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S. epidermidis strains from artisanal cheese made from unpasteurized milk in Poland - Genetic characterization of antimicrobial resistance and virulence determinants

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ABSTRACT

In Poland artisanal cheese production is an important local economic activity. Artisanal cheese is usually produced using raw cow's milk, animal rennet and salt, without the addition of starter cultures. Coagulase negative staphylococci (CoNS) are often present in artisanal cheeses. Pathogenic potential of some CoNS species, especially *S. epidermidis*, suggests that they could correspond to emerging pathogens. The identified risk factors correspond to virulence, antibiotic resistance and biofilm formation. Therefore, we aimed to characterize *S. epidermidis* isolated along the artisanal raw milk production chain. Seventy artisanal cheeses samples from unpasteurized cow milk purchased in Podlasie and Warmia and Mazury region in Poland, were included in this study. A total of 26 *S. epidermidis* isolates were obtained. Most of them were antimicrobial resistant, such as to penicillin (84,6%), clindamycin (46,2%), tetracycline (42,3%), erythromycin (42,3%) and cefoxitin (26,9%). Only one isolate was susceptible to all antibiotics used in the study. All methicillin resistant *S. epidermidis* strains (26,9%) harbored *mec*A gene. Isolates, phenotypic resistant to tetracycline, harbored at least one tetracycline resistance determinant on which *tet*(M) was most frequent. Moreover, all tetracycline resistant strains harbored Tn*916*-Tn*1545*-like integrase family gene. In the erythromycin resistant isolates, the macrolide resistance genes *erm*C, *ermB* or *msr*A/B were present. Seven strains demonstrated a strong ability to form biofilm and moderate and weak biofilm was demonstrated by 4 strains, whereas 11 of *S. epidermidis* isolates were found to be unable to form a biofilm. All strains producing strong biofilm harbored the *ica*D gene which occurred independently or in combination with the *ica*A. Insertion element IS256, was identified in 15,4% of *S. epidermidis* strains, all of which were multidrug resistant. Arginine Catabolic Mobile Element (ACME) was identified in 13 of the 26 examined strains (50%). Most common was ACME type I (26,9%), followed by type III (15,4%) and type II (7,7%). Our data indicate that *S. epidermidis* are widely present in artisanal cheeses from raw whole cow milk in Poland. Many isolated strains containing more virulence factors and antibiotic resistant and carry mobile genetic elements which represent a potential source of resistance transmission to bacteria in humans.

1. Introduction

Antibiotic resistance is currently one of the most important public health issues. For many years studies on the selection and dissemination of antibiotic resistance have focused mainly on clinical strains. Recently many investigators showed that the food chain can be considered as the main route of transmission of antibiotic resistant bacteria (Chajęcka-Wierzchowska et al., 2014, 2015; Fontes et al., 2013). More specifically, fermented ready-to-eat dairy products made from unpasteurized milk provide a vehicle for antibiotic resistant bacteria with a direct link between the animal indigenous microflora and the human gastrointestinal tract. During the production process, artisanal cheese can be contaminated with pathogenic microorganisms that are transmitted by the workers and from the environment (Pelaéz and Requema, 2005). In Poland one of the most popular kind of artisanal cheese is produced in Podlasie region. This is a rennet cheese produced from the raw cow's whole milk. During the production process raw milk is heated and treated with salt and rennet. When the whey is separated, the cheese is formed and rubbed with salt. After that the brine is dripped off and the cheese is placed onto dry shelf for the maturation process (Ołdak et al., 2017).

Staphylococci are part of the normal human and animal flora, and they are ubiquitous in dairy farming. Staphylococci are a large, heterogeneous group of bacteria, but most research efforts have been

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focused on *Staphylococcus aureus*. The safe limits for *S. aureus* in foods have been set forth by Commission Regulation No. 2073 of 15 November 2003 on microbiological criteria for foodstuffs. It is generally believed that coagulase-negative staphylococci (CoNS) have low pathogenic potential, and they do not have to be enumerated in food products on the European market. Coagulase-negative staphylococci have been generally regarded as saprophytic microflora. The recent increase in the incidence of infections caused by CoNS in humans and animals has challenged the above conviction. CoNS now represent one of the major nosocomial pathogens, with *S. epidermidis* and *S. haemolyticus* being the most significant species (Becker et al., 2014).

Several virulence factors that had been regarded as characteristics of coagulase-positive staphylococci (CPS) are presently also identified in strains that do not produce coagulase. Coagulase-negative staphylococci have a greater ability to adhere to biological materials and form biofilm than CPS. The polysaccharide intercellular antigen (PIA), a gene product encoded by the *ica*ADBC operon, plays a very important role in that process (Dunne, 2002; Nuryastuti et al., 2011). The bacterial sequence insertion element IS256 is also associated with biofilm formation, in particular in multidrug resistant strains. Insertion element IS256 can induce biofilm formation in *S. epidermidis* strains by reversible transposition into genes responsible for biofilm production (Ziebuhr et al., 1999). This insertion element can also increase antibiotic resistance in staphylococci. IS256 has been detected in the *mec* region of methicillin-resistant strains (Arciola et al., 2004). The presence of CoNS in ready-to-eat foods and its biofilm forming ability are often associated with the arginine catabolic mobile element (ACME) which promotes prolonged bacterial colonization and survival under unfavorable conditions. ACME consists of two major gene clusters: the *arc* cluster (*arc*C-*arg*R) and *opp*3 cluster (*opp*3A-*opp*3E). Three ACME types (I, II, III) have been described in *S. epidermidis* (Barbier et al., 2011; Soroush et al., 2016). ACME type I with *arc* and *opp*3 clusters, ACME type II with *arc* cluster, and ACME type III with *opp*3 cluster (Barbier et al., 2011; Diep et al., 2006). The above contributes to the pathogenicity of *S. epidermidis* and the rapid increase in the antibiotic resistance of CoNS.

CoNS in particular *S. epidermidis*, are often resistant to multiple drugs, including methicillin. Methicillin resistance has been reported in 75–90% of hospital isolates of *S. epidermidis* and in 40–60% of *S. aureus* isolates (Diekema et al., 2001). Resistance genes encoded on *S. epidermidis* plasmids are easily transferred to *S. aureus* and other pathogenic strains, which also promotes the adaptability and antibiotic resistance of typical pathogens (Forbes and Schaberg, 1983). Our previous study of a large sample of ready-to-eat foods (858 samples of cold cuts, fish, salads and cheese) demonstrated that CoNS were more often resistant to β-lactams, cefoxitins, macrolides and tetracyclines than coagulase-positive strains of *S. aureus*. Multidrug-resistant (MDR) strains which are resistant to at least two or three antibiotic classes as well as extensively drug-resistant (XDR) strains which are susceptible to only one antibiotic class were also more frequently observed in the evaluated CoNS (Chajęcka-Wierzchowska et al., 2014).

The aim of this work was to study the phenotypic and genotypic antimicrobial resistance profile and virulence factors of *S. epidermidis* isolated from artisanal cheeses from raw whole cow milk in Poland.

2. Materials and methods

2.1. Isolation of Staphylococcus spp. strains

Seventy samples of artisanal, ripening cheeses from unpasteurized cow milk (n = 70) were obtained from local shops, markets and stands in Podlasie and Warmia and Mazury region in Poland and tested for *Staphylococcus* spp. According to ISO 6887-1:1999 samples were representative, 25 g of cheese was weighed (both the skin and the middle part of the cheese were collected) and mixed. Next 10 g were cultured on double concentrated Giolitti-Cantoni broth (Merck Millipore, Germany) incubated overnight at 37 °C. After incubation a loopfull culture were streaked on Baird-Parker agar medium (Merck Millipore, Germany) for *Staphylococcus* spp. isolation. Typical 1 to 5 different colonies were taken and streaked on Rabbit Plasma Fibrinogen - RPF agar plates (Biomerieux, France) to differentiate into coagulase-positive and coagulase-negative strains. All coagulase-negative strains were taken to for further assays.

2.2. Identification of S. epidermidis strains

Total Genomic DNA of isolated strains was extracted using the Genomic Mini DNA Purification Kit (A&A Biotechnology) according to the manufacturer's instructions. Identification of strains was performed by PCR using primers and conditions described previously (Chajęcka-Wierzchowska et al., 2016a). *S. epidermidis* ATCC 49461 was used as control strain in PCR reaction.

2.3. Phenotypic antibiotic resistance

An inoculum of each *S. epidermidis* strain equivalent to 0.5 McFarland scale was swabbed onto the Mueller-Hinton agar plate (Merck, Germany) and the antibiotic disc (Oxoid, Poland) was then placed on the plate followed by 24 hour incubation at 37 °C. The inhibition zone was interpreted according to the Clinical Laboratory Standards Institute (CLSI, 2017) guidelines The tested antibiotics were erythromycin (E-15 μg), clindamycin (DA-2 μg), gentamicin (CN-10 μg), cefoxitin (FOX-30 μg), norfloxacin (NOR-10 μg), ciprofloxacin (CIP-5 μg), tetracycline (TE-30 μg), rifampicin (RD-5 μg), penicillin (P–10U), nitrofurantoin (F-300 μg), linezolid (LZD-30 μg), chloramphenicol (C-30 μg), trimetoprim (W-5 μg), trimethoprim/sulfamethoxazole (SXT-1.25/23.75 μg), tigecycline (TGC-1 μg) and quinupristin/dalfopristin (QDA-15 μg). *Escherichia co*li ATCC 25922 and *S. aureus* ATCC 29213 were used as reference strains for antibiotic disc control.

2.4. Analysis of the molecular mechanisms of antibiotic resistance

For all isolates, the presence of the *mec*A, *tet*K and *tet*L which encode efflux pumps that actively remove antimicrobials from the cell, *tet*M, *erm*B (disrupts antibiotic transport to the cell and modifies the target site for the drug) *erm*A, *erm*C, *mrs*A/B, *aac*A-*aph*D, and *int* (Tn916-Tn1545) was evaluated as described previously (Chajęcka-Wierzchowska et al., 2014, 2015, 2016a). The presence of *bla*Z gene was performed as describe by Rosato et al. (2003).

The PCR products were visualized by electrophoresis on 1.5% agarose gels (Agarose Basica LE) in 1× TBE (Tris-borate-EDTA) buffer stained by 0.5 μg/mL of ethidium bromide (0.5 mg/mL; Sigma-Aldrich) and visualized using the system for the documentation and analysis of fluorescently stained gels G-BOX F3 (Syngene) and analyzed using the program Gene Tools (Syngene).

2.5. Detection of virulence-associated genes in staphylococci and biofilm formation of S. epidermidis

The presence of ACME element and IS256 was confirmed according to Diep et al. (2008), Kozitskaya et al. (2004), and Arciola et al. (2004), respectively. Biofilm formation was evaluated using a quantitative spectrophotometric microtiter plate assay (MPA) as described previously (Chajęcka-Wierzchowska et al., 2016b). Absorbance was read using an Infinite M1000 PRO plate reader (Tecan) at 570 nm. The optical density (ODs) of each strain was obtained by the arithmetic mean of the absorbance of three wells and this value was compared with the cut-off OD (ODc) which was defined as three standard deviations above the mean OD of the negative control. The following classification was used for the determination of biofilm formation: no biofilm production (OD/ODc), weak biofilm production (ODc < OD/2 × ODc), moderate biofilm production (2 × ODc < OD/4 × ODc) and strong biofilm

Table 1
Phenotypic and genotypic antibiotic resistance of *S. epidermidis* strains isolated from artisanal cheeses.

Strains number		Antibiotic resistance genes	Tn916/ Tn1545	Antibiotic resistance phenotypes
1.	*S. epidermidis*	*blaZ*	–	DA, P
2.	*S. epidermidis*	*erm* (*C*), *blaZ*		E, DA, NOR, RD, P
3.	*S. epidermidis*	*mec*(*A*), *tet*(*M*), *msrA/B*, *blaZ*, *aacA-aphD*	*int*	FOX, TE, E, CN, NOR, P
4.	*S. epidermidis*	*mec*(*A*), *erm*(*B*), *tet*(*L*), *tet*(*M*), *blaZ*	*int*	DA, E, FOX, TE, P
5.	*S. epidermidis*	*erm*(*C*), *blaZ*	–	E, TGC, P
6.	*S. epidermidis*	*erm*(*B*), *tet*(*M*), *blaZ*	–	DA, E, TE, P
7.	*S. epidermidis*	*mec*(*A*), *tet*(*M*), *blaZ*, *aacA-aphD*	*int*	FOX, TE, CN, RD, P
8.	*S. epidermidis*	*blaZ*	–	P
9.	*S. epidermidis*	*erm*(*C*), *blaZ*	–	E, RD, NOR, P
10.	*S. epidermidis*	*tet*(*L*), *tet*(*M*), *erm*(*C*), *msrA/B*, *blaZ*	*int*	DA, TE, RD, P
11.	*S. epidermidis*	*tet*(*M*), *msrA/B*, *blaZ*	–	E, TE, SXT, CIP, P
12.	*S. epidermidis*	*blaZ*	–	DA, CIP, P
13.	*S. epidermidis*	*blaZ*	–	E, DA, P
14.	*S. epidermidis*	*blaZ*	–	P
15.	*S. epidermidis*	*erm*(*C*), *blaZ*	–	DA, E, CIP, P
16.	*S. epidermidis*	*tet*(*M*), *tet*(*K*), *aacA-aphD*	*int*	DA, TGC, TE, RD, CN
17.	*S. epidermidis*	*blaZ*	–	–
18.	*S. epidermidis*	*blaZ*	–	CIP, P
19.	*S. epidermidis*	*mec*(*A*),*tet*(*L*), *tet*(*M*), *aacA-aphD*	*int*	DA, FOX, TE, SXT, CN
20.	*S. epidermidis*	*tet*(*M*), *tet*(*K*), *erm*(*C*), *blaZ*	*int*	E, TGC, TE, P
21.	*S. epidermidis*	*mec*(*A*), *blaZ*	–	FOX, P
22.	*S. epidermidis*	*blaZ*	–	RD, NOR, P
23.	*S. epidermidis*	*mec*(*A*)*tet*(*M*), *blaZ*	*int*	DA, FOX, TE, P
24.	*S. epidermidis*	*tet*(*M*), *tet*(*L*), *tet*(*K*)	*int*	E, TE, LZD
25.	*S. epidermidis*	*blaZ*	–	SXT, P
26.	*S. epidermidis*	*mec*(*A*), *blaZ*, *aacA-aphD*	–	DA, FOX, CN, P

E – erythromycin, DA – lindamycin, CN - gentamicin, FOX – cefoxitin, NOR - norfloxacin, CIP - ciprofloxacin, TE - tetracycline, RD – rifampicin, LZD – linezolid, SXT - trimethoprim/sulfamethoxazole, TGC – tigecycline, P – penicillin.

production (4 × ODc < OD) (Stepanovic et al., 2000). Two biofilm related genes were analyzed by simplex PCR assays to detect the presence of *ica*A (intercellular adhesion gene A) and *ica*D (intercellular adhesion gene D) according to Vasudevan et al. (2003).

3. Results

Fifty-six CoNS were isolated from 70 samples of ready-to-eat unpasteurized cheese purchased in retail in Olsztyn (Poland). They were obtained from 45 cheese samples. 1–3 colonies were isolated from each of 45 positive samples. All of these colonies were characterized by different morphology and antibiotic resistance profile. From 56 CoNS strains 26 were classified as *S. epidermidis* and used in further analyses. They were isolated from 26 different cheese samples.

Nearly all (25 of 26) of the examined *S. epidermidis* strains were resistant to at least one antibiotic. Nineteen strains (73.1%) were multidrug resistant (MDR-CoNS), which mean resistant to at least three antibiotics from different classes (Table 1). The highest percentage of strains was resistant to penicillin (84.6%), clindamycin (46.2%), erythromycin and tetracycline (42.3%). Seven CoNS strains (26.9%) were resistant to methicillin (MR-CoNS), which gives cause for concern. Only one *S. epidermidis* strain was susceptible to all tested antibiotics.

The strains characterized by phenotypic resistance to cefoxitin, tetracycline, erythromycin and gentamicin were analyzed for the presence of genes encoding resistance to the above antibiotics. Tetracycline resistance was encoded by the *tet*M gene (42.3%) responsible for

Table 2
Virulence factors of *S. epidermidis* strains isolated from artisanal cheeses.

Strains number		ACME element	IS256 element	*ica*ADBC operon	Biofilm formation
1.	*S. epidermidis*	I (*arc*A, *opp*3)	–	*icaD*	Strong
2.	*S. epidermidis*	II (*arc*A)	IS256	*icaD*	Strong
3.	*S. epidermidis*	III (*opp*3)	–	*icaD*	Moderate
4.	*S. epidermidis*	–	–	–	No biofilm
5.	*S. epidermidis*	–	–	–	No biofilm
6.	*S. epidermidis*	II (*arc*A)	–	*icaD*	Moderate
7.	*S. epidermidis*	–	–	–	No biofilm
8.	*S. epidermidis*	–	–	–	No biofilm
9.	*S. epidermidis*	I (*arc*A, *opp*3)	IS256	*icaA*, *icaD*	Strong
10.	*S. epidermidis*	–	–	–	No biofilm
11.	*S. epidermidis*	–	–	–	No biofilm
12.	*S. epidermidis*	III (*opp*3)	–	*icaD*	Moderate
13.	*S. epidermidis*	–	–	icaA	Weak
14.	*S. epidermidis*	–	–	–	No biofilm
15.	*S. epidermidis*	I (*arc*A, *opp*3)	IS256	*icaA*, *icaD*	Strong
16.	*S. epidermidis*	I (*arc*A, *opp*3)	IS256	*icaA*, *icaD*	Strong
17.	*S. epidermidis*	–	–	–	No biofilm
18.	*S. epidermidis*	I (*arc*A, *opp*3)	–	*icaA*, *icaD*	Strong
19.	*S. epidermidis*	–	–	–	No biofilm
20.	*S. epidermidis*	III (*opp*3)	–	*icaD*	Strong
21.	*S. epidermidis*	III (*opp*3)	–	*icaD*	Moderate
22.	*S. epidermidis*	–	–	–	No biofilm
23.	*S. epidermidis*	–	–	–	No biofilm
24.	*S. epidermidis*	–	–	*icaA*	Weak
25.	*S. epidermidis*	I (*arc*A, *opp*3)	–	*icaD*	Weak
26.	*S. epidermidis*	I (*arc*A, *opp*3)	–	*icaD*	Weak

proteins that protect ribosomes against tetracyclines and, less frequently, by *tet*L (15.4%) and *tet*K (11.5%) genes. All tetracycline-resistant strains also harbored the Tn*916*-Tn*1545*-like integrase family gene. Resistance to macrolides was encoded by *erm*C (23.1%), *msr*A/B (11.5%) and/or *erm*B (7.7%) genes. All methicillin-resistant *S. epidermidis* (MRSE) isolates harbored the *mec*A gene. The resistance of *S. epidermidis* to gentamicin (aminoglycoside class of antibiotics) was conditioned by a gene encoding the *aac*A-*aph*D bifunctional enzyme with acetyltransferase and phosphotransferase activity (19.2%).

All 7 strains producing strong biofilm harbored the *ica*D gene which occurred independently (n = 3) or in combination with the *ica*A gene (n = 4). Strains harboring only *ica*A were characterized by weak biofilm-forming ability. The presence of *ica*ADBC operon genes was not detected in non-biofilm formers (Table 2).

Insertion element IS256, which is also associated with biofilm formation, was identified in 4 strains. All of them were multidrug resistant, and one strain was also resistant to methicillin. A large percentage of *S. epidermidis* strains harbor ACME, a novel genomic island that can increase their ability to colonize both living organisms and inanimate matter. ACME was identified in 13 of the 26 examined strains (50%). Seven *S. epidermidis* strains harbored ACME type I (26.9%), 4 strains – ACME type III (15.4%) and 2 strains – ACME type II (7.7%).

4. Discussion

The results of this study indicate that CoNS are ubiquitous in Polish artisanal cheese made from unpasteurized milk and that *S. epidermidis* accounts for a large proportion of these bacteria. Al-Khafaji and Flayyih (2015) isolated 103 CoNS strains, including 21 *S. epidermidis* strains, from 300 samples of cheese and milk purchased in Baghdad. However, the authors did not specify whether the analyzed milk was raw and what kind of milk was used for making the cheeses. In our study, a

higher proportion of *S. epidermidis* in the bacterial flora could be attributed to the fact that artisanal cheeses are made using traditional methods. Another reason for this may be the fact that the samples come from different areas and countries, in different climates, environments and cultures. According to Schlegelova et al. (2008), *S. epidermidis* is more prevalent in dairy and meat products than in the raw ingredients. The above could point to cross-contamination during the production process because *S. epidermidis* is often carried on the workers' hands and is capable of producing biofilm and surviving in production premises. The antibiotic resistance analysis revealed that 7 strains were resistant to cefoxitin, and all of them harbored the *mec*A gene. The presence of cefoxitin-resistant strains in food gives cause for concern because it is indicative of methicillin-resistant CoNS (MR-CoNS) strains. These strains are phenotypically resistant to all existing β-lactam antibiotics, including penicillins, aminopenicillins, isoxazolyl penicillins (oxacillin, cloxacillin, dicloxacillin, flucloxacillin, nafcillin, cephalosporins, penicillin-derived inhibitors, cephalosporin-derived inhibitors and carbapenems). In *S. epidermidis*, resistance to methicillin, biofilm formation and the presence of the *icaADBC* operon are regarded as important markers that differentiate commensal and pathogenic strains (Prasad et al., 2012). Methicillin-resistant strains are particularly abundant in clinical isolates of CoNS. Up to 85% of *S. epidermidis* strains isolated from this environment are classified as methicillin-resistant (MRSE) (Miragaia et al., 2005; Prasad et al., 2012). Isolates from raw cow's milk are generally characterized by lower resistance to methicillin than clinical isolates (Jaglic et al., 2010). The above seems to suggest that these strains are more likely to be transmitted by humans than raw materials. However, further research is needed to verify this hypothesis. Fontes et al. (2013) isolated a much higher percentage (81.5%) of *mec*A-positive CoNS from cheese in Brazil. In contrast, Resch et al. (2008) did not identify any MR-CoNS strains in a study of German food products.

The vast majority of CoNS isolated from artisanal cheese was resistant to penicillin and harbored the *blaZ* gene. The percentage of CoNS resistant to β-lactams was higher than that found by Sampimon et al. (2011), in whose study, only 14% of 170 staphylococci from raw milk of various ruminant species were resistant to penicillin. It is possible that contamination with resistant strains occurs at later stages of cheese processing due to improper handling and insufficient sanitation. The above would explain the difference between the prevalence of CoNS in bulk milk and dairy products.

The biofilm forming ability of bacteria has a dual significance. From the medical point of view, biofilm formation testifies to a strain's virulence. The components of the biofilm matrix protect bacteria against the host's immune responses (such as phagocytosis) and chemotherapeutic agents (antibiotics). Biofilm formation is probably associated with antibiotic resistance, and strains that are stronger biofilm formers are often multidrug resistant (Sahal and Bilkay, 2014). From the industrial point of view, bacteria that form biofilm on production surfaces pose a considerable problem. They are much more difficult to eliminate, often less sensitive to disinfectants, and they can contribute to cross-contamination in food processing plants (Shi and Zhu, 2009; Zadernowska and Chajęcka-Wierzchowska, 2017). Biofilm formation is largely a strain-specific trait, and in *S. epidermidis*, it is associated with the presence of the *ica*ABDC operon which is responsible for the synthesis of the polysaccharide intercellular adhesin (PIA) (Mack et al., 2004). In our study, all 7 strains that were strong biofilm formers harbored the *ica*D gene or, additionally, the *ica*A gene. The *ica*ABDC operon was not detected in non-biofilm formers. Michu et al. (2011) observed that *S. epidermidis* strains isolated from dairy processing plants were capable of producing biofilm and were possible sources of cross-contamination. They also observed a relationship between biofilm formation and the expression of the *ica* gene under various environmental conditions (Michu et al., 2011). Our results as well as results obtained by other authors on clinical isolates (Qin et al., 2007; Schommer et al., 2011) showed the ability to produce biofilm by *ica*-negative strains. Our data indicate that while the presence of *ica* may be positively correlated with the extent of biofilm accumulation, its presence does not necessarily mean greater biofilm accumulation than may be seen in an *ica*-negative strain.

Gram-positive bacteria are highly adapted for horizontal gene transfer, and they can acquire and spread resistance to antibiotics as well as virulence factors (Chajęcka-Wierzchowska et al., 2018; Grohmann et al., 2003). The pathogenesis of most infections involves a series of consecutive events, including colonization, adhesion to host cells, and resistance to specific and non-specific defense mechanisms. Staphylococci harboring virulence factors cause more severe infections than strains without virulence factors (Watkins et al., 2012). The presence of the *ica*ADBC operon, associated with biofilm formation, in 46.2% of *S. epidermidis* strains isolated from cheese and the presence of insertion element IS256 in 15.4% of the evaluated strains indicates their potential pathogenicity. The insertion sequence element IS256 is a mobile genetic element that often exists in multiple copies on the *S. epidermidis* chromosome (Kozitskaya et al., 2004). Thus, inserted into the host chromosome, it may affect the expression of different genes associated with pathogenesis, such as biofilm formation (Hennig and Ziebuhr, 2010) and antimicrobial resistance (Kozitskaya et al., 2004) and may thus increase the pathogenicity of *S. epidermidis* (Gu et al., 2005).

The acquisition of the genetic island ACME by the staphylococci species seems to provide advantages in terms of host colonization, rather than an enhanced pathogenicity (Miragaia et al., 2009). In the present study, one half of the analyzed *S. epidermidis* strains harbored ACME. This is in accordance with results obtained by Svensson et al. (2011) who evaluated *S. epidermidis* isolates of blood and detected this genetic element in 43% of isolates, while Granslo et al. (2010) detected it in 23% of clinical isolates. However, these authors also detected ACME in many isolates considered as contaminants and they concluded that this genetic element do not seem to be associated with increased pathogenicity of *S. epidermidis*.

5. Conclusions

The present study demonstrated that artisanal cheese made with unpasteurized milk can be a source of virulent and antibiotic-resistant strains of *S. epidermidis*. Further research is needed to analyze the transmission of *S. epidermidis* in Polish artisanal cheese and in the production environment to develop effective programs for protecting consumers against the spread of virulent and resistant bacteria.

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