scientific reports

Check for updates

OPEN

Isolation and assessment of antibiotic resistance of *Staphylococcus aureus* in the air of an underground hard coal mines

Iwona Beata Paśmionka[1], Piotr Cheluszka[2]✉, Janina Gospodarek[1], Krzysztof Chmielowski[3] & Jiří Fries[4]

Mine aerosol poses a serious health threat due to its easy access to the human respiratory tract. Damage may be caused by the chemical composition of dust and the substances adsorbed on its surface, including microorganisms that potentially affect human health. Our proposed research aimed to isolate *Staphylococcus aureus* strains from coal mine bioaerosol and to assess its sensitivity towards selected antibiotics. Bioaerosol samples were collected in three underground hard coal mines located in Upper Silesia in southern Poland. Microbiological tests of the air samples were carried out according to standard microbiological techniques. All tested strains of *Staphylococcus aureus* were sensitive to oxacillin, which indicated the lack of methicillin-resistant isolates (MRSA) in the tested group. However, antibiotic resistance from macrolide and lincosamide groups was observed among certain strains. 10% of isolates were constitutive MLS_B resistance, while 4% of strains were inductive MLS_B resistance. Less than 1% of isolates were erythromycin-resistant and clindamycin-sensitive (MS_B). Based on the Chi-square test, statistically significant differences were found in the frequency of MS_B, MLS_B inductive, and MLS_B constitutive phenotypes. Almost 30% of the identified strains showed multi-antibiotic resistance. However, the Chi-square test did not reveal any statistically significant differences in the frequency of multidrug-resistant strains in the considered research areas. The analyses carried out constituted the first study related to the isolation and assessment of drug susceptibility of *Staphylococcus aureus* in the bioaerosol of hard coal mines. Identification of bioaerosol in underground coal mines is a key issue because, due to the presence of pathogens, it plays a significant role in limiting the spread of occupational diseases. For the health of miners, research into microbial communities benefits the promotion of microbiological control of mine air.

Keywords Bioaerosol, Staphylococci, Drug sensitivity, Mining excavations, Respirable dust

The advancement of civilization and the increase in industrialization resulted in a significant increase in interest in bioaerosols in workplaces. To date, airborne microorganisms in numerous industrial plants and municipal facilities, such as breeding plants, sewage treatment plants, and waste landfills, have been comprehensively characterized[1,2]. However, microorganisms in mine aerosols are not fully understood, even though hard coal mining is one of the most dangerous industries in the world[3].

In general, mine dust is emitted into the air during the coal mining process[4–6]. However, the impact of the physical and chemical properties of mine dust on the health of miners is lacking, and the fact that this type of dust contains a huge number of microorganisms is unexplored[7,8]. Currently, little research has been conducted on the microbiome of mine dust. Current literature mostly considers microbial diversity and functional microorganisms, such as methanogens and methanotrophs, which are related to carbon bioconversion and methane metabolism[3].

[1]Department of Microbiology and Biomonitoring, Faculty of Agriculture and Economics, University of Agriculture in Krakow, Mickiewicza 21 Av., 30-120 Kraków, Poland. [2]Department of Mining Mechanization and Robotisation, Faculty of Mining, Safety Engineering and Industrial Automation, Silesian University of Technology, Akademicka 2, 44-100 Gliwice, Poland. [3]Department of Natural Gas Engineering, Oil and Gas Faculty, AGH University of Science and Technology, Al. A. Mickiewicza 30, 30-059 Kraków, Poland. [4]Department of Machine and Industrial Design, Faculty of Mechanical Engineering, VSB-Technical University of Ostrava, 17. Listopadu 2172/15, Poruba, 708 00 Ostrava, Czech Republic. ✉email: piotr.cheluszka@polsl.pl

However, our research group has shown in a previous study that bioaerosols in underground coal mines contain many pathogens, including *Staphylococcus aureus*[9].

Staphylococci are an important indicator of air pollution[10,11]. Additionally, antibiotic-resistant strains that appear due to irrational use of antibiotic therapy are of particular importance[12,13]. The acquisition of drug resistance by microorganisms is related to genes circulating in the environment. Resistance determinants can be transferred not only to pathogenic bacteria but also to environmental bacteria[14]. One of the main mechanisms responsible for the spread of antibiotic-resistant genes in the environment is horizontal gene transfer[15,16]. Drug-resistant strains significantly impact public health, and their presence in the environment has been increasingly observed[17,18]. Therefore, regular microbiological control of air quality is vital, especially in confined environments, such as underground coal mines[19]. Research on the microbiome of mine dust will enhance our understanding and supplement information on health hazards in the miners' workplace.

Occupational diseases of miners are an ongoing subject of environmental health research[20–23]. Long-term stay in underground excavations of hard coal mines causes many occupational diseases, including those related to the respiratory system[24]. They are primarily responsible for abiotic factors, e.g., coal dust, the inhalation of which can cause many diseases[25–28]. However, biological threats, which are also important factors causing infections, should not be overlooked. Potential pathogens and their metabolism products may negatively impact human health[3]. The impact of bioaerosols on the functioning of the human body has been extensively studied, but, to the best of our knowledge, the resistance to chemotherapeutics of potential pathogens occurring in bioaerosols of hard coal mines, including *Staphylococcus aureus*, has yet to be properly addressed.

Staphylococcus aureus, due to its biological properties, is considered the most pathogenic species of staphylococci, capable of infecting any tissue and organ. In addition to the various virulence factors determining the pathogenicity of this microorganism, it has developed many mechanisms of resistance to antibacterial drugs, which has made it one of the most dangerous human pathogens[29]. *Staphylococcus aureus* is one of the most important etiological factors of skin and bone infections[30]. Its enormous pathogenic potential results directly from the wide spectrum of virulence factors it possesses. The pathogenicity of these bacteria is associated with their unique ability to adhere to specific structures in the human body and grow in privileged niches[31]. They cause such common diseases as impetigo, and folliculitis, but also less common diseases, e.g. staphylococcal scalded skin syndrome[32,33]. *Staphylococcus aureus* also produces cytolytic toxins and enzymes that facilitate the spread of this microorganism in the infected organism and cause tissue damage. A special group consists of toxins with the nature of so-called superantigens, including exfoliatin, also called epidermolytic toxins, which can cause toxic shock[34]. An important mechanism of pathogenicity of *Staphylococcus aureus* is also the ability to form a biofilm[30,35], i.e. a layer of bacterial cells covered with a substance that prevents antibiotics from accessing them. A growing problem is the resistance of *Staphylococcus aureus* to β-lactam antibiotics, spreading in various environments, not only hospitals[36]. Thus, the importance of choosing the right antibiotic therapy increases.

An increasingly frequently observed phenomenon that directly affects the quality of human life is the increase in microbiological contamination in indoor air. This fact is caused, among others, by inadequate ventilation of rooms in which people stay. Microorganisms suspended in the form of bioaerosol can play an important role in the spread of infections, allergic diseases, and antibiotic resistance genes in the environment and population[37]. In closed spaces, humans are the main reservoir of *Staphylococcus aureus*, causing the transmission of this pathogen into the air. One of the sources of bioaerosol is the human microbiome[38]. The air we breathe is a place where microorganisms temporarily stay. However, biological aerosol associated with the human body as a result of coughing or sneezing is transferred with the air current and may pose a health risk. Unfortunately, microorganisms, regardless of their source, are increasingly acquiring resistance to available antibiotics, becoming a potential cause of secondary infections[39,40]. In the literature, one can find publications presenting the results of research on public utility facilities such as hospitals, ambulances, pharmaceutical facilities, kindergartens, and schools[41–43]. However, little attention has been paid to underground architectural structures, which include deep mines. Because they are a place of constant movement of people, including those affected by infections of various origins, potentially pathogenic microorganisms can be transmitted into the air, e.g. *Staphylococcus aureus*. This situation subsequently leads to the spread of diseases that cause negative health effects. Moreover, in underground hard coal mines, with a high density of people, the number of microorganisms suspended in dust increases many times over.

Underground mines are a difficult area of research due to the conditions there. The global increase in hard coal mining, with a large share of underground mines, creates a need for constant monitoring of the mine atmosphere, including the microbiome. However, bioaerosols in underground hard coal mines are very poorly understood. Taking into account the above problems, our research aimed to isolate and determine the number of *Staphylococcus aureus* in the bioaerosol of active underground hard coal mines. Moreover, our research assesses the drug sensitivity of *Staphylococcus aureus* isolates to selected antibiotics with bactericidal or bacteriostatic properties. Our research is the first analysis of mine air of this type. Due to the similar specificity of underground mines, operating conditions, and structure of ventilation networks, it can be assumed that the share of *Staphylococcus aureus* in mine bioaerosols on a global scale is at a similar level. These bacteria are dangerous biological agents spreading through mine ventilation systems. The antibiograms we perform can be used to select appropriate chemotherapy that effectively combats potential infections of mine workers caused by *Staphylococcus aureus*, without the risk of spreading drug resistance. The presented conclusions also allow us to determine ways of organizing the work stations of underground mine workers, limiting their exposure to this harmful biological factor. Our research expands knowledge, among others, on the creation and spread of microbiological contamination in mine air. Due to the insufficient knowledge on drug-resistant *Staphylococcus aureus* in such a difficult environment as underground coal mines, our research significantly expands knowledge in this area. Our research is pioneering.

Material and methods

Research site

The presented research was conducted in active underground hard coal mines located in Upper Silesia in southern Poland. For this purpose, three ventilation areas with a mining wall were selected, where hard coal mining was actively carried out. In all studied areas, a suction ventilation system was used. The system consisted of fresh air being sucked in through the downcast shaft due to the depression created by the main ventilation fans installed at the outlet of the upcast shaft. For this work, the examined areas were marked A, B, and C, respectively.

Area A was separated from the structure of mine excavations, providing access to and preparing for exploitation of a coal seam at the mining level of − 900 m (Fig. 1a). It consisted of an excavations network with a total length of approx. 5.5 km[9]. Fresh air is sucked in through the downcast shaft and reaches the ventilation area through the main crosscut. The fresh air current (solid arrows in Fig. 1a) is supplied to the coal face, splitting along the way to other excavations. The main air current flows via the conveying roadway, through the belt dip into the headgate. The air current is divided at a certain distance from the inlet to the headgate so that a certain part of it (approx. 350 m^3 min^{-1}) flows through the crosscut to the tailgate, where it joins the air waste discharged from the coal face. However, the main part of the fresh air current (approx. 1200 m^3 min^{-1}) flows through the coal face and is directed to the headway above the tailgate. At a short distance from the outlet of the coal face, the exhaust air (dashed arrows in Fig. 1a) is mixed with fresh air pumped by a duct fan system from the ventilation dip, which is sucked in from the side of the conveying roadway. The exhaust air flows further along the ventilation dip and ventilation roadway to the main crosscut at the level of − 830 m. The exhaust air is discharged to the mine surface through a downcast shaft. To collect mine air samples, 11 measurement points were selected on the ventilation paths of the considered area. The location of these points was determined based on the analysis of the airflow in the examined ventilation area. Measuring points located in the direction of airflow were marked with circles in the spatial diagram of the ventilation network (Fig. 1a). The shaft foot of the downcast shaft was the reference point constituting the background (measurement point 0).

Area B included a group of excavations forming a ventilation network at the level of − 637 m with a length of nearly 3.0 km (Fig. 1b). Fresh air (solid arrows) supplied to the considered mining level downcast shaft is directed to the main crosscut, and further through the crosscut to the conveying crosscut. From the conveying crosscut, the fresh air flow is directed to the main coal roadway and flows to the maingate. The maingate supplies air to the area of the coal face and the coal roadway during drilling carried out for the newly prepared coal face. Because the coal roadway is located above the coal face, the fresh air supplied to the headgate is mixed with the exhaust air coming from the coal roadway. The exhaust airflow (dashed arrows) from the coal face flows through the tailgate and then through the main coal roadway, isolated from the fresh air flow by ventilation dams. Air exhausted from the main coal roadway via the crosscuts group (not described in Fig. 1b) is discharged to the isolated part of the main crosscut and is directed to the upcast shaft at the − 350 m level. Based on the analysis of the spatial diagram of the above-described ventilation network, the location of 12 measurement points was determined, starting from the shaft foot of the downcast shaft (measurement point 0, constituting the background of the measurements), through the main crosscut (measurement point 1), conveying crosscut (measurement point 2), maingate (measurement points 3 and 6), the coal face (measurement points from 7 to 9), and ending with the outlet from the ventilation area in the main coal roadway, where exhaust air is discharged (measurement point 10). The location of the measurement points was to ensure the possibility of collecting samples in the current of fresh air, exhaust air, and in the area of mining works (coal face and the heading face of the coal roadway being excavation). Measurements were carried out in transport and ventilation excavations. They included the area of coal seam mining within the intersection at the coal face entry (measurement point 7) and the coal face exit (measurement point 9), as well as in the coal face, in the vicinity of the longwall shearer's workplace approx. 30 m from the intersection of the coal face (measurement point 8). At this point, air samples were taken while mining the coal face with a longwall shearer towards the headgate. Due to the fault in the coal seam, the longwall shearer mined gangue, which was a source of high dust. Measurement points 4 and 5 were located at the face of the coal roadway that was being excavated with a roadheader (drilled with a boom-type roadheader). Measurement point 4 was at the level of the shearer's position, and measurement point 5 was at the outlet of the suction duct dust collector. This tract combined the ventilation, consisting of a pressure duct sucking air from the maingate, and a suction duct equipped with a wet dust collector. The outlet from this dust collector is located a short distance behind the roadheader and transfers the dust-cleaned air to the exhaust air stream. Due to the reconstruction of the ventilation installation (extension of the pressure duct) during our research, the outlet from the dust collector was unfortunately located just before the outlet from the pressure duct. This resulted in significant disturbances in air circulation occurring at measurement point 5. While taking air samples and measuring dustiness at these points, the roadheader only mined coal. Measurement point 11 was located between two dams on the main crosscut, forming a lock isolating the fresh air flow from the exhaust airflow. Fresh air flows through this lock from the main coal roadway to a part of the excavation, where the exhaust air is discharged to the upcast shaft (in the amount of approximately 500 m^3 min^{-1}).

Area C, where mine air samples were taken, is located at the level of − 726 m, quite close to the downcast shaft (Fig. 1c). The total length of the excavations in which the mine air quality was tested was approx. 3.5 km (measured from the bottom of the downcast shaft, where measurement point 0 was located, constituting the background for the measurements). Fresh air (solid arrows) supplied to the level of − 726 m through the downcast shaft is directed to the main crosscut, where the tested ventilation area is supplied. The beginning of the considered ventilation network is located within the intersection of the main crosscut and main coal roadway (measurement point 1). A current of fresh air that flows by this route to the junction with the maingate is directed to the headgate. Fresh air is supplied from the headgate to the coal face. Due to the climatic hazard occurring in the considered ventilation area (caused by the high temperature in the mine excavations), the air

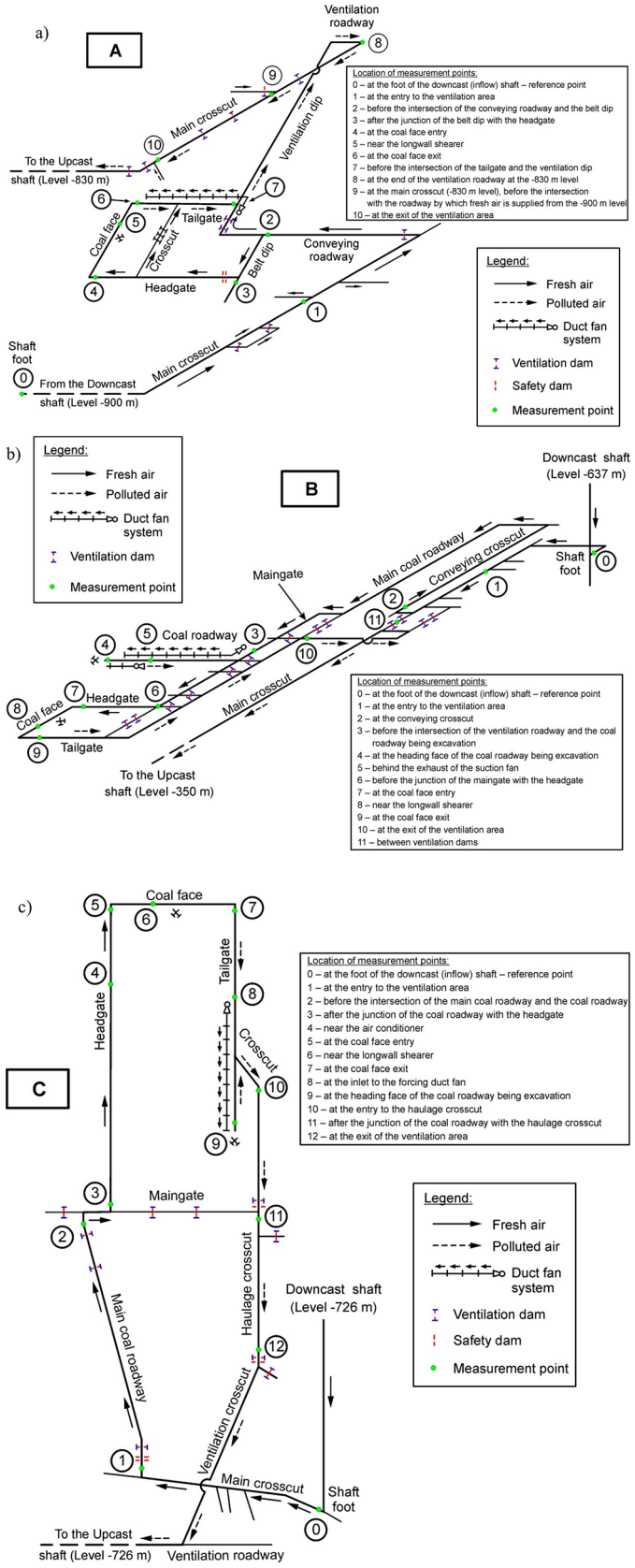

Fig. 1. Spatial diagram of the excavations networks in the studied ventilation areas of a hard coal mine with marked measurement points: (**a**) area A[9], (**b**) area B, (**c**) area C.

in front of the coal face is cooled using a local air conditioner located approx. 50 m before the intersection of the headgate with the coal face. The exhaust airflow (dashed arrows) is directed from the coal face to the tailgate and flows through the crosscut to the haulage crosscut. The exhaust air flows further through the ventilation crosscut to the ventilation roadway, which is discharged to the upcast shaft. In the area of the intersection of the tailgate and the crosscut, during the measurements, mining works were carried out to extend the tailgate. This excavation was drilled in old workings created after previous coal seam exploitation, filled with power plant dust. The drilling was carried out using a roadheader. Air was supplied to the face of this working from the tailgate using a duct fan equipped with an air cooler (air conditioner) to reduce its temperature. The exhaust air returned through the same excavation to the crosscut and was combined with the mainstream of exhaust air from the coal face. Based on the analysis of airflow in the considered ventilation area (area C), 13 measurement points were selected where the mine air parameters were measured, and air samples were taken for microbiological tests. In addition to the previously mentioned measurement points (0 – shaft foot, constituting the background of the measurements, and 1, the beginning of the tested ventilation area, located behind the intersection of the main crosscut with the main coal roadway), further measurement points were located following the direction of the mine airflow (Fig. 1c). Point 2 was on the main coal roadway before the junction with the maingate. Points between 3 and 8 were located within the coal face and associated excavations (headgate and tailgate). Point 3 was located at the entrance to the headgate, point 4 near the air conditioner, and point 5 at the intersection with the coal face. Measuring point 6 was located near the workplace of the longwall shearer, approx. 200 m from the intersection with the headgate. During the measurements, normal coal mining was carried out using a longwall shearer. Measuring points from 7 to 12 were located in the exhaust airflow. Point 7 was located just behind the intersection of the coal face with the tailgate, and point 8 was located in front of the duct fan, forcing air to the heading face excavated with a roadheader. Measurement points from 10 to 12 were located in the haulage crosscut, i.e., on the way of hauling spoil from the coal face and from the heading face where the roadheader was working. Point 12 was located at the outlet of the examined ventilation area. Additionally, measurements were also carried out at the extended tailgate, i.e., at the working site of the roadheader (point 9). Unlike area B, where a roadheader mined coal, area C reconsolidated backfill material (power plant dust) was mined, which was used to fill the post-mining void during previous mining. The physicochemical properties of the backfill material differ from those of the rocks. Moreover, the area around the face of the excavation being drilled was characterized by high waterlogging. These factors may influence the obtained results.

At individual measurement points in each location (areas A, B, and C), the values of air parameters characterizing its quality were measured, and air samples were taken for microbiological analyses.

Measurements of air parameters in hard coal mines

To determine the correlation between the concentration of *Staphylococcus aureus* in the tested bioaerosol and the mine air parameters, the following analyses were conducted at the measurement points:

- Wet and dry bulb temperature (T_W and T_D) using an Assmann PM-821L aspiration psychrometer (Merazet, Poland),
- Relative humidity (φ) using a hand-held BAR-TH barometer (JMD, Poland),
- Airflow velocity (v) using a 1468 vane anemometer (Lambrecht Meteo, Germany),
- Concentration of the respirable dust fraction (in the grain size range from 1 to 8 µm—Johannesburg curve) contained in the mine air (C), using a TM Data dust meter (Hund, Germany). During the measurements at all research points, the concentration courses of the respirable fraction of dust contained in the air current flowing through a given excavation were recorded in the device's memory (with a frequency of 1 Hz). For the adopted recording time interval ($\Delta t = 10$ min.), the average value of the concentration of the respirable dust fraction was read[9].

Additionally, the cross-sectional area of the excavation (S) was determined at the measurement points, and on its basis, the airflow rate (Q) was determined in connection with the airflow speed (v).

Air sampling

Mine air samples were collected using the aspiration method with a MAS-100 ECO air sampler (Merck, Germany). MAS-100 ECO is designed to monitor the concentration of microorganisms in the air and is adapted to work with standard Petri dishes with a diameter of 90 mm. The automation of the device allows for the collection of air samples in the range of 1–1000 dm^3. The sampler head has 400 holes, which increases the efficiency of collecting microorganisms from the air. Chapman's medium (BLT, Poland) was used to isolate staphylococci. This medium is dedicated to the isolation and differentiation of staphylococci. Strains that degrade mannitol produce yellow colonies, and the color of the medium also changes from pink to yellow (phenol red is the indicator). Strains that cannot degrade mannitol do not change the color of the colonies. According to the procedure, a precisely defined volume of air (V) was sucked into the Petri dish through the head of the apparatus, adjusted to the location of the measurement point, the size of the cross-section of the excavation (S), and the airflow speed (v). Samples were taken three times at a height of approx. 1.5 m from the floor, i.e., in the human respiratory zone. The collected bioaerosol samples were incubated at 37 °C for 48 h, then the grown staphylococci colonies were converted into colony-forming units in 1 m^3 of tested air using the procedure provided by the sampler manufacturer[9]. The cultured staphylococci colonies were counted twice after 24 and 48 h of incubation using an eCount Colony Counter (Heathrow Scientific, USA). To test the statistical significance of differences in the number of *Staphylococcus aureus* strains to all bacteria in the measurement points of individual areas, mesophilic bacteria were isolated in parallel from the mine air on TSA (Trypticase-Soy Agar, bioMerieux, France) using the same

procedure. For statistical purposes, the isolated colonies of bacteria were only counted, without being converted into colony-forming units in 1 m^3 of tested air (Table 3S, supplementary materials).

Identification of *Staphylococcus aureus*

To identify *Staphylococcus aureus*, staphylococci colonies that were isolated from the mine's bioaerosol were transferred to the chromogenic medium chromID™ *Staphylococcus aureus* Elite (BioMérieux, France). This was intended for the selective isolation and direct identification of *Staphylococcus aureus*. The cultures were incubated at 37 °C for 48 h. Bacterial colonies that, according to the specifications developed by the culture medium manufacturer, met the criteria for *Staphylococcus aureus* (pink color, smooth, shiny, and convex surface) were selected for further analysis to confirm the species, and then their resistance to selected antibiotics was determined. The species was identified based on the morphological features described above, where potential *Staphylococcus aureus* isolates were selected, and a biochemical test was performed for each sample to check their ability to produce coagulase (Pastorex™ Staph-Plus, BIO-RAD, France). Isolates of coagulase-negative strains were eliminated from further analysis, while coagulase-positive strains were identified using the MALDI-TOF MS technique (Bruker Daltonics, Germany). This method uses a technique to generate protein spectra profiles for bacterial proteins. The obtained spectra were characteristic of a given species, and through comparison with the spectra collected in the database (MALDI-Biotyper 3.0, Bruker Daltonics, Germany), species affiliation was confirmed. Isolates were prepared by protein extraction with ethanol and formic acid[9]. According to the manufacturer, the following identification scores were used: < 1.7 – no reliable identification,1.7–1.999 – probable identification to the genus level; 2.0–2.299 – safe identification of the genus and probable identification of the species; 2.3–3.0 – highly probable species identification. Only strains with highly probable identification to the species *Staphylococcus aureus* were selected for further research.

Testing the sensitivity of *Staphylococcus aureus* to selected antibiotics

After the identification of the species of the collected isolates, their sensitivity to antibiotics and the occurrence of various resistance mechanisms were tested. The sensitivity of *Staphylococcus aureus* to selected antibiotics was determined by the disk diffusion method. The tests were performed under EUCAST recommendations[44]. For this purpose, the tested isolates were suspended in sterile physiological saline, obtaining a suspension with a density of 0.5 on the McFarland scale. The inoculum density was verified using a Densimat densitometer (BioMérieux, France). Then, the suspension was evenly spread on Mueller–Hinton II medium (BTL, Poland) using a sterile swab. Paper discs soaked in antibiotics were placed on the plates following the principles of microbiological purity, within 15 min of inoculation. The cultures prepared in this manner were incubated at 35 °C for 18–20 h. After incubation, the growth inhibition diameters around individual discs were measured, and the results were compared to the breakpoint values provided by EUCAST. According to these guidelines, the tested strains were classified as resistant or susceptible. The standard strain *Staphylococcus aureus* ATCC 29,213 was used as a quality control. The susceptibility of *Staphylococcus aureus* to antibiotics was tested using: oxacillin (OX, 6 µg per disc), ciprofloxacin (CIP, 5 µg per disc), tetracycline (TE, 30 µg per disc), gentamicin (GM, 10 µg per disc), clindamycin (CC, 2 µg per disc), erythromycin (E, 15 µg per disc) and penicillin (*P*, 10 units = 6 µg per disc). The study used 6.5 mm diameter discs (Bio-Rad, France). These are discs made of high-quality filter paper impregnated with antibiotic solutions of precisely defined concentrations. The discs are clearly marked on each side with a letter symbol, followed by the concentration of the antibiotic being tested in each disc, as presented above.

Statistical analysis

Statistical processing was performed in Statistica 13.3 (StatSoft, Inc., Tulsa, OK, USA). First, a correlation was sought between the number of *Staphylococcus aureus* strains isolated from the mine's air at individual measurement points and the parameters of the mine ventilation networks in the considered areas (Table 1). The analysis included 12 measurement points where *Staphylococcus aureus* was identified. To increase the size of the examined statistical sample, observations obtained in subsequent repetitions of air sampling at individual measurement points were considered as separate data sets. To determine the significance of the relationship between the studied features, non-parametric tests were used: Spearman's rank correlation, τ (Kendall) and γ (Goodman and Kruskal). These are non-parametric tests used in the case of outlier observations, non-linear dependencies in the data set, and situations where the assumption of normality of the distribution of the analyzed data may not be met. Additionally, the γ statistic is applicable when the data contain many related observations representing the same variant of a trait[45]. For statistically significant correlations between the amounts of the considered microorganisms and the parameters of the mine's ventilation networks, the predictive capabilities of simple linear regression models (their statistical significance) were examined.

Based on the test for two structure indicators, the statistical significance of differences in the number of *Staphylococcus aureus* strains in the corresponding measurement points of the ventilation network of individual areas was also examined. Due to the differences in these networks, it was possible to select four pairs of corresponding measurement points in which *Staphylococcus aureus* strains were isolated. The Z test statistic was as follows:

$$Z = \frac{\frac{m_1}{n_1} - \frac{m_2}{n_2}}{\sqrt{\frac{\overline{p}(1-\overline{p})}{n}}} \quad (1)$$

wherein:

No. of the measurement point	V [dm^3]	D [m]	T_D [°C]	T_W [°C]	φ [%]	S [m^2]	Q [$m^3 \cdot min^{-1}$]	v [$m \cdot s^{-1}$]	C [$mg \cdot m^{-3}$]	Staphylococci [$CFU \cdot m^{-3}$]	*Staphylococcus aureus* [$CFU \cdot m^{-3}$]
Area A											
0	100	0	14.0	9.0	51	21.0	1700	1.35	0.71	0	0
1	100	1770	15.4	13.2	79	13.2	3120	3.94	1.57	0	0
2	50	2070	19.6	17.2	79	15.0	2200	2.44	1.72	2840	0
3	20	2220	25.8	24.0	86	13.6	960	1.18	2.25	3350	0
4	20	2841	25.0	22.8	83	12.8	1200	1.56	4.23	100	0
5	20	2991	28.2	26.8	90	12.4	1200	1.61	4.49	7400	0
6	20	3091	32.0	31.4	96	12.0	1500	2.08	10.02	5150	5100
7	50	3780	29.2	27.6	88	17.8	2200	2.06	8.09	100	80
8	50	4030	28.4	26.8	89	18.0	2200	2.04	5.25	20	0
9	50	4250	27.6	25.8	86	13.0	2520	3.23	3.42	4420	0
10	50	5450	27.4	26.0	90	14.0	2520	3.00	2.73	38	0
Area B											
0	20	20	19.6	18.2	88	7.6	330	0.72	0.26	0	0
1	20	220	19.8	18.4	88	15.0	1920	2.14	0.27	0	0
2	20	420	19.8	18.8	91	10.3	990	1.60	0.34	600	0
3	20	1730	23.8	21.2	80	17.5	1150	1.10	0.73	150	0
4	20	2594	26.4	23.2	76	16.4	520	0.53	4.39	1300	0
5	20	2574	26.4	23.4	78	16.4	570	0.58	7.6	7350	0
6	20	2060	25.8	23.2	80	18.2	980	0.90	0.8	1400	650
7	20	2115	26.0	23.6	82	16.1	600	0.62	1.28	4250	1150
8	20	2235	26.2	24.6	88	10.0	600	1.00	66.11	7900	1600
9	20	2325	25.8	25.0	94	15.0	600	0.67	28.36	4450	350
10	20	2880	23.8	22.2	87	18.6	1690	1.52	1.71	450	0
11	20	380	20.2	18.8	88	15.3	530	0.58	0.35	1000	0
Area C											
0	20	0	17.80	15.20	76	14.50	331	0.38	0.60	0	0
1	20	315	19.20	16.20	74	15.00	1521	1.69	0.62	2050	400
2	20	745	20.00	16.20	68	14.50	1244	1.43	0.72	350	0
3	20	865	24.80	21.00	71	14.00	546	0.65	1.37	800	150
4	20	1590	25.40	24.00	89	10.50	548	0.87	0.88	3250	300
5	20	1640	25.80	24.80	92	9.00	545	1.01	4.50	1750	650
6	20	1725	28.80	28.00	94	9.00	545	1.01	11.90	1950	1100
7	20	1875	30.40	29.60	94	11.00	548	0.83	6.26	400	0
8	20	2175	30.80	30.00	94	11.00	548	0.83	2.17	900	0
9	20	2675	26.80	24.80	85	13.00	351	0.45	11.12	2450	750
10	20	2265	27.20	26.20	92	11.00	548	0.83	1.46	0	0
11	20	2715	29.00	28.00	93	11.00	733	1.11	1.22	0	0
12	20	3005	29.40	28.60	94	15.00	864	0.96	1.60	0	0

Table 1. Air parameters at individual measurement points and concentrations of staphylococci, including *Staphylococcus aureus,* in designated areas (A, B, and C). V, the volume of air taken by the aeroscope; D, distance of the measurement point from the foot of the downcast shaft (point 0); T_D, temperature measured with a dry bulb thermometer; T_W, temperature measured with a wet bulb thermometer; φ, relative humidity; S, cross-sectional area of the air current; Q, airflow rate; v, airflow speed; C, concentration of respirable dust; CFU, colony-forming units.

$$\overline{p} = \frac{m_1 + m_2}{n_1 + n_2} \tag{2}$$

$$n = \frac{n_1 \cdot n_2}{n_1 + n_2} \tag{3}$$

The quantities involved in the definition of the Z statistic will be discussed in Chapter 3.1.

In the second stage of the study, extensive statistical studies were carried out to determine the significance of the impact of the antibiotics used on the resistance of *Staphylococcus aureus* strains. The following statistical tests were used in these studies:

- Non-parametric Kruskal–Wallis test – to test the statistical significance of the impact of the antibiotics used on the number of resistant strains,
- Multiple comparison mean rank test – to check the differences in the effects of individual antibiotics,
- Chi-square test – to check the statistical significance of the resistance of *Staphylococcus aureus* strains to individual antibiotics and their multi-resistance in individual areas,
- Non-parametric Spearman, Kendall, and Goodman–Kruskal rank correlation tests – to find statistical relationships between the resistance of *Staphylococcus aureus* strains to individual antibiotics and their multi-resistance with mine air parameters,
- Chi-square test – to determine the statistical significance of differences in the frequency of MS_B, MLS_B inductive, and MLS_B constitutive phenotypes in the studied population of *Staphylococcus aureus* strains.

Results

Development of basic air parameters in selected measurement points of individual areas (A, B, and C) and the concentration of isolated staphylococci, including *Staphylococcus aureus*

The characteristics of the tested air at individual measurement points located in three areas (A, B, and C) are summarized in Table 1.

According to the obtained results, the amount of staphylococci in the mine bioaerosol varied and ranged from 0 to 7900 CFU·m^{-3}. The concentration of these microorganisms depended on both the examined area and the location of the measurement point within the area. The highest concentrations of staphylococci (above 7000 CFU·m^{-3}) were observed in area A (Fig. 1a), near the longwall shearer (measurement point 5), and in area B (Fig. 1b), behind the exhaust of the dust collector of the suction fan (measurement point 5) and near the longwall shearer (measurement point 8).

In selected areas, 233 strains of *Staphylococcus aureus* were isolated from among all staphylococci. In area A (Fig. 1a), these strains were found in two out of eleven measurement points (point 6: 90 strains and point 7: 4 strains), in area B (Fig. 1b) in four out of twelve measurement points (point 6: 13 strains, point 7: 22 strains, point 8: 31 strains and point 9: 7 strains), and in area C (Fig. 1c) in six of the thirteen selected measurement points (point 1: 8 strains, point 3: 3 strains, point 4: 6 strains, point 5: 13 strains, point 6: 21 strains and point 9: 15 strains). The largest number of strains identified as *Staphylococcus aureus* occurred at the outlet of the coal face (point 6) in area A and the vicinity of the longwall shearer operation in areas B (point 8) and C (point 6) (Table 1).

Examination of the correlation of the number of *Staphylococcus aureus* strains within the mine air parameters and the route of its flow revealed their statistical significance (Table 1S, supplementary materials). Statistically significant values of correlation coefficients were $\alpha = 0.05$ (marked in red). All three tests confirmed a significant statistical dependence of the number of *Staphylococcus aureus* strains on the length of the airflow path (D) through the mine excavations (the distance of measurement points from the foot of the downcast shaft, through which fresh air is introduced into the mine underground), its temperature (T_D and T_W) and relative humidity (φ). The relationship between the number of *Staphylococcus aureus* strains and the concentration of respirable dust was statistically significant (C). Additionally, the Spearman test showed a statistically significant relationship between the number of bacteria and the cross-sectional area of the air current (S) flowing through the network of mine excavations. However, for the remaining two statistical tests, no such relationship was found because the test probability was higher than the assumed significance level ($p > \alpha$). Therefore, in these cases, there were no grounds to reject the null hypothesis that there was no correlation between the considered variables.

The correlation between the number of *Staphylococcus aureus* strains and the distance from the downcast shaft and the mine air parameters (temperature and relative humidity) was positive. Statistically speaking, the number of the detected microorganisms increased as the distance from the downcast shaft increased and as the temperature and humidity of the mine air increased. This was justified because the further from the downcast shaft, the worse the quality of the mine air (its physical and chemical parameters deteriorated), which certainly favored the development of microorganisms, leading to a deterioration of the microbiological quality of the mine atmosphere. The observed increase in air dustiness, especially in the proximity of cutting machines, favored this scenario (the process of mechanical cutting of coal and surrounding rocks was a source of high dustiness). Also, there was a positive correlation between the number of *Staphylococcus aureus* strains and the concentration of respirable dust. The largest amounts of these bacteria and dust were recorded at measurement points 6 (area A), 8 (area B), and 6 and 9 (area C). All these points were located in the coal faces (or directly next to them) and in the roadway being excavation with a road header.

The values of the correlation coefficients obtained by the three methods considered are varied and ranged from approx. 0.3 to nearly 0.6 (Table 1S, supplementary materials). The highest coefficient values were recorded for the relationship between the number of tested bacteria and the temperature measured with a dry thermometer (T_D) (strong correlation). In the case of other parameters characterizing the atmospheric conditions prevailing in the underground hard coal mines, the correlation was rather moderate. The values of correlation coefficients ranged from ~ 0.3 to ~ 0.4.

The statistically significant relationships between the number of *Staphylococcus aureus* strains and mine air parameters were approximated and represented by straight lines to illustrate their nature (Fig. 2). The exception was the temperature dependence. The confidence interval $P = 0.95$ was marked with dashed lines. Due to the relatively small number of data points obtained for these analyses, the raw data were not cleaned to remove outliers. The points are shown on the charts. To determine the usefulness of the regression models based on the lines described by the equations in Fig. 2, the statistical significance of the regression coefficients was tested (whether they differ significantly from zero). The analytical results are summarized in Table 2S (supplementary materials), with statistically significant coefficients highlighted in red.

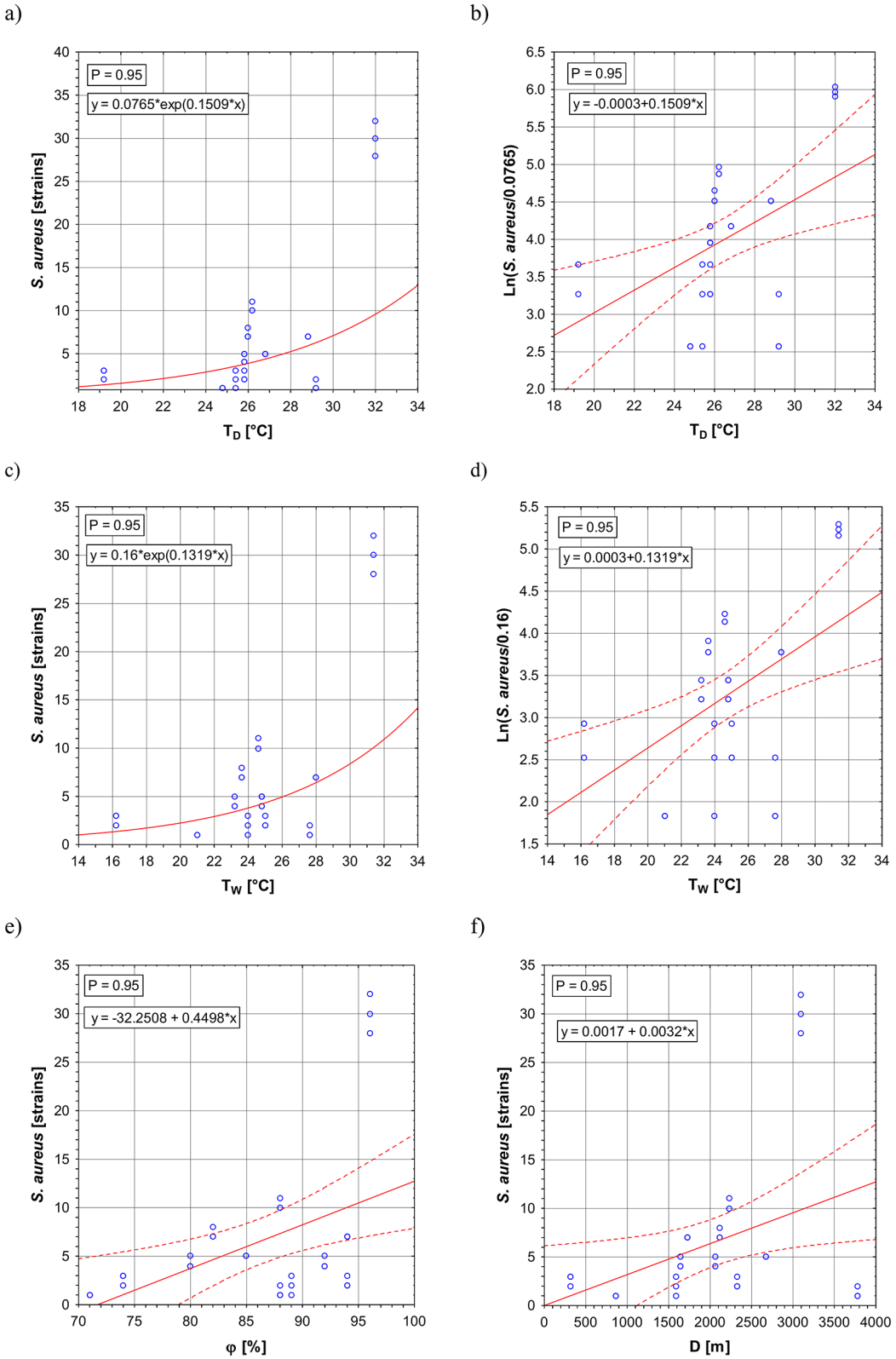

Fig. 2. Scatterplots and regression line equations of the number of *Staphylococcus aureus* strains as a function of mine air parameters: (**a**) temperature measured with a dry bulb thermometer, (**b**) temperature measured with a dry bulb thermometer – after conversion to a linear model, (**c**) temperature measured with a wet bulb thermometer, (**d**) temperature measured with a wet bulb thermometer – after conversion to a linear model, (**e**) relative humidity, (**f**) distance from the downcast shaft, (**g**) respirable dust concentration – raw data, (**h**) respirable dust concentration – outlier observations removed.

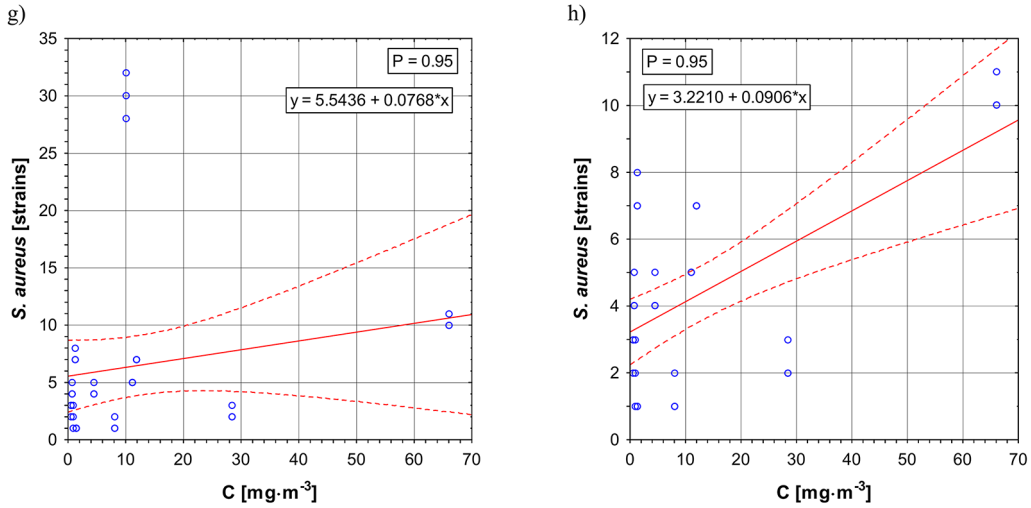

Figure 2. (continued)

In the case of all air parameters except temperature, the tested relationships were well described by linear regression models (Fig. 2e–h). The dependence of the number of *Staphylococcus aureus* strains on the temperature measured with a dry and wet bulb thermometer was non-linear (Fig. 2a,c). To determine the statistical significance of the regression coefficients, the exponential equations were transformed into a linear form by taking their logarithm. The result of this operation and the equations of the regression lines are shown in Fig. 4b for the dependence on the temperature measured with a dry bulb thermometer and in Fig. 4d for the dependence on the temperature measured with a wet bulb thermometer.

The results revealed that only for relative humidity (φ), both the intercept (b_0) and the slope coefficient (b_1) differed significantly from zero. The values of the test probability *p* were lower than the assumed significance level $\alpha = 0.05$ (rejection of the null hypothesis: H_0: $\beta_i = 0$ in favor of the alternative hypothesis: H_1: $\beta_i \neq 0$). The error in estimating the values of parameters ε was approx. 1/3 of their values. The situation was different for the regression model that described the dependence of the number of *Staphylococcus aureus* strains on the temperature measured with a dry bulb thermometer (T_D), the temperature measured with a wet bulb thermometer (T_W), the length of the airflow path (distance from the downcast shaft D), and the concentration of respirable dust (C). In the case of the influence of distance from the downcast shaft (D), the statistical significance of the intercept term (b_0) was lacking due to the value of the test statistic $t << t_{crit}$ and the test probability $p = 0.999$ being higher than the assumed significance level $\alpha = 0.05$ (there were no grounds for rejection of the null hypothesis: H_0: $\beta_0 = 0$). This effect is shown in Fig. 2f, where the regression line passes through the point (0.0) of the graph. The values of the intercept (b_0) in the linear regression equations for temperature were also statistically insignificant (logarithmized models) – Fig. 2b,d. However, such significance was demonstrated by the slope coefficients of these equations (b_1), which proved the significance of the parameters of the non-linear regression models used (exponential) to describe the examined dependencies.

In the case of the dependence of the number of *Staphylococcus aureus* strains on the concentration of respirable dust C (Fig. 2g), the slope coefficient (b_1) differed insignificantly from zero. The value of the test statistic $t = 1.0771$ was below the critical value $t_{crit} = 2$, and the test probability $p = 0.289$ was greater than the assumed significance level $\alpha = 0.05$. Therefore, although previously performed tests indicated the statistical significance of the relationship between the number of *Staphylococcus aureus* strains and the amount of air dust, the linear regression model may not explain this relationship. It was uncertain that the concentration of dust in the air (predictor) may not impact the number of *Staphylococcus aureus* strains (dependent variable). However, removing outlier observations changed this situation (Fig. 2h). It was found that both regression parameters: intercept (b_0) and slope coefficient (b_1), differed significantly from zero. The test probability *p* was lower than the assumed significance level α (Table 2S – line marked with the number 2 in the superscript).

Individual analyzed areas were characterized by a different specificity of the ventilation network resulting from the arrangement of the mine's excavation, through which mine air was supplied to the coal faces and roadway during drilling. Hence, the question arises, are the obtained numbers of *Staphylococcus aureus* strains compared to the total number of all isolated bacteria and, therefore, the share of their unit concentration comparable? To examine the statistical significance of differences in the number of *Staphylococcus aureus* strains that were identified in the corresponding measurement points of individual areas, a test was performed for two structure indicators. Despite differences in the structure of the ventilation network of individual areas, common points were identified, resulting from the method of ventilating longwall workings. These points were arranged as follows:

- Inlet to the coal face ventilation area,
- The intersection of the headgate and the coal face,
- Coal face (longwall working),

- The intersection of the coal face with the tailgate,
- The outlet from the coal face ventilation area,
- Heading face of excavation during drilling.

Since *Staphylococcus aureus* strains were isolated from the air collected only in some measurement points of individual areas (Table 1), for this analysis, data from four pairs of measurement points located in areas A, B, and C were considered (Table 3S, supplementary materials). These points were located as follows:

- The inlet to the coal face ventilation area in areas B and C (line no. 1, Table 3S, supplementary materials),
- The intersection of the headgate with the coal face in areas B and C (line no. 2, Table 3S, supplementary materials),
- Coal face in areas B and C (line no. 3, Table 3S, supplementary materials),
- The intersection of the coal face with the tailgate in areas A and B (line no. 4, Table 3S, supplementary materials).

The tested null hypothesis was that the number of identified *Staphylococcus aureus* strains was equal to the total number of bacteria isolated from the mine air at the corresponding measurement points in individual areas (H_0: $p_1 = p_2$).

The values of the Z statistic, described by formulas (1)–(3), the quantities included in its definition, and the test probability *p* are listed in Table 3S (supplementary materials). It was found that only two of the four cases examined had no grounds to reject the null hypothesis ($|Z| < Z_\alpha$). Cases for which the test probability *p* was greater than the adopted significance level $\alpha = 0.05$ were marked in red. Therefore, it was not possible to state statistically significant differences in the share of *Staphylococcus aureus* strains in the total number of bacteria isolated in the corresponding measurement points of areas B and C located at the inlet to the coal face ventilation area and in the coal face. The shares of *Staphylococcus aureus* strains in the total number of bacteria in the first-mentioned places of the ventilation network were: 2.9% (area B) and 1.1% (area C), respectively. Area B had almost three times more bacteria than area C. In turn, at the coal face, these shares were at a similar level (2.7% – area B and 3.4% – area C). Therefore, it was assumed that under similar operating conditions and a similar structure of the ventilation network used to ventilate coal faces, the share of *Staphylococcus aureus* strains in the total number of bacteria isolated from the air was at a similar level.

Unfortunately, the analyzed measurement data did not allow for the determination of a statistically significant similarity in the share of *Staphylococcus aureus* strains in the total number of isolated bacteria in the area of the intersection of the coal face with the headgate and the coal face with the tailgate in the considered areas. Possible confirmation of the statistical significance of the share of *Staphylococcus aureus* strains in the total number of bacteria isolated from mine air in the above-mentioned places requires further research.

Assessment of antibiotic resistance of *Staphylococcus aureus*

211 highly probable strains identified as a species of *Staphylococcus aureus* were tested for sensitivity to selected antibiotics. 22 strains were rejected due to lack of reliable identification. The tested strains were grouped into two categories: sensitive or resistant to a given antibiotic. Categorization was based on the inhibition zone limits established by EUCAST[44]. The obtained results are presented in Table 2.

Oxacillin was the most effective antibiotic for all 211 tested strains. Also, ciprofloxacin was a very active antibiotic, to which only 7% of the tested strains showed resistance. 12% of *Staphylococcus aureus* strains were resistant to tetracycline, whereas 15% were resistant to gentamicin. For clindamycin, 25% of *Staphylococcus aureus* strains were resistant. 31% of the strains were resistant to erythromycin. However, penicillin was the least effective, where 82% showed resistance and only 18% were sensitive (Table 2). This indicates the presence of strains resistant to β-lactam antibiotics in the tested pool. The ratio of resistant to susceptible strains among *Staphylococcus aureus* isolates, to the tested antibiotics, is illustrated in Fig. 3.

The sensitivity of *Staphylococcus aureus* isolates to selected antibiotics in individual areas is presented in Table 3.

The largest number of ciprofloxacin-resistant strains was found in area B (Fig. 1b), at measurement point 7. At the intersection of the coal face entry and the headgate, the percentage of strains resistant to this antibiotic was 30%. The highest concentration of tetracycline-resistant strains was also found in area B. In the vicinity of the longwall shearer's workplace resistant strains accounted for 27% of the isolates (measurement point 8). However, the highest resistance to gentamicin was observed in area A (Fig. 1a), at the outlet of the coal face. At measurement point 6, the percentage of isolates resistant to this antibiotic was 13%. The highest resistance to clindamycin was detected in areas A and C (Fig. 1a,c). In measurement point 5 (area C, at the coal face entry), the percentage of resistant strains was 77%, while in area A, at the coal face exit – 12% (measurement point 6). Erythromycin-resistant strains were mostly isolated in area B, near the operating longwall shearer (measuring

***Staphylococcus aureus* strains**	**OX**	**CIP**	**TE**	**GM**	**CC**	**E**	**P**
Sensitive	211	197	186	179	159	145	37
Resistant	0	14	25	32	52	66	174
Sum	211	211	211	211	211	211	211

Table 2. Sensitivity of *Staphylococcus aureus* strains to selected antibiotics. OX, oxacillin; CIP, ciprofloxacin; TE, tetracycline; GM, gentamicin; CC, clindamycin; E, erythromycin; P, penicillin.

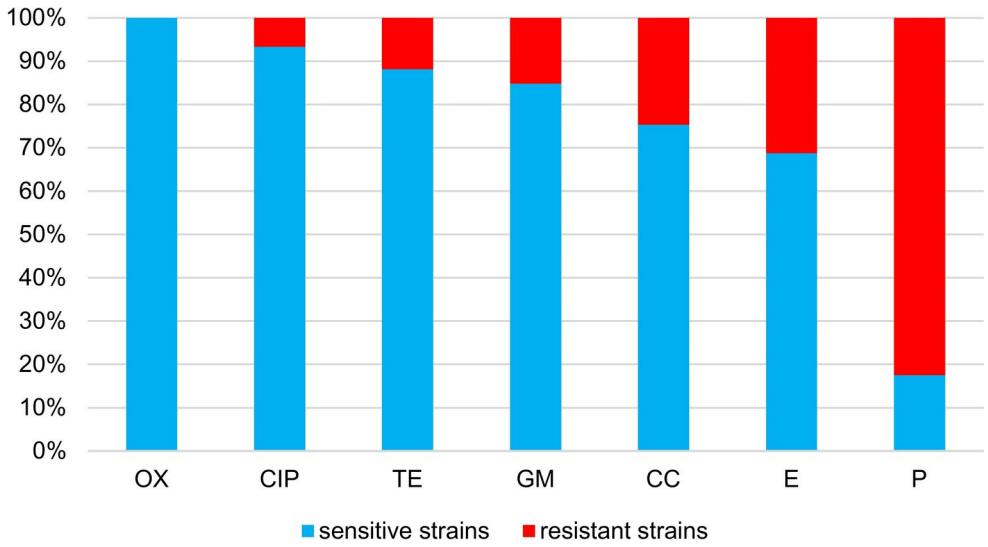

Fig. 3. Percentage of resistant and sensitive strains to the tested antibiotics.

	OX		CIP		TE		GM		CC		E		P	
No. of the measurement point	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Area A														
6	82	0	80	2	76	6	71	11	72	10	66	16	6	76
7	2	0	2	0	1	1	0	2	2	0	0	2	0	2
Sum	84	0	82	2	77	7	71	13	74	10	66	18	6	78
Area B														
6	11	0	11	0	10	1	10	1	11	0	8	3	0	11
7	20	0	14	6	19	1	18	2	20	0	8	12	3	17
8	30	0	29	1	22	8	25	5	24	6	9	21	10	20
9	5	0	5	0	2	3	5	0	5	0	5	0	0	5
Sum	66	0	59	7	53	13	58	8	60	6	30	36	13	53
Area C														
1	6	0	6	0	5	1	6	0	0	6	6	0	2	4
3	3	0	3	0	3	0	3	0	0	3	2	1	1	2
4	6	0	6	0	6	0	6	0	0	6	5	1	1	5
5	13	0	11	2	10	3	10	3	3	10	12	1	4	9
6	20	0	17	3	19	1	14	6	12	8	12	8	8	12
9	13	0	13	0	13	0	11	2	10	3	12	1	2	11
Sum	61	0	56	5	56	5	50	11	25	36	49	12	18	43

Table 3. Sensitivity of *Staphylococcus aureus* strains to tested antibiotics in selected areas. S,sensitive strain; R, resistant strain.

point 8) at 70%. The greatest number of penicillin-resistant strains (93%) were found in area A, at measurement point 6, i.e., at the coal face exit (Table 3).

To determine the statistical impact of the antibiotics used on the resistance of *Staphylococcus aureus*, we first examined whether the analysis of variance regarding the normality of distribution and the homogeneity of variance of the analyzed data regarding the antibiotic resistance of *Staphylococcus aureus* strains isolated from mine air were met. The Shapiro–Wilk tests showed that only two data sets corresponding to antibiotics: erythromycin and penicillin, had features of normal distribution. The results of the homogeneity of variance test (Bartlett's test) also gave a negative result, which was the basis for rejecting the hypothesis of homogeneity of variance.

Due to the assumptions of the analysis of variance were not met, further research was carried out using the non-parametric Kruskal–Wallis test. The results of this test highlighted a statistically significant relationship between the number of resistant *Staphylococcus aureus* strains isolated from the mine air and the type of antibiotics tested. The value of the test statistic H was equal to 41.78, and the test probability $p = 0.000$ was lower than the assumed significance level α. Therefore, the null hypothesis that there was no difference in the

effectiveness of individual antibiotics was rejected due to the statistically similar share of *Staphylococcus aureus* strains resistant to their action. The test showed that the studied antibiotics worked with varying effects, which was reflected in the different average shares of resistant *Staphylococcus aureus* strains (Fig. 4). The least resistance was shown by *Staphylococcus aureus* strains to oxacillin, ciprofloxacin, tetracycline, and gentamicin. The share of resistant strains was practically reduced to zero for oxacillin and ciprofloxacin. Tetracycline and gentamicin were reduced by 8.2(± 11.2)% and 12(± 10)%, respectively (Table 4S, supplementary materials). The average share of strains resistant to penicillin was many times higher than for the above-mentioned antibiotics and reached 84(± 14.9)%. This proved the very weak effect of this antibiotic on *Staphylococcus aureus* strains isolated from the mine air. The other two antibiotics: clindamycin and erythromycin, had moderate to similar effects. The average share of strains resistant to these antibiotics was approx. 22% (± quartile deviation).

Since the Kruskal–Wallis test showed statistically significant differences in the average share of strains resistant to individual antibiotics ($p < \alpha = 0.05$), an additional multiple comparison test of mean ranks was performed for all samples (Dunn's test). The test probability values for individual pairs of antibiotics are listed in Table 5S (supplementary materials). Statistically significant differences in the share of *Staphylococcus aureus* strains resistant to individual antibiotics are marked in red. As shown in Table 5S (supplementary materials), significant differences in the average share of resistant *Staphylococcus aureus* strains were noted for oxacillin, clindamycin, erythromycin, and penicillin. The test probability p was lower than the assumed significance level α. Similarly, the shares of resistant *Staphylococcus aureus* strains differed statistically significantly when comparing ciprofloxacin, tetracycline, and gentamicin with penicillin. The lower the value of the test probability, the greater the diversity of the effects of the compared antibiotics on the studied bacteria. The antibiotics for which no statistically significant differences were found in the share of resistant *Staphylococcus aureus* strains isolated in the mine air ($p > \alpha$) to oxacillin (which has 100% effectiveness) included ciprofloxacin, tetracycline, and gentamicin. High values of test probability p, up to 1, may indicate a similar strength of the impact of the compared antibiotics on *Staphylococcus aureus*.

The Chi-square test showed statistically significant differences in the frequency of strains resistant to the tested antibiotics in areas A, B, and C. The test statistic χ^2 was equal to 63.8, and the test probability $p = 0.00$ was lower than the assumed significance level α. Environmental conditions that vary in individual areas and the different specificity of the ventilation network in these regions may significantly impact the spread of drug resistance in underground hard coal mines.

There was no statistically significant correlation of resistance to the tested antibiotics of *Staphylococcus aureus* strains isolated from mine air with the air parameters (Table 6S, supplementary materials). The determined values of correlation coefficients were statistically insignificant for the significance level $\alpha = 0.05$ (test probability $p > \alpha$)..

Some of the isolates selected for testing were characterized by multi-antibiotic resistance. The obtained results are summarized in Table 4.

Among the 84 *Staphylococcus aureus* strains isolated in area A, 69% showed sensitivity to most of the antibiotics tested. Their presence was detected at the coal face exit (measurement point 6, Fig. 1a). However, 31% of the

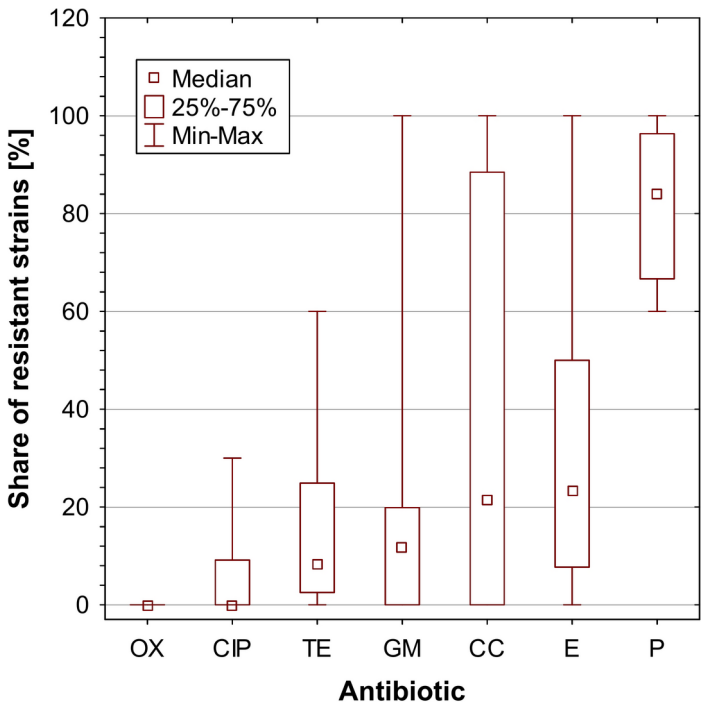

Fig. 4. Range of variability, median, and quartile deviation of the share of *Staphylococcus aureus* strains resistant to the antibiotics used.

No. of the measurement point	Number of strains	MS	MR
Area A			
6	82	58	24
7	2	0	2
Sum	84	58	26
Area B			
6	11	8	3
7	20	5	15
8	30	26	4
9	5	3	2
Sum	66	42	24
Area C			
1	6	6	0
3	3	3	0
4	6	6	0
5	13	10	3
6	20	14	6
9	13	9	4
Sum	61	48	13

Table 4. The number of multi-susceptible and multi-resistant *Staphylococcus aureus* strains isolated in individual areas. MS, multi-antibiotic susceptible strains, MR, multi-antibiotic resistant strains.

isolates were multi-antibiotic resistant strains, of which 92% were isolated at the coal face exit (measurement point 6, Fig. 1a), and only 8% before the intersection of the tailgate and the ventilation dip (measurement point 7, Fig. 1a). In area B, 66 strains were used for testing, where 64% of the isolates were susceptible to most antibiotics. The remaining 36% of the strains were multi-antibiotic resistant. The most sensitive strains of *Staphylococcus aureus* (87%) originated from the area around the longwall shearer workplace (measurement point 8, Fig. 1b). At this point, only 13% of the isolates were resistant to most of the studied antibiotics. Among the strains isolated before the junction of the maingate with the headgate (measurement point 6, Fig. 1b), 73% were sensitive to most of the tested antibiotics, and only 27% were multi-antibiotic resistant. The greatest number of multi-antibiotic resistant strains (75%) were isolated in the area where the coal seam was mined, at the junction of the coal face with the headgate (measurement point 7, Fig. 1b), while the number of susceptible strains at this point was only 25%. In area B, the least strains of *Staphylococcus aureus* were isolated at measurement point 9, at the intersection of the coal face exit and the tailgate. At this measurement point, among the 5 isolates, 60% were multi-antibiotic sensitive strains, and 40% were multi-antibiotic resistant. In area C, 61 strains of *Staphylococcus aureus* were used in the tests. Multi-antibiotic sensitive strains were found at the beginning of the studied ventilation area, located after the main crosscut with the main coal roadway (measurement point 1, Fig. 1c), at the inlet to the headgate (measurement point 3, Fig. 1c) and in the vicinity of the air conditioner (measurement point 4, Fig. 1c). At the intersection the headgate with the coal face (measurement point 5, Fig. 1c), in the vicinity of the longwall shearer's workplace (measuring point 6, Fig. 1c) and at the heading face of the roadway being excavation (measurement point 9, Fig. 1c) there were both multi-antibiotic sensitive and resistant strains. At measurement points 5 and 9, the percentage of multiantibiotic-sensitive strains was approx. 70%, and slightly more were observed at measurement point 6 (77%) (Table 4).

Significant statistical relationships were found between the share of multi-antibiotic resistant *Staphylococcus aureus* strains and three parameters of mine air, i.e., the length of its flow path from the downcast shaft (D) and the temperature measured with a dry-bulb thermometer (T_D) and a wet-bulb thermometer (T_W) – Table 7S in supplementary materials (coefficients with statistically significant correlations at the significance level $\alpha = 0.05$ are marked in red). The correlations of the share of multi-resistant strains in the total number of isolated *Staphylococcus aureus* with the above-mentioned mine air parameters were determined as positive. Hence, an increase in air temperature and its flow path measured from the foot of the downcast shaft, increased the resistance of *Staphylococcus aureus* to many antibiotics. The values of correlation coefficients determined by three tests (Spearman, Kendall, and Goodman–Kruskal) were high and ranged from 0.46 to 0.78. Therefore, it was assumed that the correlations between these values were strong, despite the relatively large dispersion of points reflecting these relationships (Fig. 5).

The Chi-square test did not show statistically significant differences in the frequency of multi-resistant strains in the considered areas (A, B, and C). The test statistic χ^2 was equal to 3.51, and the test probability $p = 0.173$ was higher than the assumed significance level α.

Prevalence of the MLS_B phenotype

All tested strains were sensitive to oxacillin, which indicated the lack of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in the tested group. However, according to the reaction of the macrolides (erythromycin) and lincosamides (clindamycin) groups to antibiotics, different phenotypes were distinguished among the tested

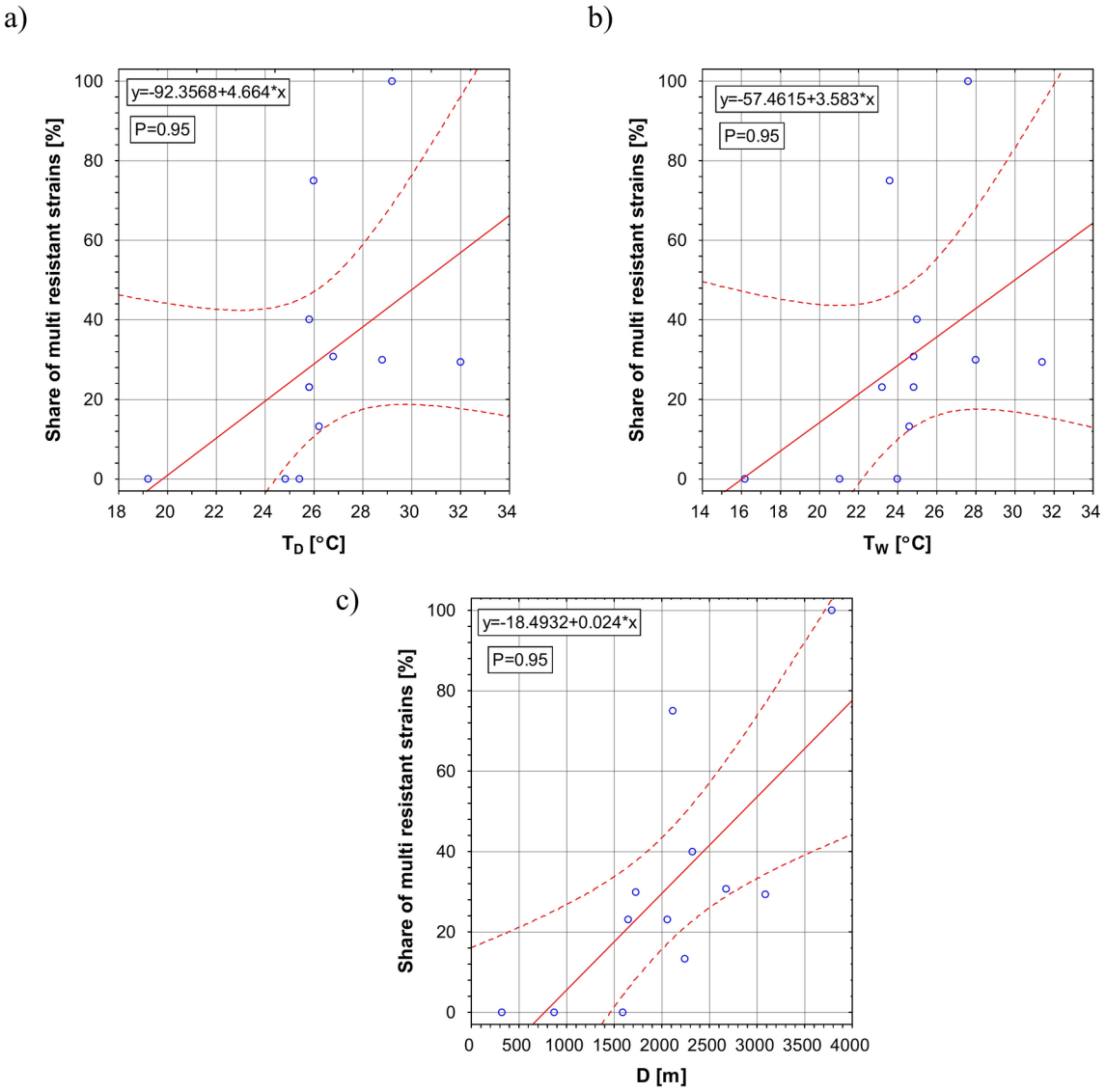

Fig. 5. Scatterplots and regression line equations of the share of multi-resistant *Staphylococcus aureus* strains as a function of mine air parameters: (**a**) temperature measured with a dry-bulb thermometer, (**b**) temperature measured with a wet-bulb thermometer, (**c**) distance from the downcast shaft.

E		CC		Resistance phenotype	Number of strains
A	B	A	B		
25	S	25	S	No resistance	Control strain *Staphylococcus aureus* ATCC 29213
0	R	26	S	MS_B	2
13	R	20	S	MLS_B inductive	8
8	R	12	R	MLS_B constitutive	22

Table 5. Resistance phenotypes of *Staphylococcus aureus* to macrolides and lincosamides. E, erythromycin; CC, clindamycin; A, growth inhibition zone [mm]; B, antibiotic susceptibility; R, resistant strain; S, sensitive strain.

isolates (Table 5). Determination of susceptibility to macrolides and lincosamides is performed using two disks: erythromycin and clindamycin. Only the disk diffusion method allows the detection of the inductive mechanism of resistance to macrolides and lincosamides. In the case of erythromycin resistance, attention should be paid to the flattening of the growth inhibition zone around the clindamycin disk on the side of the erythromycin disk (D-shape), indicating an inductive mechanism of MLS_B resistance. Constitutive MLS_B is associated with simultaneous resistance to both antibiotics.

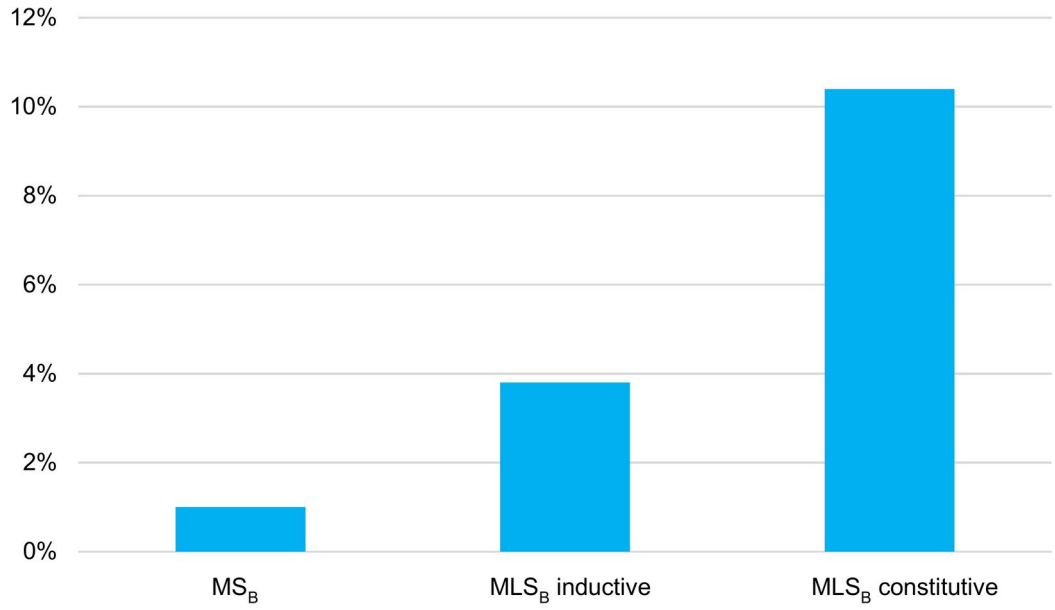

Fig. 6. Percentage of resistance phenotypes in the tested *Staphylococcus aureus* strains.

Among the *Staphylococcus aureus* strains isolated from the examined mine bioaerosol, the majority were sensitive to antibiotics from the macrolides (erythromycin) and lincosamides (clindamycin) groups. However, in the tested pool of 211 strains, constitutive MLS_B resistance was observed in 10% of the isolates, and inductive MLS_B resistance was found among 4% of the isolates (flattening of the growth inhibition zone in 8 strains). Only 2 strains of *Staphylococcus aureus* isolated in area C (measurement points 3 and 4, Fig. 1c, Table 3) were resistant to erythromycin and sensitive to clindamycin (MS_B), which was less than 1% of all isolates tested (Fig. 6).

Based on the Chi-square test, statistically significant differences were found in the frequency of MS_B, MLS_B inductive, and MLS_B constitutive phenotypes. The test statistic χ^2 was equal to 16.44, and the test probability $p = \sim 0.000$ was lower than the assumed significance level $\alpha = 0.05$.

Discussion

The presence of microorganisms in the air is influenced by many factors that may determine their number in various ways[46]. The presence of microorganisms in underground hard coal mines may be correlated with the number of people, temperature, relative humidity, and the amount of dust particles. The number of bacteria in such areas can reach several thousand or even several tens of thousands of CFU m^{-3} of air. Furthermore, among these microorganisms, there are also pathogenic forms, which include some staphylococci[9,47]. In our research, numerous units forming staphylococci colonies were found in three underground hard coal mines (Fig. 1a–c). *Staphylococcus aureus* strains were identified in 12 of the measurement points. The highest concentrations of these pathogens were observed (Table 1) at the coal face exit in area A (5100 CFU m^{-3}), near the longwall shearer in area B (1600 CFU m^{-3}) and C (1100 CFU m^{-3}). The recorded number of *Staphylococcus aureus* increased the risk of potentially harmful biological agents in the miners' workplace. This phenomenon is particularly dangerous for workers with reduced immunity.

Nowadays, the acquisition of resistance to antibiotics by microorganisms is widely discussed. The ease of biofilm formation by *Staphylococcus aureus* cells that promotes antibiotic resistance can pose a health threat, causing serious infections[48–51]. In our studies, all analyzed strains of staphylococci were sensitive only to oxacillin. The least effective antibiotic was penicillin, to which as many as 82% of isolates showed resistance (Table 2). These results differed from those obtained in other research facilities. Gandara et al.[52] examined the antibiotic resistance of *Staphylococcus aureus* strains isolated in single-family houses in Texas (USA) and found 100% penicillin resistance in only 3 cases. Taking into account all the strains analyzed by this research group at individual measurement points, approx. 60% of the isolates were resistant to penicillin. Zhou and Wang[53] examined the effect of various antibiotics on staphylococci strains isolated from waiting rooms at a metro station in Shanghai and found penicillin resistance in only 28%. None of the analyzed staphylococci showed resistance to gentamicin, and the most effective antibiotic was erythromycin. These results were not confirmed in our presented research (Fig. 3).

The drug susceptibility of staphylococci isolated from different environments may vary significantly. This applies primarily to isolates from clinical materials, which are usually characterized by greater resistance[54–56]. Many authors highlight the rapidly decreasing methicillin sensitivity of *Staphylococcus aureus* strains. Resistance to this antibiotic indicates resistance to β-lactam antibiotics[57,58]. In our study, all isolates were sensitive to oxacillin, an antibiotic belonging to the β-lactam group. This proved the absence of MRSA strains in the underground hard coal mines selected for research (Table 2). However, owing to the observed effect of erythromycin and

clindamycin, antibiotics belonging to the group of macrolides and lincosamides, strains characterized by both constitutive and inductive MLS_B resistance were found (Table 5, Fig. 6).

Most literature data concerning hospital strains of *Staphylococcus aureus* are often insensitive to tetracyclines, macrolides, lincosamides, and other chemotherapeutics. However, strains isolated from other sources are more or less sensitive to most antibiotics, and resistant only to methicillin and tetracyclines[59–62]. This was not confirmed by our research, which showed the presence of staphylococci resistant to many antibiotics in the mine bioaerosol, of which 30% were multi-antibiotic resistant strains (Tables 2 and 4).

Our research, together with the antibiogram, is the first analysis of this type of air from coal mines to determine the presence of drug-resistant *Staphylococcus aureus*. The majority of studies focus on the analysis of bioaerosols in various environments, taking into account only the total number of microorganisms[63–65]. An important problem in examining the threats posed by air pollution is the precise determination of the causes of human infections and to determine an antibiogram so that appropriate therapy can be applied. Our obtained results indicated a significant problem of mine air pollution by staphylococci. Therefore, it is worth investigating the possibility of infections caused by these microorganisms for various ailments reported by miners.

The high percentage of antibiotic-resistant staphylococci proves that systematic microbiological air inspections in deep coal mines and further research are necessary to help identify biological factors that may affect the quality of human health in such workplaces. This applies especially to mining excavations where, due to high air dustiness, *Staphylococcus aureus* concentrations may be high, which was confirmed by our research. The World Health Organization and the European Commission have also highlighted the need to monitor and actively combat drug resistance. There are indications that the process of emergence and spread of antibiotic-resistant microorganisms can be inhibited not only by the prudent use of drugs but also by continuous monitoring of resistance in various environments. As our research shows, this phenomenon is also widespread in hard-to-reach environments such as deep coal mines.

Conclusions

Based on our analyses, we found that the highest concentration of staphylococci occurred in underground mining areas with the highest dustiness, during mining works using longwall shearers and roadheaders. The analysis of the taxonomic diversity of staphylococci showed the identification of *Staphylococcus aureus* species. A statistically significant correlation between the number of *Staphylococcus aureus* strains and the mine air parameters and its flow path was also observed. Therefore, we assumed that under similar operating conditions and a similar structure of the ventilation network used to ventilate coal faces, the share of *Staphylococcus aureus* strains in the total number of bacteria isolated from the air would be at a comparable level. The analyses performed showed a significant statistical impact of the tested antibiotics on the number of resistant *Staphylococcus aureus* strains isolated from mine air at measurement points in individual areas. Among the 211 *Staphylococcus aureus* strains tested, over 82% showed resistance to penicillin. The analyzed isolates were 100% sensitive to oxacillin, which indicated the absence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains in the examined mine aerosol. Therefore, in the case of potential infections caused by pathogenic isolates of *Staphylococcus aureus*, the use of antibiotics related to the β-lactam group will be effective. Various sensitivity to antibiotics from the macrolide and lincosamide groups was found among the tested *Staphylococcus aureus* strains. 10% of the isolates showed constitutive MLS_B resistance, while 4% were characterized by inductive MLS_B resistance. Less than 1% of the isolates were resistant to erythromycin and sensitive to clindamycin (MS_B). Based on the Chi-square test, statistically significant differences were found in the frequency of MS_B, MLS_B inductive, and MLS_B constitutive phenotypes. In the tested group of *Staphylococcus aureus* isolates, 30% of the strains were characterized by multi-resistance to the tested antibiotics. However, the Chi-square test did not show statistically significant differences in the frequency of multi-resistant strains in the considered areas (A, B, and C). Underground coal mines, as permanent workplaces for miners, may be a potential source of pathogenic, drug-resistant bacteria, therefore, they should be subject to periodic monitoring of the microbiological quality of the air. The antibiograms performed will help select appropriate chemotherapy aimed at effectively combating infections among mine workers without the risk of spreading drug resistance[36,37,42,44].

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Received: 9 October 2024; Accepted: 17 March 2025
Published online: 04 April 2025

References

1. Paśmionka, I. Assessment of microbial contamination of atmospheric air in a selected wastewater treatment plant. *Arch. Environ. Prot.* **45**(4), 60–67 (2019).
2. Paśmionka, I. Evaluation of microbiological quality of atmospheric air in a selected sewage treatment plant in Lesser Poland. *Aerobiologia* **36**, 249–260. https://doi.org/10.1007/s10453-020-09627-x (2020).
3. Xue, S. et al. Pathogenic bacterial communities of dust in a coal mine. *Front. Environ. Sci.* **10**, 857744. https://doi.org/10.3389/fenvs.2022.857744 (2022).
4. Li, S. et al. Research on the evaluation of air quality in underground coal mines based on a generalized contrastive weighted comprehensive scale index method. *Atmosphere* **14**(6), 1021. https://doi.org/10.3390/atmos14061021 (2023).
5. Trechera, P. et al. Comprehensive evaluation of potential coal mine dust emissions in an open-pit coal mine in Northwest China. *Int. J. Coal Geol.* **235**, 103677. https://doi.org/10.1016/j.coal.2021.103677 (2021).

6. Xia, N., Hai, W., Song, G. & Tang, M. Identification and monitoring of coal dust pollution in Wucaiwan mining area, Xinjiang (China) using Landsat derived enhanced coal dust index. *PLoS ONE* **17**(4), e0266517. https://doi.org/10.1371/journal.pone.0266517 (2022).
7. Luo, H., Zhou, W., Jiskani, I. M. & Wang, Z. Analyzing characteristics of particulate matter pollution in open-pit coal mines: Implications for green mining. *Energies* **14**(9), 2680. https://doi.org/10.3390/en14092680 (2021).
8. Zeng, F. & Jiang, Z. Spatial and temporal evolution of mine dust research: Visual knowledge mapping analysis in Web of Science from 2001 to 2021. *Environ. Sci. Pollut. Res.* **30**, 62170–62200. https://doi.org/10.1007/s11356-023-26332-7 (2023).
9. Cheluszka, P., Paśmionka, I. B., Gospodarek, J. & Vieira, F. M. C. V. The spread of microbiota in the air of an underground hard coal mine - A case study. *Build. Environ.* **242**, 110495. https://doi.org/10.1016/j.buildenv.2023.110495 (2023).
10. Cuny, C., Layer-Nicolaou, F., Werner, G. & Witte, W. A look at staphylococci from the one health perspective. *IJMM* **314**, 151604. https://doi.org/10.1016/j.ijmm.2024.151604 (2024).
11. Mata, T. M. et al. Indoor air quality in elderly centers: Pollutants emission and health effects. *Environments* **9**(7), 86. https://doi.org/10.3390/environments9070086 (2022).
12. Yan, X. et al. Characteristics of airborne bacterial communities and antibiotic resistance genes under different air quality levels. *Environ. Int.* **161**, 107127. https://doi.org/10.1016/j.envint.2022.107127 (2022).
13. Zhou, Z. et al. Association between particulate matter (PM)2·5 air pollution and clinical antibiotic resistance: A global analysis. *Lancet Planet Health* **7**(8), e649–e659. https://doi.org/10.1016/S2542-5196(23)00135-3 (2023).
14. Mutuku, C., Gazdag, Z. & Melegh, S. Occurrence of antibiotics and bacterial resistance genes in wastewater: Resistance mechanisms and antimicrobial resistance control approaches. *World. J. Microbiol. Biotechnol.* **38**, 152. https://doi.org/10.1007/s11274-022-03334-0 (2022).
15. Larsson, D. G. J. & Flach, C. F. Antibiotic resistance in the environment. *Nat. Rev. Microbiol.* **20**(5), 257–269. https://doi.org/10.1038/s41579-021-00649-x (2022).
16. Ray, S., Das, S. & Suar, M. Molecular Mechanism of Drug Resistance. Drug Resistance in Bacteria, Fungi, Malaria, and Cancer. 47–110. https://doi.org/10.1007/978-3-319-48683-3_3 (2017).
17. Bengtsson-Palme, J., Kristiansson, E. & Larsson, D. G. J. Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiol. Rev.* https://doi.org/10.1093/femsre/fux053 (2018).
18. Bengtsson-Palme, J. et al. Towards monitoring of antimicrobial resistance in the environment: For what reasons, how to implement it, and what are the data needs?. *Environ. Int.* **178**, 108089. https://doi.org/10.1016/j.envint.2023.108089 (2023).
19. Singer, A. C., Shaw, H., Rhodes, V. & Hart, A. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Front. Microbiol.* **7**, 1728. https://doi.org/10.3389/fmicb.2016.01728 (2016).
20. Ayaaba, E., Li, Y., Yuan, J. & Ni, C. Occupational respiratory diseases of miners from two gold mines in Ghana. *IJERPH* **14**(3), 337. https://doi.org/10.3390/ijerph14030337 (2017).
21. Donoghue, A. M. Occupational health hazards in mining: An overview. *Occup. Med.* **54**, 283–289. https://doi.org/10.1093/occmed/kqh072 (2004).
22. Hall, N. B., Blackley, D. J., Halldin, C. N. & Laney, A. S. Current review of pneumoconiosis among US coal miners. *Curr. Environ. Health Rep.* **6**(3), 137–147. https://doi.org/10.1007/s40572-019-00237-5 (2019).
23. Vanka, K. S. et al. Understanding the pathogenesis of occupational coal and silica dust-associated lung disease. *ERR* **31**, 210250. https://doi.org/10.1183/16000617.0250-2021 (2022).
24. Stewart, A. G. Mining is bad for health: a voyage of discovery. *Environ. Geochem. Health* **42**(4), 1153–1165. https://doi.org/10.1007/s10653-019-00367-7 (2020).
25. Naghadehi, M. Z., Sereshki, F. & Mohammadi, F. Pathological study of the prevalence of silicosis among coal miners in Iran: A case history. *Atmos. Environ.* **83**, 1–5. https://doi.org/10.1016/j.atmosenv.2013.10.053 (2014).
26. Perret, J. L. et al. Respiratory surveillance for coal mine dust and artificial stone exposed workers in Australia and New Zealand: A position statement from the Thoracic Society of Australia and New Zealand*. *Respirology* **25**(11), 1193–1202. https://doi.org/10.1111/resp.13952 (2020).
27. Yang, L., Birhane, G. E., Zhu, J. & Geng, J. Mining employees safety and the application of information technology in coal mining: Review. *Front. Public Health* **9**, 709987. https://doi.org/10.3389/fpubh.2021.709987 (2021).
28. Zhou, L.-j, Cao, Q.-g, Yu, K., Wang, L.-l & Wang, H.-b. Research on occupational safety, health management and risk control technology in coal mines. *IJERPH* **15**(5), 868. https://doi.org/10.3390/ijerph15050868 (2018).
29. Brdová, D., Ruml, T. & Viktorová, J. Mechanism of staphylococcal resistance to clinically relevant antibiotics. *Drug Resist. Updates* **77**, 101147. https://doi.org/10.1016/j.drup.2024.101147 (2024).
30. Masters, E. A. et al. Evolving concepts in bone infection: Redefining "biofilm", "acute vs. chronic osteomyelitis", "the immune proteome" and "local antibiotic therapy". *Bone Res.* **7**, 20. https://doi.org/10.1038/s41413-019-0061-z (2019).
31. Yamazaki, Y. et al. The role of *Staphylococcus aureus* quorum sensing in cutaneous and systemic infections. *Inflamm. Regener.* **44**, 9. https://doi.org/10.1186/s41232-024-00323-8 (2024).
32. Cheung, G. Y. C., Bae, J. S. & Otto, M. Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence* **12**(1), 547–569. https://doi.org/10.1080/21505594.2021.1878688 (2021).
33. Gehrke, A.-K.E., Giai, C. & Gómez, M. I. *Staphylococcus aureus* adaptation to the skin in health and persistent/recurrent infections. *Antibiotics* **12**, 1520. https://doi.org/10.3390/antibiotics12101520 (2023).
34. Ahmad-Mansour, N. et al. *Staphylococcus aureus* toxins: An update on their pathogenic properties and potential treatments. *Toxins* **13**, 677. https://doi.org/10.3390/toxins13100677 (2021).
35. Song, M. et al. *Staphylococcus aureus* and biofilms: Transmission, threats, and promising strategies in animal husbandry. *J. Anim. Sci. Biotechnol.* **15**, 44. https://doi.org/10.1186/s40104-024-01007-6 (2024).
36. Liu, Z.-H., Qi-Ying, Wu., Feng, Xu., Zhang, X. & Liao, X.-B. Biofunction and clinical potential of extracellular vesicles from methicillin-resistant *Staphylococcus aureus*. *Microbiol. Res.* **266**, 127238. https://doi.org/10.1016/j.micres.2022.127238 (2023).
37. Qiu, Y. et al. Five major two components systems of *Staphylococcus aureus* for adaptation in diverse hostile environment. *Microb. Pathog.* **159**, 105119. https://doi.org/10.1016/j.micpath.2021.105119 (2021).
38. Hardy, B. L. et al. Antimicrobial activity of clinically isolated bacterial species against *Staphylococcus aureus*. *Front. Microbiol.* **10**, 2977. https://doi.org/10.3389/fmicb.2019.02977 (2020).
39. Girma, A. *Staphylococcus aureus*: Current perspectives on molecular pathogenesis and virulence. *Cell Surf.* **13**, 100137. https://doi.org/10.1016/j.tcsw.2024.100137 (2025).
40. Nikolic, P. & Mudgil, P. The cell wall, cell membrane and virulence factors of *Staphylococcus aureus* and their role in antibiotic resistance. *Microorganisms* **11**, 259. https://doi.org/10.3390/microorganisms11020259 (2023).
41. Arunachalam, K., Pandurangan, P., Shi, C. & Lagoa, R. Regulation of *Staphylococcus aureus* virulence and application of nanotherapeutics to eradicate *S. aureus* infection. *Pharmaceutics* **15**, 310. https://doi.org/10.3390/pharmaceutics15020310 (2023).
42. Venkataraman, R. & Yadav, U. Ravi Kurikempannadoddi Shivalingegowda, Yogendra Shrestha, Vaccination strategies to combat nosocomial infections. *Vacunas (English Edition)* **24**(1), 60–67. https://doi.org/10.1016/j.vacune.2023.02.005 (2023).
43. Watkins, K. E. & Unnikrishnan, M. Chapter Three - Evasion of host defenses by intracellular *Staphylococcus aureus*. In *Advances in Applied Microbiology* (eds Gadd, G. M. & Sariaslani, S.) 105–141 (Academic Press, 2020).
44. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. http://www.eucast.org (Access date: 03 February 2024)
45. Goodman, L. A. & Kruskal, W. H. Measures of association for cross classifications. *J. Am. Stat. Assoc.* **49**(268), 732–764 (1954).

46. Chen, X., Kumari, D. & Achal, V. A review on airborne microbes: The characteristics of sources, pathogenicity and geography. *Atmosphere* **11**(9), 919. https://doi.org/10.3390/atmos11090919 (2020).
47. Rdzanek, M., Pusz, W., Gębarowska, E. & Pląskowska, E. Airborne bacteria and fungi in a coal mine in Poland. *J. Cave Karst Stud.* **77**(3), 177–182. https://doi.org/10.4311/2015MB0102 (2015).
48. Dutt, Y. et al. The association between biofilm formation and antimicrobial resistance with possible ingenious bio-remedial approaches. *Antibiotics* **11**(7), 930. https://doi.org/10.3390/antibiotics11070930 (2022).
49. Sharma, D., Misba, L. & Khan, A. U. Antibiotics versus biofilm: An emerging battleground in microbial communities. *Antimicrob. Resist. Infect. Control.* **8**, 76. https://doi.org/10.1186/s13756-019-0533-3 (2019).
50. Sharma, S. et al. Microbial biofilm: A review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms* **11**(6), 1614. https://doi.org/10.3390/microorganisms11061614 (2023).
51. Zhao, A., Sun, J. & Liu, Y. Understanding bacterial biofilms: From definition to treatment strategies. *Front. Cell. Infect. Microbiol.* **13**, 1137947. https://doi.org/10.3389/fcimb.2023.1137947 (2023).
52. Gandara, A. et al. Isolation of *Staphylococcus aureus* and antibiotic-resistant *Staphylococcus aureus* from residential indoor bioaerosols. *Environ. Health Perspect.* **114**(12), 1859–1864. https://doi.org/10.1289/ehp.9585 (2006).
53. Zhou, F. & Wang, Y. Characteristics of antibiotic resistance of airborne staphylococcus isolated from metro stations. *IJERPH* **10**(6), 2412–2426. https://doi.org/10.3390/ijerph10062412 (2013).
54. Linz, M. S., Mattappallil, A., Finkel, D. & Parker, D. Clinical impact of *Staphylococcus aureus* skin and soft tissue infections. *Antibiotics* **12**(3), 557. https://doi.org/10.3390/antibiotics12030557 (2023).
55. Monistero, V. et al. Different distribution of antimicrobial resistance genes and virulence profiles of *Staphylococcus aureus* strains isolated from clinical mastitis in six countries. *JDS* **103**(4), 3431–3446. https://doi.org/10.3168/jds.2019-17141 (2020).
56. Yang, F. et al. Antimicrobial resistance and virulence profiles of staphylococci isolated from clinical bovine mastitis. *Front. Microbiol.* **14**, 1190790. https://doi.org/10.3389/fmicb.2023.1190790 (2023).
57. Kmiha, S. et al. Methicillin-resistant *Staphylococcus aureus* Strains Isolated from burned patients in a Tunisian Hospital: Molecular typing, virulence genes, and antimicrobial resistance. *Antibiotics* **12**(6), 1030. https://doi.org/10.3390/antibiotics12061030 (2023).
58. Wang, Y. et al. Rapid and ultrasensitive detection of methicillin-resistant *Staphylococcus aureus* based on CRISPR-Cas12a combined with recombinase-aided amplification. *Front. Microbiol.* **13**, 903298. https://doi.org/10.3389/fmicb.2022.903298 (2022).
59. El Mammery, A. et al. An increase in erythromycin resistance in methicillin-susceptible Staphylococcus aureus from blood correlates with the use of macrolide/lincosamide/streptogramin antibiotics (2004–2020). *EARS-Net Spain. Front. Microbiol.* **14**, 1220286. https://doi.org/10.3389/fmicb.2023.1220286 (2023).
60. Miklasińska-Majdanik, M. Mechanisms of resistance to macrolide antibiotics among *Staphylococcus aureus*. *Antibiotics* **10**(11), 1406. https://doi.org/10.3390/antibiotics10111406 (2021).
61. Stefańska, I., Kwiecień, E., Kizerwetter-Świda, M., Chrobak-Chmiel, D. & Rzewuska, M. Tetracycline, macrolide and lincosamide resistance in Streptococcus canis strains from companion animals and its genetic determinants. *Antibiotics* **11**(8), 1034. https://doi.org/10.3390/antibiotics11081034 (2022).
62. Urban-Chmiel, R. et al. Antibiotic resistance in bacteria - a review. *Antibiotics* **11**(8), 1079. https://doi.org/10.3390/antibiotics11081079 (2022).
63. Can-Güven, E. The current status and future needs of global bioaerosol research: A bibliometric analysis. *Int. J. Environ. Sci. Technol.* **19**, 7857–7868. https://doi.org/10.1007/s13762-021-03683-7 (2022).
64. Jabeen, R., Kizhisseri, M. I. & Mayanaik, S. N. Bioaerosol assessment in indoor and outdoor environments: A case study from India. *Sci. Rep.* **13**, 18066. https://doi.org/10.1038/s41598-023-44315-z (2023).
65. Sajjad, B., Hussain, S., Rasool, K., Hassan, M. & Almomani, F. Comprehensive insights into advances in ambient bioaerosols sampling, analysis and factors influencing bioaerosols composition. *Environ. Pollut.* **336**, 122473. https://doi.org/10.1016/j.envpol.2023.122473 (2023).

Author contributions

I. B. P.: Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization, Project administration, Funding acquisition, Data curation. P. C.: Writing – original draft, Conceptualization, Methodology, Investigation, Formal analysis, Data curation. J. G.: Writing – review & editing, Data curation, Resources. K. C.: Writing – review & editing, Supervision, Resources. J.F.: Writing – review & editing, Supervision, Resources.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-94630-w.

Correspondence and requests for materials should be addressed to P.C.