and surface runoff from agriculture and the animal industry [35]. Thus, the source of the AMR CoNS observed in the examined samples was most probably located upstream of the water intakes for technical snow production.

Finally, the isolation and identification of staphylococcal opportunistic pathogens in technical snow, with which skiers (especially beginners and young children) have frequent contact, is worth further examination. One aspect of technical snow production that has not yet been unexplored is the fact that, during technical snow production, there are forced transport mechanisms whereby water—after filtration or without it—is aerosolized by snow cannons, thus contributing to bioaerosol formation. Only one study to date has explored microbial and non-microbial ice nucleation particles, their distribution and their impact on the ice nucleation of water [36]. For this reason, future experiments could focus on the detection and microbial composition of bioaerosols formed during the technical snowmaking processes.

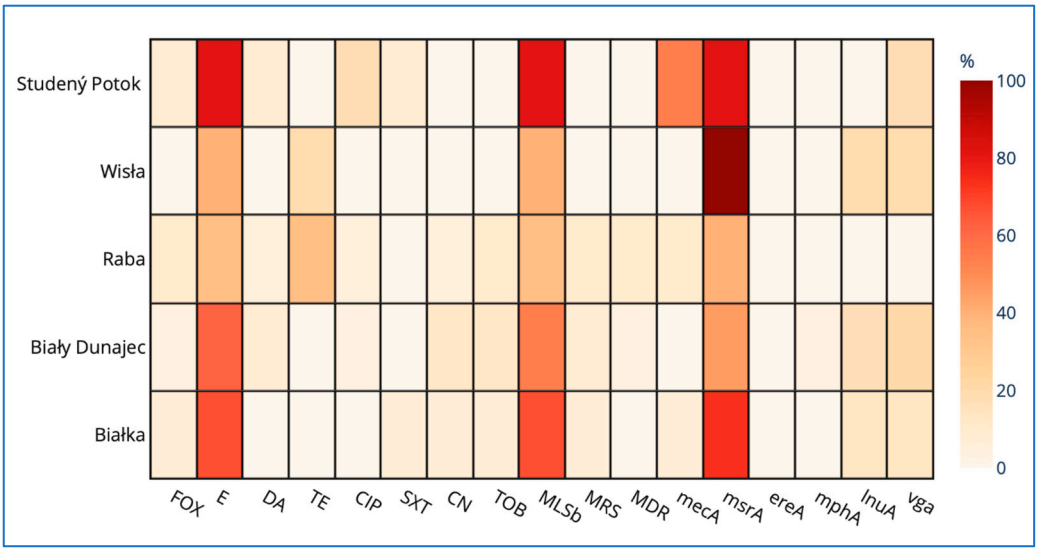


Figure 5. Heatmap showing the antibiotic resistance phenotypes, MLSb and MRS types of resistance and the genetic determinants of antibiotic resistance in the catchments of five rivers: Białka, Biały Dunajec, Raba and Wisła in Poland, and Studený Potok in Slovakia. FOX—cefoxitin; E—erythromycin; DA—clindamycin; TE—tetracycline; CIP—ciprofloxacin; SXT—trimethoprim/sulfamethoxazole; CN—gentamycin; TOB—tobramycin; MLSb—macrolide/lincosamid/streptogramin b type of resistance; MRS—methicillin resistance; MDR – multidrug resistance. The differences in tetracycline resistance as well as in *mecA* and *msrA* detection rates were statistically significant (F = 5.88, 7.89 and 3.26, respectively; p = 0.0004, 0.00003 and 0.016, respectively).

4. Conclusions

Our study is a pioneering investigation of the technical snowmaking process in ski resorts. It contributes not only to understanding the environmental impact of technical snow production from water of varying quality, but also to the identification of the potential health risks to workers and tourists associated with bacteriologically contaminated technical snow. Only when such risks are identified and understood will it be possible to implement preventive measures.

This study demonstrated that the following:

- Technical snow produced from microbiologically contaminated water may frequently contain coagulase-negative staphylococci (CoNS), since as many as 60% of technical snowmelt water samples proved positive for the presence of *Staphylococcus* spp.;
- The CoNS counts in technical snowmelt water reached high values, in some cases exceeding those observed in water used for the production of technical snow. If maintenance and cleaning of snowmaking devices is conducted too rarely, staphylococci-containing water microbiota may form biofilms within the devices, resulting in increased concentrations of these microorganisms in technical snow;
- Ten CoNS species were identified in the study, including opportunistic pathogens such as *S. haemolyticus*, *S. warneri* and *S. lugdunensis*. Among them, *S. lugdunensis* shares some clinical features with *S. aureus*, with several virulence factors already demonstrated, while *S. warneri* has been recently recognized as a new emerging pathogen responsible for severe invasive infections;
- Resistance to the antibiotic erythromycin (macrolide) was the most frequent in all three types of samples—the same as the MLSb type of resistance—probably due to the fact that erythromycin is one of the "oldest" antibiotics used in medicine. This was coupled with the most frequent detection of *msrA* gene, which encodes the erythromycin efflux pump.

Further studies are needed to examine as yet unexplored aspects of the technical snow production, which has recently become essential for winter sports to continue in lower altitudes due to the global climate warming. One such aspect and future direction of research may be the exploration of bioaerosol formation during the technical snowmaking process.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w17020185/s1. Table S1: Data analyzes; Table S2: Correlation between resistance phenotypes and genes.

Author Contributions: Conceptualization, K.S. and A.L.-B.; methodology, A.L.-B.; software, K.S. and A.L.-B.; validation, K.S. and A.L.-B.; formal analysis, K.S.; investigation, K.S.; resources, A.L.-B.; data curation, A.L.-B.; writing—original draft preparation, A.L.-B.; writing—review and editing, K.S.; visualization, K.S.; supervision, A.L.-B.; project administration, A.L.-B.; funding acquisition, A.L.-B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the statutory measures of the University of Agriculture in Kraków.

Data Availability Statement: Data are available on request from the corresponding author.

Acknowledgments: The authors would like to thank Justyna Prajsnar for her support in the analyses of antimicrobial content in the water and snow samples.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Becker, K.; Both, A.; Weißelberg, S.; Heilmann, C.; Rohde, H. Emergence of Coagulase-Negative Staphylococci. *Expert Rev. Anti-Infect. Ther.* **2020**, *18*, 349–366. [CrossRef] [PubMed]
2. Becker, K.; Heilmann, C.; Peters, G. Coagulase-Negative Staphylococci. *Clin. Microbiol. Rev.* **2014**, *27*, 870–926. [CrossRef] [PubMed]
3. Faria, C.; Vaz-Moreira, I.; Serapicos, E.; Nunes, O.C.; Manaia, C.M. Antibiotic Resistance in Coagulase Negative Staphylococci Isolated from Wastewater and Drinking Water. *Sci. Total Environ.* **2009**, *407*, 3876–3882. [CrossRef] [PubMed]
4. Santos, G.A.C.; Dropa, M.; Martone-Rocha, S.; Peternella, F.A.S.; Veiga, D.P.B.; Razzolini, M.T.P. Microbiological Monitoring of Coagulase-Negative Staphylococcus in Public Drinking Water Fountains: Pathogenicity Factors, Antimicrobial Resistance and Potential Health Risks. *J. Water Health* **2023**, *21*, 361–371. [CrossRef]
5. Fowoyo, P.T.; Ogunbanwo, S.T. Antimicrobial Resistance in Coagulase-Negative Staphylococci from Nigerian Traditional Fermented Foods. *Ann. Clin. Microbiol. Antimicrob.* **2017**, *16*, 4. [CrossRef]
6. Grünewald, T.; Wolfsperger, F. Water Losses During Technical Snow Production: Results From Field Experiments. *Front. Earth Sci.* **2019**, *7*, 435385. [CrossRef]
7. Vanham, D.; Fleischhacker, E.; Rauch, W. Technical Note: Seasonality in Alpine Water Resources Management—A Regional Assessment. *Hydrol. Earth Syst. Sci.* **2008**, *12*, 91–100. [CrossRef]
8. Kulik, K.; Lenart-Boroń, A.; Wyrzykowska, K. Impact of Antibiotic Pollution on the Bacterial Population within Surface Water with Special Focus on Mountain Rivers. *Water* **2023**, *15*, 975. [CrossRef]
9. Stankiewicz, K.; Boroń, P.; Prajsnar, J.; Żelazny, M.; Heliasz, M.; Hunter, W.; Lenart-Boroń, A. Second Life of Water and Wastewater in the Context of Circular Economy—Do the Membrane Bioreactor Technology and Storage Reservoirs Make the Recycled Water Safe for Further Use? *Sci. Total Environ.* **2024**, *921*, 170995. [CrossRef]
10. Hijazin, M.; Hassan, A.A.; Alber, J.; Lämmler, C.; Timke, M.; Kostrzewa, M.; Prenger-Berninghoff, E.; Zschöck, M. Evaluation of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) for Species Identification of Bacteria of Genera Arcanobacterium and Trueperella. *Vet. Microbiol.* **2012**, *157*, 243–245. [CrossRef]
11. Bauer, A.W.; Kirby, W.M.; Sherris, J.C.; Turck, M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am. J. Clin. Pathol.* **1966**, *45*, 493–496. [CrossRef] [PubMed]
12. EUCAST European Committee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters. 2023, pp. 0–77. Available online: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf (accessed on 5 June 2023).
13. Magiorakos, A.-P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Harbarth, S.; Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance. *Clin. Microbiol. Infect.* **2012**, *18*, 268–281. [CrossRef] [PubMed]

14. Fiebelkorn, K.R.; Crawford, S.A.; McElmeel, M.L.; Jorgensen, J.H. Practical Disk Diffusion Method for Detection of Inducible Clindamycin Resistance in *Staphylococcus aureus* and Coagulase-Negative Staphylococci. *J. Clin. Microbiol.* **2003**, *41*, 4740–4744. [CrossRef] [PubMed]
15. EUCAST. *EUCAST_RefStaemme_Sollwerte*; European Committee On Antimicrobial Susceptibility Testing: Basel, Switzerland, 2024.
16. Geha, D.J.; Uhl, J.R.; Gustaferro, C.A.; Persing, D.H. Multiplex PCR for Identification of Methicillin-Resistant Staphylococci in the Clinical Laboratory. *J. Clin. Microbiol.* **1994**, *32*, 1768–1772. [CrossRef]
17. Lina, G.; Quaglia, A.; Reverdy, M.E.; Leclercq, R.; Vandenesch, F.; Etienne, J. Distribution of Genes Encoding Resistance to Macrolides, Lincosamides, and Streptogramins among Staphylococci. *Antimicrob. Agents Chemother.* **1999**, *43*, 1062–1066. [CrossRef]
18. Sutcliffe, J.; Grebe, T.; Tait-Kamradt, A.; Wondrack, L. Detection of Erythromycin-Resistant Determinants by PCR. *Antimicrob. Agents Chemother.* **1996**, *40*, 2562–2566. [CrossRef]
19. Lenart-Boroń, A.; Prajsnar, J.; Boroń, P. Survival and Antibiotic Resistance of Bacteria in Artificial Snow Produced from Contaminated Water. *Water Environ. Res.* **2017**, *89*, 2059–2069. [CrossRef]
20. Lenart-Boroń, A.; Boroń, P.; Kulik, K.; Prajsnar, J.; Żelazny, M.; Chmiel, M.J. Anthropogenic Pollution Gradient along a Mountain River Affects Bacterial Community Composition and Genera with Potential Pathogenic Species. *Sci. Rep.* **2022**, *12*, 18140. [CrossRef]
21. Williams, M.M.; Domingo, J.W.S.; Meckes, M.C.; Kelty, C.A.; Rochon, H.S. Phylogenetic Diversity of Drinking Water Bacteria in a Distribution System Simulator. *J. Appl. Microbiol.* **2004**, *96*, 954–964. [CrossRef]
22. Michels, R.; Last, K.; Becker, S.L.; Papan, C. Update on Coagulase-Negative Staphylococci—What the Clinician Should Know. *Microorganisms* **2021**, *9*, 830. [CrossRef]
23. Kosecka-Strojek, M.; Wolska-Gębarzewska, M.; Podbielska-Kubera, A.; Samet, A.; Krawczyk, B.; Międzobrodzki, J.; Michalik, M. May *Staphylococcus lugdunensis* Be an Etiological Factor of Chronic Maxillary Sinuses Infection? *Int. J. Mol. Sci.* **2022**, *23*, 6450. [CrossRef] [PubMed]
24. Ravaioli, S.; De Donno, A.; Bottau, G.; Campoccia, D.; Maso, A.; Dolzani, P.; Balaji, P.; Pegreffi, F.; Daglia, M.; Arciola, C.R. The Opportunistic Pathogen *Staphylococcus warneri*: Virulence and Antibiotic Resistance, Clinical Features, Association with Orthopedic Implants and Other Medical Devices, and a Glance at Industrial Applications. *Antibiotics* **2024**, *13*, 972. [CrossRef] [PubMed]
25. Lu, Y.; Lu, Q.; Cheng, Y.; Wen, G.; Luo, Q.; Shao, H.; Zhang, T. High Concentration of Coagulase-Negative Staphylococci Carriage among Bioaerosols of Henhouses in Central China. *BMC Microbiol.* **2020**, *20*, 21. [CrossRef] [PubMed]
26. Ruiz-Ripa, L.; Gómez, P.; Alonso, C.A.; Camacho, M.C.; Ramiro, Y.; de la Puente, J.; Fernández-Fernández, R.; Quevedo, M.Á.; Blanco, J.M.; Báguena, G.; et al. Frequency and Characterization of Antimicrobial Resistance and Virulence Genes of Coagulase-Negative Staphylococci from Wild Birds in Spain. Detection of Tst-Carrying *S. sciuri* Isolates. *Microorganisms* **2020**, *8*, 1317. [CrossRef]
27. Nahaei, M.R.; Shahmohammadi, M.R.; Ebrahimi, S.; Milani, M. Detection of Methicillin-Resistant Coagulase-Negative Staphylococci and Surveillance of Antibacterial Resistance in a Multi-Center Study from Iran. *Jundishapur J. Microbiol.* **2015**, *8*, e19945. [CrossRef]
28. Lenart-Boroń, A.; Wolny-Koładka, K.; Juraszek, K.; Kasprowicz, A. Phenotypic and Molecular Assessment of Antimicrobial Resistance Profile of Airborne *Staphylococcus* spp. Isolated from Flats in Kraków. *Aerobiologia* **2017**, *33*, 435–444. [CrossRef]
29. Nor Amdan, N.A.; Shahrulzamri, N.A.; Hashim, R.; Mohamad Jamil, N. Understanding the Evolution of Macrolides Resistance: A Mini Review. *J. Glob. Antimicrob. Resist.* **2024**, *38*, 368–375. [CrossRef]
30. Yeamans, S.; Gil-de-Miguel, Á.; Hernández-Barrera, V.; Carrasco-Garrido, P. Self-Medication among General Population in the European Union: Prevalence and Associated Factors. *Eur. J. Epidemiol.* **2024**, *39*, 977–990. [CrossRef]
31. OECD. *Fighting Antimicrobial Resistance in EU and EEA Countries*; OECD: Paris, France, 2023.
32. European Centre for Disease Prevention and Control. *Antimicrobial Consumption in the EU/EEA (ESAC-Net)—Annual Epidemiological Report for 2022*; European Centre for Disease Prevention and Control: Stockholm, Sweden, 2023; pp. 1–27.
33. ECDC. *Country Summaries—Antimicrobial Resistance in the EU/EEA 2022*; European Centre for Disease Prevention and Control: Stockholm, Sweden, 2023.
34. Stankiewicz, K.; Boroń, P.; Prajsnar, J.; Lenart-Boroń, A. Is our winter experience safe? Micropollutant risks for artificial snowing. *Sci. Tot. Environ.* **2024**; *submitted*.
35. Nava, A.R.; Daneshian, L.; Sarma, H. Antibiotic Resistant Genes in the Environment-Exploring Surveillance Methods and Sustainable Remediation Strategies of Antibiotics and ARGs. *Environ. Res.* **2022**, *215*, 114212. [CrossRef]
36. Baloh, P.; Els, N.; David, R.O.; Larose, C.; Whitmore, K.; Sattler, B.; Grothe, H. Assessment of Artificial and Natural Transport Mechanisms of Ice Nucleating Particles in an Alpine Ski Resort in Obergurgl, Austria. *Front. Microbiol.* **2019**, *10*, 2278. [CrossRef]