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ORIGINAL PAPER

OCCUPATIONAL EXPOSURE TO *STAPHYLOCOCCUS AUREUS* IN THE WASTEWATER TREATMENT PLANTS ENVIRONMENT

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Abstract

Background: The aim of the study was to assess the occupational exposure to *Staphylococcus aureus* bacteria, including methicillin-resistant *S. aureus* (MRSA) and other antibiotic-resistant strains in the municipal wastewater treatment plants (WWTPs) environment. **Material and Methods:** In 16 WWTPs in Poland, 33 wastewater and 253 air samples were collected in the spring–summer season. The microbiological analysis was carried out using a chromogenic medium. Species identification was carried out using the matrix assisted laser desorption ionization time-of-flight method, while the antibiotic-resistance analysis was performed with an automatic method. **Results:** Among 2805 bacterial isolates from the air and wastewater, 574 were identified as *S. aureus* species (20.5%). The presence of *S. aureus* species was found in 11 WWTPs (69%), among them in 11 WWTPs in raw wastewater and in 1 WWTP additionally in treated wastewater. The concentrations of *S. aureus* in wastewater ranged 2–1215 colony-forming units per milliliter (CFU/ml). In the air, 2 *S. aureus* isolates were identified in concentrations of 5 and 10 CFU/m^3; both samples were collected at the stage of mechanical wastewater treatment. The results revealed the following trend: the higher the outdoor temperature, the bigger the number of WWTPs with confirmed *S. aureus* presence. Among 149 *S. aureus* isolates (2 from the air and 147 from wastewater, including 2 MRSA), 100 isolates were resistant only to penicillin, while 34 isolates showed multi-antibiotic resistance (to penicillin and other drugs). It was found that isolated bacteria were resistant almost strictly to critical and highly important antibiotics in veterinary medicine. **Conclusions:** In general, WWTPs workers are occupationally exposed to *S. aureus*, including MRSA, and other antibiotic- and multi-antibiotic-resistant strains. The highest risk of infection concerns the activities carried out in direct contact with wastewater or devices through which wastewater flows, particularly at the stage of mechanical treatment. A significant source of *S. aureus* seems to be intensive livestock farming located in the area of the WWTPs under analysis. The study confirms the necessity to disinfect the wastewater discharging into WWTPs. Med Pr. 2020;71(3):265–78

Key words: occupational exposure, antibiotic resistance, bioaerosol, MRSA, *S. aureus*, WWTP

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INTRODUCTION

Staphylococcus aureus is a Gram-positive bacteria which is quite common in the environment. This species is a part of the natural bacterial microflora of humans and animals; it particularly often colonizes the skin and mucous membranes of the upper respiratory tract. Staphylococci (especially pathogenic strains) are highly resistant to various physical and chemical agents, e.g., when drying. These bacteria can survive for many weeks (or even months) outside the living organism, particularly in such environments in which protein is accessible but there is no sunlight [1,2]. In addition, *S. aureus* bacteria can form biofilms on surfaces. This ability, in combination with the capability of forming persisters, considerably inhibits the treatment of infections induced by biofilm-forming strains [3].

The research conducted in the wastewater treatment plants (WWTPs) environment confirmed the presence of pathogenic bacteria in bioaerosol where their source is wastewater subjected to treatment processes [4,5]. The presence of airborne pathogenic bacteria, including *S. aureus*, was also confirmed in the immediate environment of WWTPs [6].

Notably, *S. aureus* bacteria are very invasive and may cause slow-healing infections of the skin and soft tis-

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sues, endocarditis, osteitis and myelitis, cerebral meningitis, bacteremia, and pneumonia (including pneumonia resulting from flu), with chronic and recurrent infections induced by persisters [3,7]. The cause of such a high invasiveness of the bacteria is their ability to synthesize:
- enzymes responsible for the cytolytic effect,
- toxins inducing the inflammatory effect,
- exotoxins inducing toxic shock syndrome,
- surface proteins which bind with cells, proteins and blood cells in the attacked organism [7].

It has been estimated that approx. 20–40% of the general population in developed countries are carriers, whereas among the medical staff – even as many as 90%. The nasal colonization constitutes a significant risk factor of the subsequent *S. aureus* infection [6]. The bacteria are common members of the human microbiome [8]. However, it has been indicated that in at least 80% of the cases of bacteremia induced by *S. aureus*, in the carriers of those bacteria, the infecting strain is genetically identical to the nasal colonizing strain identified before the development of bacteremia [6,8,9]. The highest risk of the *S. aureus* infection is connected with the direct hand–face (mouth or nose) transmission [10]. However, the contaminated air is considered to be another possible route of transmission [10,11].

Presently, literature points expressly to the public health increasing problem of spreading bacterial genes of resistance to medicinal drugs beyond the hospital environment [12]. In intensive livestock farming (ILF), antibiotics are commonly used to prevent the dissemination of infectious diseases inside a herd. As a consequence of the transfer of the antibiotic-resistance genes between bacterial species and the selective pressure phenomena (the survival of bacteria with natural and acquired resistance), the antibiotic-resistant and multi-antibiotic-resistant strains prevail in the microflora of animals [13]. In the wastewater delivered to WWTPs, the presence of methicillin-resistant *S. aureus* (MRSA) was confirmed empirically [14–16]. The wastewater discharged to WWTPs is a potential source of MRSA bacteria, including those from hospitals [14] and from the areas where ILF is conducted [17]. This situation generates a significant problem for public health, and poses a real risk that WWTPs workers, due to their occupational exposure, become carriers of MRSA and participate in the spreading of antibiotic resistance in the environment [18,19].

The main aim of the study was to assess the occupational exposure to pathogenic *S. aureus* bacteria, including the antibiotic-resistant strains in the work environment of the municipal WWTPs.

MATERIAL AND METHODS

Sample site

The study was carried out in 16 WWTPs with different treatment technologies:
- 5 biological WWTPs with increased biogen removal,
- 5 mechanical and biological WWTPs,
- 3 biological WWTPs,
- 2 mechanical, chemical and biological WWTPs with increased biogen removal,
- 1 biological WWTP with increased biogen removal, and with chemical support of the dephosphatation process.

A detailed description of the investigated WWTPs is presented in Table 1. The studied WWTPs were located in different regions of Poland.

Sampling strategy

In total, 286 samples were collected, including 253 from the air and 33 from wastewater, of which 17 samples were collected from raw wastewater and 16 from treated wastewater discharged to the environment. The air samples were taken using the impact method (94 samples) and the filtration method (159 samples). Calibration certificates for the devices used in both methods were issued just before the start of the sampling process.

Air samples

Air samples for the research were collected from many different WWTPs points determined so as to cover the whole process of municipal wastewater treatment and the workstations with the highest risk of forming bioaerosol from wastewater in the treatment process. The air sampling parameters were empirically established based on an independent pilot study.

Impact method

Air samples were collected using a 1-step portable air sampler made by Burkard (Burkard Manufacturing Company Ltd, UK), working with a flow rate of 20 l/min for 10 min, directly onto the plates with the culture medium. Each time before sampling, the air sampler was disinfected with a 70% ethanol solution.

Filtration method

Air samples were collected using the measuring sets consisting of a GilAir-5 pump (Sensidyne, USA) and an open-faced aerosol sampler (Two-Met, Poland), with a gelatin filter (Whatman International Ltd, UK) of a 37 mm

Table 1. Characteristics of the wastewater treatment plants (WWTPs) involved in the study on the *Staphylococcus aureus* presence in bioaerosol in the WWTPs environment in 2017 in Poland (N = 16)

Code	Wastewater treatment technology	T_{OUT} range [°C]	Wastewater discharged from the sewage system [m³/year]	Wastewater delivered by slurry tankers [m³/year]	Hospital wastewater discharge	Area of ILF
1	biological	≤10	26 875	4 800	no	MD
2	biological with increased biogen removal	≤10	179 000	60 000	no	MD
3	mechanical and biological	11–19	27 330	0	no	no
4	biological with increased biogen removal	11–19	2 148 497	48 560	yes	no
5	mechanical and biological	≤10	249 000	5 100	yes	MD
6	biological with increased biogen removal, and with chemical support of the dephosphatation process	11–19	727 000	25 000	yes	MD
7	biological	11–19	50 700	11 000	yes	HD
8	biological	≥20	39 212	400	no	HD
9	mechanical, chemical and biological with increased biogen removal	≥20	1 460 000	7 860 000	yes	no
10	with chemical support of the dephosphatation process	11–19	438 480	18 000	no	MD
11	biological, mechanical and chemical	≥20	630 000	166 400	yes	HD
12	mechanical and biological	≥20	739 989	7 522	yes	MD
13	with chemical support of the dephosphatation process	11–19	970 700	15 100	yes	MD
14	biological	≥20	117 084	6 500	no	MD
15	mechanical and biological with increased biogen removal	≥20	10 421 000	40 700	yes	MD
16	mechanical and biological	≥20	740 184	66 399	yes	MD

ILF – intensive livestock farming, HD – high density of ILF, MD – medium density of ILF, T_{OUT} – outdoor temperature.

in diameter and 3 μm pores at a flow rate of 3 l/min during 1.5–2 h. The measuring sets were calibrated before each sampling procedure, using a Gillibrator-2 calibrator with a high accuracy of measurement ±1% (Sensidyne, USA). Heads of the aerosol samplers, disinfected with a 70% ethanol solution under a laminar chamber, were filled with sterile gelatin filters and packed one by one into sterile pre-stressed bags in the laboratory, directly before going on the field study. The heads were fitted into the unit with an air aspirator directly before the start of the sampling procedure in a given WWTP. The gelatin filters, directly after sampling, were put onto the culture medium and transported in a refrigerator to the microbiological laboratory, where bioaerosol samples were incubated and analyzed according to the procedure described below.

Wastewater sampling

In each WWTP, 2 samples of wastewater were analyzed, containing raw wastewater discharged into the WWTP and treated wastewater (water flowing into the tank, where the stabilization process takes place, which is necessary before discharging the treated wastewater into the environment). In WWTP No. 4, an additional sample of wastewater was collected after the end of the mechanical treatment process. In total, 33 samples of wastewater were collected (16 samples of raw wastewater, 1 sample of wastewater after mechanical treatment, and 16 samples of treated wastewater). The samples were transported in a refrigerator to the microbiological laboratory. Then, according to a good microbiological practice, the cultures were carried out using a method of a serial dilution from 10^{-1} to 10^{-10} in a sterile buffered solution of physiological saline (BTL Sp. z o.o., Poland) on the culture medium, and then subjected to further analysis according to the procedure described below.

Microbiological analysis

The culture medium used at all stages of the laboratory analysis was the chromogenic substrate CHROMID® S. aureus Elite agar (BioMérieux, France). The samples of bioaerosol and wastewater on the culture medium were incubated at 37°C for 48 h. The counting of grown bacterial colonies was conducted twice after 24 h and 48 h of the microbiological culture. In the case of the samples collected using the impact method, the results were supplemented with a statistical amendment according to the procedure developed by the air sampler producer. The bacterial colonies which, according to the specification developed by the culture medium producer, met the criteria for *S. aureus* species (pink color, smooth, shiny and convex surface) were selected for further analysis to confirm the species and then to analyze antibiotic resistance.

To identify the species, the colonies selected based on the above-described morphological traits were isolated and for each of them a biochemical test was carried out to check the capability to produce coagulase (the latex test). The isolates of coagulase-negative strains were eliminated from further analysis, whereas coagulase-positive strains were identified using the mass spectrometry technique (matrix assisted laser desorption ionization time-of-flight – MALDI-TOF). This method uses the technique of generating protein spectra profiles for bacterial proteins. These spectra are characteristic of a given species and, by comparison of the obtained spectra with those collected in the database (library), the species affiliation can be confirmed.

Table 2. List of antibiotics included in the analysis of the antibiotic resistance of *Staphylococcus aureus* isolates from the wastewater treatment plants (WWTPs) involved in the study on the *S. aureus* presence in bioaerosol in the WWTPs environment in 2017 in Poland

Groups of antibiotics	Antibiotics
β-lactams	ampicillin cefadroxil cefaclor cefalexin cefazolin cefoxitin cefuroxime cloxacillin methicillin penicillin
Aminoglycosides	amikacin gentamicin
Macrolides	erythromycin
Lincosamides	clindamycin
Fluoroquinolones	ciprofloxacin levofloxacin
Tetracycline	tetracycline
Glycopeptides	teicoplanin vancomycin
Oxazolidinones	linezolid
Sulfonamides	co-trimoxazole

Analysis of antibiotic resistance

The *S. aureus* isolates were analyzed in terms of their sensitivity to different antibiotic groups which are most often used in human and animal treatment, including β-lactams, using an automatic method (the WalkAway system), according to current recommendations [20]. In total, the strains of *S. aureus* isolated from WWTPs were analyzed in terms of their resistance to 21 antimicrobial drugs from 9 groups of antibiotics, as shown in Table 2.

Microclimatic parameters

The basic microclimatic parameters were measured in each sample collection point, including temperature (T) [°C], relative humidity (RH) [%], the concentration of CO_2 [ppm] and air flow velocity [m/s]. The measurements were carried out using a multifunctional microclimate meter Testo 435-2 (Testo AG, Germany), equipped with 2 connectable probes. The measurements were carried out at a height of 1.5 m above the ground/floor, for 10 min, and the readouts were taken at 1-min intervals, and then the result was averaged for each measurement point.

Questionnaire study

The WWTPs data were collected using a tool prepared in a form of an interview questionnaire. The questionnaire containing a set of questions about the WWTPs characteristics was developed based on literature, experience of the study team in the previous studies conducted in other WWTPs, and the questionnaire filled in by WWTPs as part of the annual reporting for statistical purposes to the Environmental Protection Inspection in Poland. The questionnaire consisted of 21 questions, including those related to the environment (the area around the plant), type (a municipality or a town), number of employees, treatment technology, and characteristics of the discharged wastewater. Information from the questionnaires was entered to the database prepared specifically for this purpose (using MS Office Excel), and it was then used for the analysis.

Results presentation

The results are presented as the concentrations of *S. aureus* in wastewater, expressed in the colony-forming units per milliliter (CFU/ml), calculated as the average value from a series of dilutions. Analyses were carried out using MS Office Excel sheets (Microsoft, USA).

RESULTS

The microclimatic parameters of the WWTPs included in the analysis were divided into 3 categories (Table 3), taking into account the values of outdoor temperature on the measurement day:
- ≤10°C,
- 11–19°C,
- ≥20°C.

The lowest indoor and outdoor temperatures were recorded for WWTP No. 2 (7.5°C) and WWTP No. 1 (3.8°C), whereas the highest indoor and outdoor temperatures were measured in WWTP No. 12 (28.3°C and 29.5°C, respectively).

The relative humidity in indoor air ranged 41–72%, with the highest value (71.6%) being noted in WWTP No. 15 and the lowest (41.3%) in WWTP No. 4. The RH values recorded indoor were at a similar level as outdoor.

When analyzing the CO_2 concentration in indoor air, the highest value was recorded in WWTP No. 10, where it reached 1572 ppm. This concentration was almost 4 times higher than in the other WWTPs, where these values ranged 375–667 ppm.

The indoor airflow values ranged 0.020–0.855 m/s; but only in 3 WWTPs these values exceeded 0.150 m/s. The range of the outdoor airflow values varied between 0.250–1.415 m/s.

Table 4 presents the occurrence of *S. aureus* bacteria in the wastewater samples and the results of the antibiotic-resistance analyses of these isolates. The presence of *S. aureus* bacteria was confirmed in 12 samples: in 11 samples of raw wastewater and in 1 sample of treated wastewater.

In the study, a total of 2805 bacteria isolates from the air and wastewater samples were analyzed to confirm the species, and 574 isolates were identified as *S. aureus*

Table 3. Microclimatic parameters in the indoor and outdoor environment in the wastewater treatment plants (WWTPs), and presented by outdoor temperature, in the study on the *Staphylococcus aureus* presence in bioaerosol in the WWTPs environment in 2017 in Poland (N = 16)

Range T_{OUT} [°C]	WWTP code	Month of sampling	Air samples [n]	Indoor parameters				Outdoor parameters			
				T [°C]	RH [%]	CO_2 [ppm]	AF [m/s]	T [°C]	RH [%]	CO_2 [ppm]	AF [m/s]
≤10	1	April	11	7.9	56.2	667	0.105	3.8	60.6	519	0.625
	2	April	11	7.5	61.2	592	0.150	6.7	46.0	487	0.835
	5	May	15	–	–	–	–	7.8	49.5	476	0.590
11–19	3	April	10	16.0	42.5	444	0.140	15.5	45.2	440	0.860
	4	April	17	17.2	41.3	502	0.100	16.7	40.5	467	0.630
	6	May	17	11.5	53.0	484	0.135	13.0	39.1	465	1.750
	7	May	14	–	–	–	–	19.5	45.2	364	1.415
	10	June	16	18.6	60.7	1572	0.150	17.3	62.8	512	0.940
	13	June	12	–	–	–	–	18.2	57.9	420	0.605
≥20	7	May	4	20.1	47.7	375	0.435	–	–	–	–
	8	May	15	21.6	48.0	398	0.020	21.3	41.8	380	0.440
	9	May	21	23.9	61.8	446	0.025	28.0	53.3	380	1.450
	11	June	15	20.9	50.0	508	0.855	21.5	44.5	357	0.175
	12	June	17	28.3	49.6	509	0.265	29.5	50.4	423	1.025
	13	June	6	20.2	58.1	436	0.070	–	–	–	–
	14	June	13	–	–	–	–	22.7	44.9	390	1.110
	15	July	21	21.0	71.6	444	0.050	21.9	70.4	432	0.250
	16	August	18	–	–	–	–	20.1	49.0	470	0.310

AF – air flow, RH – relative humidity, T – temperature, T_{OUT} – outdoor temperature.
In WWTPs No. 5, 14 and 16, the whole installation was organized outdoor (no indoor environment).

Table 4. Concentrations and antibiotic resistance of the *Staphylococcus aureus* isolates in wastewater samples collected from the wastewater treatment plants (WWTPs) involved in the study on the *S. aureus* presence in bioaerosol in the WWTPs environment in 2017 in Poland (N = 33)

WWTP code	Type of wastewater	*S. aureus* concentration* [CFU/ml]	Antibiotic-resistant isolates [n]	Antibiotics	Isolates resistant to the given antibiotics [%]**	MRSA isolates [n]
5	raw	73	2	penicillin	2.7	–
6	raw	26	2	penicillin, ampicillin	7.7	–
7	raw	28	1	cefoxitin	14.3	1
			1	tetracycline		–
8	raw	36	n/p	n/p	0.0	–
9	raw	54	16	penicillin	33.3	–
			2	penicillin, erythromycin, clindamycin		
10	raw	36	6	penicillin	100	–
			12	penicillin, tetracycline		
11	raw	1215	70	penicillin	4.7	–
			5	gentamicin, amikacin		
			10	penicillin and medium-sensitive to clindamycin		
12	raw	4	2	penicillin, erythromycin, clindamycin	100	–
13	raw	10	2	penicillin, erythromycin, clindamycin	100	–
			1	penicillin		
			1	penicillin, erythromycin, clindamycin, tetracycline		
			1	penicillin, erythromycin, clindamycin, ciprofloxacin		
14	raw	15	4	penicillin	40.0	–
			1	penicillin, gentamicin, amikacin		
			1	penicillin, tetracycline		
	treated	2	1	penicillin	100	–
16	raw	14	1	cefoxitin, erythromycin, clindamycin, tetracycline	85.7	1
			4	erythromycin, clindamycin		
			1	clindamycin		

* The concentration is given as the arithmetic mean from a series of dilutions.
** Calculated on the basis of the number of *S. aureus* isolates in the sample of wastewater.
MRSA – methicillin-resistant *S. aureus*, "–" – not present.

(20.5%). The highest concentration of *S. aureus* bacteria (1215 CFU/ml) was found in raw wastewater from WWTP No. 11. This value is by 2, or in some cases even by 3, orders of magnitude higher than the concentrations found in the other samples of wastewater (ranging 2–73 CFU/ml).

The presence of *S. aureus* was identified in wastewater samples from 11 WWTPs. Among these, in 10 WWTPs in each sample of raw wastewater sample at least 1 antibiotic-resistant isolate was found. Table 5 presents detailed results of *S. aureus* antibiotic resistance in the wastewater and air samples from the WWTPs under analysis. The antibiotic resistance was found for 147 isolates of *S. aureus* from wastewater samples, which accounts for 25.6% of all strains with confirmed species affiliation. The methicillin-resistant strains were identified in 2 WWTPs, in both cases in samples from wastewater (WWTPs No. 7 and 16).

Table 5. The *Staphylococcus aureus* isolates (N = 574) in wastewater and air samples collected from the wastewater treatment plants (WWTPs), and presented by antibiotic resistance, in the study on the *S. aureus* presence in bioaerosol in the WWTPs environment in 2017 in Poland

Type of the sample/antibiotic resistance	*S. aureus* isolates resistant to the given antibiotics (N = 149)		
	n	%	of all *S. aureus* isolates [%]
Wastewater			
penicillin	100	67.1	17.6
penicillin, tetracycline	13	8.7	2.3
penicillin, clindamycin	10	6.7	1.7
penicillin erythromycin, clindamycin	6	4.0	1.0
gentamicin, amikacin	5	3.4	0.9
erythromycin, clindamycin	4	2.7	0.7
penicillin, amikacin	2	1.3	0.3
clindamycin	1	0.7	0.3
cefoxitin, erythromycin, clindamycin, tetracycline	1	0.7	0.2
penicillin, gentamicin, amikacin	1	0.7	0.2
penicillin, erythromycin, clindamycin, ciprofloxacin	1	0.7	0.2
penicillin, erythromycin, clindamycin, tetracycline	1	0.7	0.2
tetracycline	1	0.7	0.2
cefoxitin	1	0.7	0.2
Air			
penicillin, ampicillin	1	0.7	0.2
clindamycin	1	0.7	0.2

Table 6. Concentrations and antibiotic resistance of the *Staphylococcus aureus* isolates in air samples collected from the wastewater treatment plants (WWTPs) involved in the study on the *S. aureus* presence in bioaerosol in the WWTPs environment in 2017 in Poland

WWTP code	*S. aureus* concentration [CFU/m^3]	Antibiotics	*S. aureus* isolates resistant to the given antibiotics [n]
6	10	penicillin, ampicillin	1
7	5	clindamycin	1

Among 149 isolates of *S. aureus* which were found to be antibiotic resistant in wastewater samples, as many as 100 were resistant only to penicillin, whereas 34 isolates were multi-antibiotic-resistant (to penicillin and other antibiotics). Furthermore, isolates of penicillin-resistant *S. aureus* were revealed in the samples of air and treated wastewater (in 2 different WWTPs).

In the WWTPs under analysis, 2 MRSA isolates were found in raw wastewater samples, 1 in WWTP No. 7, and 1 in WWTP No. 16. In the case of the MRSA isolated in WWTP No. 16, the strain exhibited the constitutive macrolide-lincosamide-streptogramin B (MLS_B) resistance.

When analyzing the seasonality impact, it was shown that *S. aureus* strains were not found in those WWTPs where sampling was conducted in early spring (April). But in May, despite persistently low temperatures (even <10°C), the presence of these pathogens was confirmed.

Table 6 presents detailed results of the microbiological analysis including antibiotic resistance of air samples from the WWTPs under analysis. Although MRSA strains were not found in the air samples, the presence of antibiotic-resistant bacteria was confirmed: 1 to penicillin and ampicillin, and 1 to clindamycin.

In both WWTPs, antibiotic-resistant bacteria were isolated from the air at the initial stages of wastewater treatment (during mechanical treatment). The first sample was collected in a grating building with a wastewater discharge point (a pumping station) on the outflow to the sand catcher, whereas the second sample was collected in a wastewater pumping station in the external uncovered well (sampling performed during wastewater discharge). Sampling in the first WWTP was performed in temperatures of 11°C and 13°C, and in the second WWTP in temperatures of 20°C and 19°C, indoor and outdoor, respectively. Both samples in which *S. aureus* was identified were collected using the impact method. Considering the amounts of wastewater flowing in (the wastewater system) and delivered to (the wastewater tanker) the WWTP throughout the year, both WWTPs were within the mean values for all studied plants. To both WWTPs wastewater was discharged from hospitals (at least 1), and from multi- and single-family buildings situated in the areas with ILF. It is worth noting that WWTP No. 7 was situated in the area of a particularly dense ILF.

Table 7 presents an analysis of the relationship between outdoor temperature and the presence of the *S. aureus* isolates, including antibiotic-resistant bacteria in wastewater samples from the WWTPs under analysis.

Table 7. Concentration and antibiotic resistance of the *Staphylococcus aureus* isolates in wastewater samples collected from the wastewater treatment plants (WWTPs), and presented by outdoor temperature, in the study on the *S. aureus* presence in bioaerosol in the WWTPs environment in 2017 in Poland

T_Z [°C]	Sampling month	WWTP code	Type of wastewater	*S. aureus* (N = 33)				WWTPs [%]	
				concentration [CFU/ml]	antibiotic-resistant isolates [n (%)]	antibiotic resistance	MRSA isolates [n]	*S. aureus*	antibiotic-resistant *S. aureus*
≤10								33.3	33.3
3.8	IV	1	s	–	–	–	–		
			o	–	–	–	–		
6.7	IV	2	s	–	–	–	–		
			o	–	–	–	–		
7.8	V	5	s	73	2 (2.7)	penicillin	–		
			o	–	–	–	–		
11–19								66.7	66.7
13.0	V	6	s	26	2 (7.7)	penicillin, ampicillin	–		
			o	–	–	–	–		
15.5	IV	3	s	–	–	–	–		
			o	–	–	–	–		
16.7	IV	4	s	–	–	–	–		
			s^m	–	–	–	–		
			o	–	–	–	–		
17.3	VI	10	s	36	18 (100)	– 12 to penicillin, tetracycline – 6 to penicillin	–		
			o	–	–	–	–		
18.2	VI	13	s	10	5 (100)	– 2 to penicillin, erythromycin, clindamycin – 1 to penicillin erythromycin, clindamycin – 1 to penicillin, erythromycin, clindamycin, ciprofloxacin	–		
			o	–	–	–	–		
19.5	V	7	s	28	2 (14.3)	– 1 to tetracycline – 1 to cefoxitin	1		
			o	–	–	–	–		
≥20								85.7	71.4
20.1	VIII	16	s	14	6 (100)	– 4 to erythromycin, clindamycin	1		

						– 1 to cefoxitin, erythromycin, clindamycin, tetracycline – 1 to clindamycin	
			o	–	–	–	–
21.3	V	8	s	36	–	–	–
			o	–	–	–	–
21.5	VI	11	s	1215	85 (4.7)	– 70 to penicillin – 10 to penicillin and medium–sensitive to clindamycin – 5 to 5 gentamicin, amikacin	–
			o	–	–	–	–
21.9	VII	15	s	–	–	–	–
			o	–	–	–	–
22.7	VI	14	s	15	6 (40.0)	– 4 to penicillin – 1 to penicillin, gentamicin, amikacin – 1 to penicillin, tetracycline	–
			o	2	1 (100)	penicillin	–
28.0	V	9	s	54	18 (33.3)	– 16 to penicillin – 2 to penicillin, erythromycin, clindamycin	–
			o	–	–	–	–
29.5	VI	12	s	4	2 (100)	penicillin, erythromycin, clindamycin	–
			o	–	–	–	–

o – treated wastewater, s – raw wastewater, s^m – wastewater after the mechanical cleaning process, T_{OUT} – outdoor temperature, "–" – not present.

The analysis of the obtained results did not show any relationship between the season or month and the presence of *S. aureus* bacteria. However, higher outdoor temperatures can positively correlate with the presence of *S. aureus* in the WWTPs under analysis, and with the antibiotic resistance exhibited by the species. The higher the outdoor temperature, the more WWTPs with the *S. aureus* bacteria presence.

In 3 WWTPs (No. 1, 2 and 5) in which sampling was carried out in the outdoor temperature of ≤10°C, only in 1 sample of raw wastewater (from WWTP No. 5, with the outdoor air temperature of 7.8°C) *S. aureus* was identified in a concentration of 73 CFU/ml, and 3 isolates exhibited resistance to penicillin.

In 6 WWTPs (No. 3, 4, 6, 7, 10 and 13) in which sampling was carried out in the outdoor temperatures ranging 11–19°C, only in 2 plants (No. 3 and 4) *S. aureus* was not found. In the other raw wastewater samples, *S. aureus* was present in a concentration of 10–36 CFU/ml. The highest number of antibiotic-resistant isolates was found in the raw wastewater sample from WWTP No. 10, where 6 isolates resistant to penicillin were found, along with 12 isolates exhibiting multi-antibiotic resistance to penicillin and tetracycline. The presence of MRSA was shown in the sample of raw wastewater collected from WWTP No. 7 in the outdoor air temperature of 19.5°C.

Sampling in 7 plants (No. 8, 9, 11, 12, 14, 15 and 16) was carried out in the outdoor temperature of >20°C. In this group, only in the case of the samples of wastewater coming from 1 WWTP (No. 15) *S. aureus* bacteria were not found. In the sample of raw wastewater from WWTP No. 16, where the outdoor air temperature reached 20.1°C, 1 isolate of MRSA and 6 other antibiotic-resistant isolates were identified. The highest concentration of *S. aureus* (1215 CFU/ml) was found in the sample of raw wastewater from WWTP No. 11, where the outdoor air temperature reached 21.5°C. In this sample, the highest amount of antibiotic-resistant isolates were found, including: 70 resistant to penicillin, 5 multi-antibiotic-resistant to gentamicin and amikacin, as well as 10 isolates resistant to penicillin and medium-sensitive to clindamycin.

DISCUSSION

Notably, WWTPs constitute a significant link in the chain of antibiotic-resistance genes transmission in the environment [16]. The workers who become carriers as a result of their occupational exposure transmit pathogenic and antibiotic-resistant bacteria to their homes. This, according to Davis et al. [21], can be crucial for the further environmental fate of these strains. In the next step, both workers and their family members can transmit the pathogen to the hospital environment (e.g., during hospitalization), where they may constitute a source of nosocomial infections. Another route of transmission of these bacteria in the environment are WWTPs, in which the treated water is usually discharged to natural water basins and wastewater sludge used in agriculture to fertilize the cultivated fields. Both treated water and sludge can be contaminated by viable bacteria and antibiotic-resistance genes.

The analysis of the seasonality impact did not, on the one hand, indicate any relationship between the season or the sampling month and the presence of *S. aureus* bacteria. On the other hand, it was shown that outdoor temperatures could positively correlate with the presence of *S. aureus* bacteria. Of note is the fact that *S. aureus* was not found in those WWTPs where sampling was conducted in early spring (April) while in May, despite persistently low temperatures (even <10°C), the pathogens were found in wastewater samples. This is probably due to the wide range of temperatures (7–48°C, with the optimum temperature of 37°C) in which the *S. aureus* strains exhibit a high survival rate. Besides, the municipal wastewater pH usually ranges 6.5–9, while *S. aureus* is capable of surviving in the pH of 4–10, with the optimum pH of 6–7 [22]. Although in 1 WWTP where sampling was conducted in a warm outdoor temperature (July) *S. aureus* was not isolated either, that case may, with a high probability, be considered as accidental. Fracchia et al. [23], in the study aimed to assess bacterial aerosol in WWTPs, revealed the presence of *S. aureus* in the air in the summer and winter seasons, but no correlation was found between the season and the bacteria airborne concentration.

The analysis of the concentration of CO_2 in 1 of the studied WWTPs showed a relatively high result (>1500 ppm), which points to a wrong operation of the ventilation system and the lack of the proper air exchange. The lack of correct ventilation, apart from increased concentrations of chemical compounds which may be toxic to humans, also causes high airborne concentrations of microorganisms, which considerably enhances the risk of workers' infections [24,25].

Airflow is another significant microclimatic parameter. A strong airflow around the treated wastewaters induces the formation of bioaerosol in the workers' breathing zone. When analyzing this parameter, apart from single cases, no particularly intense air movement was noted.

This fact may lead to the following conclusions: 1) bioaerosol formation is not particularly intensive, and 2) there is poor ventilation on these premises.

Airborne antibiotic-resistant strains of *S. aureus* were isolated only in 2 WWTPs. In both cases, the bacteria were found at the initial stage of wastewater treatment, at their flow to the plant at the mechanical stage of wastewater treatment. This indicates that the most exposed workers of the plants are those employed in mechanical treatment of wastewater flowing to the WWTP (pumping stations, grids, sand catchers) and those carrying out repair works in the area of the whole installation. Sampling in both WWTPs was performed in indoor and outdoor temperatures ranging 10–20°C. Both samples in which *S. aureus* was identified were collected using the impact technique. However, considering the amounts of wastewater flowing in, and delivered to, the WWTP throughout the year, both plants did not differ from the mean values obtained for all the studied WWPTs. Based on literature, the significant source of antibiotic-resistant *S. aureus* are effluents from hospitals and ILF [13,15,26]. Wastewater from hospitals, as well as from multi- and single-family buildings situated in areas with ILF, was discharged to both WWTPs in which airborne antibiotic-resistant strains of *S. aureus* were identified. It is worth noting that WWTP No. 7 is located in the area of a particularly dense ILF.

The presence of antibiotic-resistant *S. aureus* in wastewater in WWTPs has been confirmed around the world [14,15,18,19]. The analysis of the antibiotic sensitivity of isolated *S. aureus* bacteria, as performed in this study, revealed that the bacteria were most often resistant to penicillin. Penicillin is a drug belonging to the group of β-lactam antibiotics, similarly to ampicillin and cefoxitin to which the *S. aureus* bacteria isolated from wastewater were also resistant [27–29]. In 1 of the samples of raw wastewater, the MRSA strain was isolated, which exhibited a constitutive MLS_B resistance mechanism. This means that macrolids, lincosamides and streptogramins of group B should not be used for the bacteria [30,31]. According to the study on antibiotics which are critically important for medicine, conducted by the World Health Organization (WHO) and published in 2016 [32], antibiotics used in veterinary medicine are divided into 3 categories:

- critically important,
- highly important,
- important.

The bacteria isolated from the air and wastewater in the WWTPs under analysis were resistant to the antibiotics which belong – according to WHO's classification – to the first (6 drugs: amikacin, ampicillin, ciprofloxacin, erythromycin, gentamicin, linezolid, penicillin) or second category (3 drugs: tetracycline, clindamycin, cefoxitin).

The study seems to be particularly important in view of the increasing number of livestock farms in Poland, which pose a major problem for public health. According to the EU legislation [33], municipal wastewater is defined as domestic wastewater or a mixture of domestic wastewater with industrial wastewater, or rain/snow water, discharged to the WWTP through a district/municipal sewage system. Domestic wastewater mostly contains excretions of human and animal origin, fragments of epidermis, leftovers of foodstuffs, sand, soap and other cosmetics, washing agents and paper. These consist of a lot of organic matter and microorganisms of human and animal origin, acting as nutrient mediums for microorganisms. The composition of microflora present in wastewater depends on many factors, including the basic physical parameters such as temperature, pH, chemical composition, the area which it comes from, and the current epidemiological situation in a given area. In the WWTPs environment, bioaerosol is present both inside buildings and around outdoor installations (in outdoor air). High amounts of bioaerosol are formed in open sewers where wastewater flows to further stages of treatment and, first of all, during mechanical mixing and aeration. Humid and seasonally warm environments create favorable conditions for the accumulation of microorganisms and increase the survival rate of pathogens, especially bacteria [3,34]. In addition, staphylococci are particularly characterized by a high survival rate in unfavorable environmental conditions [35].

Majchrzycka et al. [36] carried out a study on the survival rate of various species of bacteria on the non-woven fabric from filters used in devices protecting the respiratory system. That study indicated that, of all the studied microorganisms at the mass humidity of 40–200%, the *S. aureus* species exhibited the longest survival rate, which reached the level of 2083–15 796%. These results confirm the presumption that in the WWTPs environment a high availability of humidity, combined with the presence of organic matter, contributes to a long survival rate of *S. aureus*. This study confirmed that these bacteria are present in the WWTPs environment and workers are exposed to this pathogen.

According to the data published by Statistics Poland, 3268 municipal WWTPs were in operation in Poland in 2015 [37]. The WWTPs which are qualified by Central Statistical Office to the "the supply of water; wastewater and waste management; reclamation" category, employed approx. 137 000 people in total in 2016 [38]. Although the present study monitored 16 WWTPs, accounting for <0.5% of all installations of this type in Poland, some important outcomes were obtained. Among the 16 WWTPs under analysis, the presence of pathogenic species was confirmed in the discharged wastewater in 11 plants (69%); in 10 WWTPs (62%) the antibiotics resistance of *S. aureus* was found, and in 2 plants (12%) the bacteria were also present in bioaerosol at the workstations. It was also shown that in 2 WWTPs the MRSA strains were present in the samples of raw wastewater. When approximating the prevalence of *S. aureus* from this study on the national scale, exposure to the species, including antibiotic-resistant strains, can be seen as a health risk factor even for several dozen thousand employees of WWTPs. Additionally, among the occupational groups exposed to antibiotic-resistant *S. aureus* strains, a hardly estimable number of self-employed people should be also taken into account, who empty cesspools and household sewage treatment plants, and deliver wastewater and sludge to WWTPs. In view of the alerts published in scientific literature all over the world, relating to the increasing number of hard-to-treat infections caused by antibiotic-resistant *S. aureus* strains, especially MRSA strains [35,39,40], activities aimed at strengthening the prevention of adverse health effects, e.g., involving the disinfection of wastewater discharged to WWTPs, should be undertaken.

Summarizing the discussion, it should be pointed out that it is necessary to continue the study related to the presence of *S. aureus* in the occupational environment of WWTPs in an extended scope. The study should be extended so as to analyze surface swabs in terms of the presence of biofilms formed by the bacteria, and primarily to perform biomonitoring among WWTPs workers. The *S. aureus* strains isolated from workers' nasal swabs, as well as the strains isolated from the air, surface and wastewater, should be sequenced and then compared to identify clones. Only the genetic methods of analysis provide an opportunity to confirm the origin of the clones isolated from that environment.

CONCLUSIONS

Although the low number of WWTPs included in the analysis constitutes some limitation, the results lead to the following conclusions:

1. The results of the study confirm the necessity to include workers' exposure to *S. aureus* bacteria, and also to MRSA strains present in wastewater and bioaerosol, in the occupational risk assessment of WWTPs.
2. The highest health risk refers to the workers employed at the stage of mechanical treatment of wastewater delivered to WWTPs (wastewater pumping stations, grids, sand catchers), workers doing repairs all over the plant, and workers delivering wastewater and sludge from households.
3. The presence of *S. aureus* bacteria, including those drug-resistant, in both raw wastewater and the air, was confirmed in outdoor temperatures of >15°C.
4. There are premises to conclude that the presence of strains resistant mostly to the antibiotics which are critically and highly important in veterinary medicine indicates that the source of such bacteria is probably ILF located in the area of WWTPs.

REFERENCES

1. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis. 2006;6:130, https://doi.org/10.1186/1471-2334-6-130.
2. Gupta M, Bisesi M, Lee J. Comparison of survivability of *Staphylococcus aureus* and spores of *Aspergillus niger* on commonly used floor materials. Am J Infect Control. 2017;45(7):717–22, https://doi.org/10.1016/j.ajic.2017.02.014.
3. Conlon BP. *Staphylococcus aureus* chronic and relapsing infections: Evidence of a role for persister cells: An investigation of persister cells, their formation and their role in *S. aureus* disease. BioEssays. 2014;36(10):991–6, https://doi.org/10.1002/bies.201400080.
4. Korzeniewska E. Emission of bacteria and fungi in the air from wastewater treatment plants – a review. Front Biosci (Schol Ed). 2011;1(3):393–407, https://doi.org/10.2741/s159.
5. Seetha N, Bhargava R, Gurjar BR. Gaseous and bioaerosol emissions from municipal wastewater treatment plants. J Environ Sci Eng. 2013;55(4):517–36.
6. Vantarakis A, Paparrodopoulos S, Kokkinos P, Vantarakis G, Fragou K, Detorakis I. Impact on the Quality of Life When Living Close to a Municipal Wastewater Treatment Plant. J Environ Public Health. 2016;8467023, https://doi.org/10.1155/2016/8467023.
7. Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace N. The Human Nasal Microbiota and *Staphylococcus aureus* Carriage. PLoS One. 2010;5(5):10598, https://doi.org/10.1371/journal.pone.0010598.

8. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet. 2010;375(9725):1557–68, https://doi.org/10.1016/S0140-6736(09)61999-1.
9. Von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N Engl J Med. 2001;344(1):11–6, https://doi.org/10.1056/NEJM200101043440102.
10. Bos ME, Verstappen KM, van Cleef BA, Dohmen W, Dorado-García A, Graveland H., et al. Transmission through air as a possible route of exposure for MRSA. J Expo Sci Environ Epidemiol. 2016;26(3):263–9, https://doi.org/10.1038/jes.2014.85.
11. Masclaux FG, Sakwinska O, Charrière N, Semaani E, Oppliger A. Concentration of airborne *Staphylococcus aureus* (MRSA and MSSA), total bacteria, and endotoxins in pig farms. Ann Occup Hyg. 2013;57:550–7, https://doi.org/10.1093/annhyg/mes098.
12. Chipolombwe J, Török ME, Mbelle N, Nyasulu P. Methicillin-resistant *Staphylococcus aureus* multiple sites surveillance: a systemic review of the literature. Infect Drug Resist. 2016;12(9):35–42, https://doi.org/10.2147/IDR.S95372.
13. Karam G, Chastre J, Wilcox MH, Vincent JL. Antibiotic strategies in the era of multidrug resistance. Crit Care. 2016;20(1):136, https://doi.org/10.1186/s13054-016-1320-7.
14. Rosenberg Goldstein RE, Micallef SA, Gibbs SG, Davis JA, George A, Kleinfelter LM, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) detected at four U.S. wastewater treatment plants. Environ Health Perspect. 2012;120(11):1551–8, https://doi.org/10.1289/ehp.1205436.
15. Thompson JM, Gündoğdu A, Stratton HM, Katouli M. Antibiotic resistant *Staphylococcus aureus* in hospital wastewaters and sewage treatment plants with special reference to methicillin-resistant *Staphylococcus aureus* (MRSA). J Appl Microbiol. 2013;114(1):44–54, https://doi.org/10.1111/jam.12037.
16. Naquin A, Shrestha A, Shrestha A, Sherpa M, Nathaniel R, Boopathy R. Presence of antibiotic resistance genes in a sewage treatment plant in Thibodaux, Louisiana, USA. Bioresour. Technol. 2015;188:79–83, https://doi.org/10.1016/j.biortech.2015.01.052.
17. Ferguson D, Smith T, Hanson B, Wardyn S, Donham K. Detection of airborne methicillin – resistant *Staphylococcus aureus* inside and downwind of a swine building, and in animal feed: Potential occupational, animal health, and environmental implications. J. Agromedicine. 2016;21(2):149–53, https://doi.org/10.1080/1059924X.2016.1142917.
18. Kessler R. Superbug hideout: finding MRSA in U.S. Wastewater Treatment Plants. Environ Health Perspect. 2012;120(11):437, https://doi.org/10.1289/ehp.120-a437a.
19. Börjesson S, Matussek A, Melin S, Löfgren S, Lindgren PE. Methicillin-resistant *Staphylococcus aureus* (MRSA) in municipal wastewater: an uncharted threat? J Appl Microbiol. 2010;108(4):1244–51, https://doi.org/10.1111/j.1365-2672.2009.04515.x.
20. Khan ZA, Siddiqui MF, Park S. Current and Emerging Methods of Antibiotic Susceptibility Testing. Diagnostics (Basel). 2019;9(2):E49, https://doi.org/10.3390/diagnostics9020049.
21. Davis M, Iverson S, Baron P, Vasse A, Silbergeld EK, Lautenbach E, et al. Household transmission of meticillin – resistant *Staphylococcus aureus* and other staphylococci. Lancet Infect Dis. 2012;12(9):703–16, https://doi.org/10.1016/S1473-3099(12)70156-1.
22. Stewart CM. *Staphylococcus aureus* and staphylococcal enterotoxins. In: Hocking AD, editors. Foodborne microorganisms of public health significance. 6th ed. Sydney: Australian Institute of Food Science and Technology (NSW Branch): 2003. p. 359–80.
23. Fracchia L, Pietronave S, Rinaldi M, Giovanna Martinotti M. Site – related airborne biological hazard and seasonal variations in two wastewater treatment plants. Water Res. 2006;40(10):1985–94, https://doi.org/10.1016/j.watres.2006.03.016.
24. ASHRAE ANSI/ASHRAE Standard 62.1-2016. Ventilation for Acceptable Indoor Air Quality. Atlanta: American Society of Heating, Refrigerating, and Air-Conditioning Engineers; 2016.
25. Maula H, Hongisto V, Naatula V, Haapakangas A, Koskela H. The effect of low ventilation rate with elevated bioeffluent concentration on work performance, perceived indoor air quality, and health symptoms. Indoor Air. 2017;27(6):1141–53, https://doi.org/10.1111/ina.12387.
26. Friese A, Schulz J, Zimmermann K, Tenhagen B, Fetsch A, Hartung J, et al. Occurrence of livestock – associated methicillin – resistant *Staphylococcus aureus* in turkey and broiler barns and contamination of air soil surfaces in their vicinity. Appl Environ Microbiol. 2013;79(8):2759–66, https://doi.org/10.1128/AEM.03939-12.
27. Chambers HF, DeLeo FR. Waves of Resistance: *Staphylococcus aureus* in the Antibiotic Era Nat Rev Microbiol. 2009;7(9):629–41, https://doi.org/10.1038/nrmicro2200.
28. Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin Invest. 2003;111(9):1265–73, https://doi.org/10.1172/JCI18535.
29. Foster TJ. Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. FEMS Microbiol. Rev. 2017;41(3):430–49, https://doi.org/10.1093/femsre/fux007.
30. Teodoro CRS, Mattos CS, Cavalcante FS, Pereira EM, dos Santos KRN. Characterization of MLSb resistance among

Staphylococcus aureus and *Staphylococcus epidermidis* isolates carrying different SCCmectypesn. Microbiol Immunol. 2012;56:647–50, https://doi.org/10.1111/j.1348-0421.2012.00481.x.
31. Cetin ES, Gunes H, Kaya S, Aridogan BC, Demirci M. Macrolide–lincosamide–streptogramin B resistance phenotypes in clinical Staphylococcal isolates. Int J Antimicrob Agents. 2008;31(4):364–8, https://doi.org/10.1016/j.ijantimicag.2007.11.014.
32. World Health Organization. Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2016–2017 [Internet]. Geneva: The Organization; 2017 [cited 2018 Feb 26). Available from: https://www.who.int/docs/default-source/searo/amr/global-antimicrobial-resistance-surveillance-system-(glass)-report-early-implementation-2016-2017.pdf?sfvrsn=ea19cc4a_2.
33. EUR-Lex [Internet]. EUR-Lex; 2020 [cited 2020 Feb 27]. Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX-:31991L0271&from=EN.
34. Michałkiewicz M. Comparison of wastewater treatment plants based on the emissions of microbiological contaminants. Environ Monit Assess. 2018;190:640, https://doi.org/10.1007/s10661-018-7035-2.
35. Boyce JM. Environmental contamination makes an important contribution to hospital infection. J Hosp Infect. 2007;65(2):50–4, https://doi.org/10.1016/S0195-6701(07)60015-2.
36. Majchrzycka K, Okrasa M, Skóra J, Gutarowska B. Evaluation of the Survivability of Microorganisms Deposited on Filtering Respiratory Protective Devices under Varying Conditions of Humidity. Int J Environ Res Public Health. 2016;13(1):98, https://doi.org/10.3390/ijerph13010098.
37. Central Statistical Office. Employment Protection 2016. Warsaw: The Office; 2016.
38. Central Statistical Office. Employment and remunerations in national economy 2016. Warsaw: The Office; 2016.
39. Agostino JW, Ferguson JK, Eastwood K, Kirk MD. The increasing importance of community-acquired methicillin-resistant *Staphylococcus aureus* infections. Med J Aust. 2017;207(9):388–93, https://doi.org/10.5694/mja17.00089.
40. Chen LF. The changing epidemiology of methicillin-resistant *Staphylococcus aureus*: 50 years of a superbug. Am J Infect Control. 2013;41(5):448–51, https://doi.org/10.1016/j.ajic.2012.06.013.

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