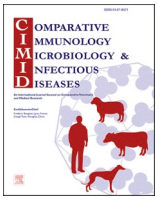
Comparative Immunology, Microbiology and Infectious Diseases 84 (2022) 101785

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid



Occurrence of emerging multiresistant pathogens in the production chain of artisanal goat coalho cheese in Brazil

Breno Bezerra Aragão [a,*], Sabrina Cândido Trajano [a], Raylson Pereira de Oliveira [a], Maria da Conceição Carvalho [a], Rafaella Grenfell de Carvalho [b], Maria Aparecida Juliano [b], José Wilton Pinheiro Junior [a], Rinaldo Aparecido Mota [a]

[a] Federal Rural University of Pernambuco - UFRPE, Department of Veterinary Medicine - R. Manuel de Medeiros, s/n - Dois Irmãos, CEP. 52171-900 Recife, PE, Brazil
[b] Federal University of São Paulo - UNIFESP, Institute of Pharmacology and Molecular Biology - Rua Três de Maio, 100 - Vila Clementino, CEP. 04044-020 São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Handling
Contamination
MALDI-TOF
Multidrug resistance
One health

ABSTRACT

Sanitary-hygienic failures in cheese making can pose health risks to consumers. This study aimed to identify multiresistant pathogens in different production stages of artisanal goat coalho cheese in Brazil and characterize their phenotypic and genotypic resistance. Eleven properties in the state of Pernambuco, Brazil, participated in the study. Samples were obtained from different stages of production and the humans involved. The samples obtained were submitted to microbiological culture, then all the isolated microorganisms were submitted to the Matrix Associated Laser Desorption-Ionization - Time of Flight technique for the microbiological identification of the species. Subsequently, *Staphylococcus* spp., *Enterococcus* spp. and *Macrococcus caseolyticus* were subjected to polymerase chain reaction to search for resistance genes and disc diffusion technique to evaluate the resistance profile. A total of 111 isolates were obtained and 31 species were identified, with the frequency of *Staphylococcus* spp. (62.20%; 69/111), *Enterococcus* spp. (11.60%; 13/111), *Macrococcus caseolyticus* (10%; 11/111), *Bacillus* spp. (3.60%; 4/111), *Enterobacter* spp. (3.60%; 4/111), *Aureobasidium pullulans* (1.80%; 2/111), *Corynebacterium camporealensis* (1.80%; 2/111), *Issatchenkia occidentalis* (1.80%; 2/111), *Kocuria kristinae* (1.80%; 2/111), *Aerococcus viridans* (0.90%; 1/111) and *Filifactor villosus* (0.90%; 1/111). Phenotypic and genotypic resistance was also detected with the occurrence of 15.90% (7/44) of the *mecA* gene, 4% (1/25) *vanA*, and 4% (1/25) *vanB* in *Staphylococcus* spp. and 20% (2/10) *vanB* in and *Enterococcus* spp. Emerging multiresistant pathogens are present in the production chain of artisanal goat cheese and humans, who exert an important role in disseminating these bacteria with imminent risks to human health.

1. Introduction

There is a wide variety of cheeses and production techniques in Brazil, reflecting the historical and cultural aspects of the country [1]. The goat coalho cheese is an example of a typical cheese from the Northeast region of Brazil, manufactured with raw or pasteurized milk. It is a culturally and economically important product of artisanal production which manufacturing technology comes from knowledge passed down from generation to generation following a family tradition. Despite this, the lack of hygiene and production in inadequate environments increases the risk of contamination by pathogenic microorganisms [2].

The quality of goat milk and its derivatives is related to microbiological safety in the different stages of obtaining and processing. Contamination during production stages can pose risks to the consumer [3]. Studies carried out in other countries have reported pathogenic microorganisms at different stages of production and/or in the goat cheese itself, such as *Enterococcus* spp. [4]; *Listeria monocytogenes* [5]; *Staphylococcus* spp. [6] and *Salmonella* sp. [7].

In Brazil, there is little information on the microbiological quality of the steps for obtaining and processing goat milk and cheese and, usually, only the *Staphylococcus* genus [8] or the *S. aureus* specie [9] are identified. However, there is already evidence of multiresistant microorganism's transmission by goat milk and coalho cheese [10].

Studies on the epidemiology and microbiological diversity of multiresistant pathogens in the production chain of artisanal goat coalho

* Corresponding author.
E-mail address: breno.aragao100@hotmail.com (B.B. Aragão).

https://doi.org/10.1016/j.cimid.2022.101785
Received 6 November 2021; Received in revised form 28 February 2022; Accepted 3 March 2022
Available online 4 March 2022

cheese are important for food safety. These studies contribute to a better understanding of measures to control and prevent the spread of pathogens.

This study aimed to identify emerging multiresistant pathogens at different stages of goat artisanal cheese production and characterize their phenotypic and genotypic resistance.

2. Materials and methods

2.1. Sampling

The sampling was of the non-probabilistic type by convenience. The collections were carried out in farms producing artisanal coalho cheese made with raw goat's milk in the Pernambuco state, Brazil. In the period from March to December 2019, samples were collected from swabs from milkers and handlers (nasal fossae and surface of the hands) and also from utensils used to obtain milk (milking buckets and sieves used in milk filtration) and preparation of the coalho cheese (form surfaces and pressing tables) in 11 properties located in seven municipalities (Fig. 1).

For sample collections, sterile swabs soaked in Muller-Hinton broth (Difco Laboratories Inc., Detroit, United States) added with 0.3% sodium chloride were used. These swabs were passed on the surfaces of the hands and nostrils of milkers and handlers, and on utensils used for obtaining milk and manufacturing the coalho cheese. A total of 118 surface swab samples were collected (Table 1).

After collection, the samples were placed in isothermal boxes with recyclable ice and transported to the laboratory for microbiological and molecular analysis.

Table 1
Distribution of samples by analyzed property and sampled steps.

Property	MB	MFS	Milker		CM	CMT	Handler of cheese	
			MH	MNC			HH	HNC
1	2	1	2	2	2	1	1	1
2	2	1	1	1	2	1	1	1
3	1	1	1	1	3	1	1	1
4	1	1	1	1	2	1	1	1
5	2	1	2	2	2	1	1	1
6	1	1	1	1	2	1	1	1
7	1	1	1	1	2	1	1	1
8	2	1	1	1	4	1	2	2
9	1	1	1	1	2	1	1	1
10	2	1	1	1	4	1	2	2
11	2	1	1	1	2	1	1	1
TOTAL	17	11	13	13	27	11	13	13

MB (milking bucket); MFS (milk filtration sieve); MH (milker hands); MNC (milker nasal cavity); CM (cheese molds); CMT (cheese making table); HH (handler hands) and HNC (handler nasal cavity).

2.2. Isolation and bacterial identification

Microbiological isolation of samples from milkers, handlers, and utensils was carried by plating them on streaks on Base agar (Difco Laboratories Inc., Detroit, United States) supplemented with 5% of sheep blood. After plating, the plates were incubated in a microbiological incubator at 37°C for 24–48 h. After this time, plate reading was performed to verify the isolated colonies. For bacterial identification, the *Matrix Associated Laser Desorption-Ionization - Time of Flight* (MALDI-

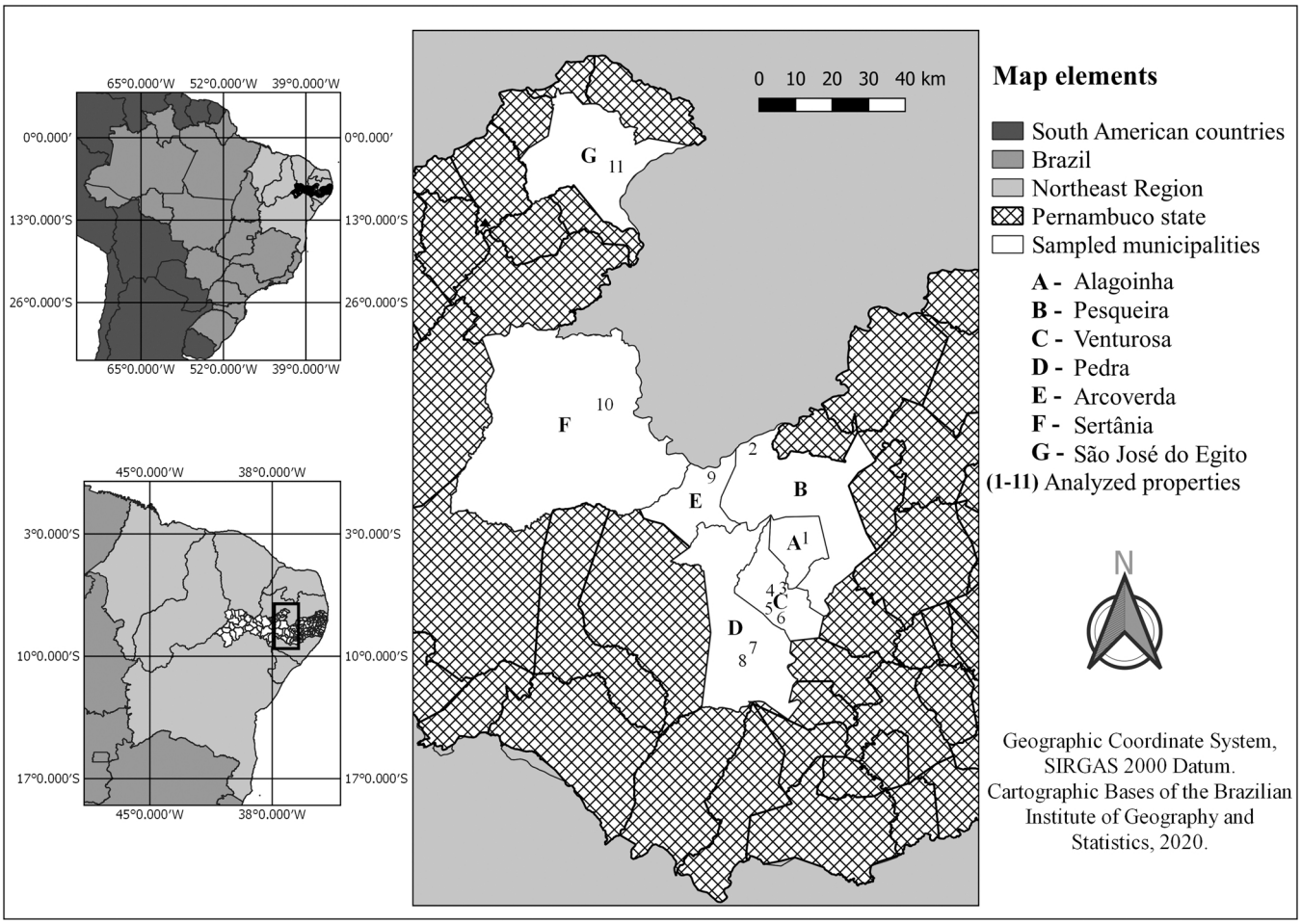


Fig. 1. Geographic distribution of sampled properties by municipalities in the state of Pernambuco.

TOF) technique (Bruker Daltonics®) was used [11]. Raw spectrums were processed using the MALDI Biotyper 3.1 program (Bruker Daltonics®) with default settings.

2.3. Thermal extraction of bacterial DNA

After identifying the microorganisms by MALDI-TOF, the colonies were plated again on Base agar with 5% sheep blood and incubated in a bacteriological incubator at 37ºC for 24 h. After this period, the thermal extraction of the genetic material began. With the aid of a platinum loop, a loop of colonies was collected then transferred to a microtube containing 200 μL of DNA-Free Water (QIAGEN, Hilden, Germany). The content with the bacteria was homogenized and subjected to a temperature of 90 °C for 15 min and, after this period, subjected to a temperature of − 20° for 40 min, after this time it was thawed, homogenized, and centrifuged at 21,000 x g rotation for five minutes. The supernatant was collected and placed in another microtube free of DNA, DNase, and RNase. The genetic material obtained was quantified and analyzed for purity degree in a spectrophotometer with absorbance readings at 260 nm, 230 nm, and 280 nm.

2.4. Analysis of the phenotypic resistance profile

To assess the *in vitro* antimicrobial resistance profile, the Mueller-Hinton agar (Difco Laboratories Inc., Detroit, United States) disc diffusion technique was used [12]. To perform the test on the *Staphylococcus* genus, were used disks impregnated with Penicillin G (10 IU), Amoxicillin + Clavulanic Acid (20/10 μg), Cefoxitin (30 μg), Oxacillin (1 μg), Tetracycline (30 μg), Enrofloxacin (5 μg), Erythromycin (15 μg), Vancomycin (30 μg), and Linezolid (30 μg). For *Enterococcus* spp. disks impregnated with Penicillin G (10 UI), Gentamicin (10 μg), Tetracycline (30 μg), Neomycin (30 μg), Rifampicin (5 μg), Enrofloxacin (5 μg), Erythromycin (15 μg), Vancomycin (30 μg), and Linezolid (30 μg). For *M. caseolyticus* isolates, were used disks impregnated with Penicillin G (10 UI), Cefoxitin (30 μg), and Oxacillin (1 μg). The zone of inhibition was interpreted after 24 h of incubation according to the Clinical and Laboratory Standards Institute [12].

2.5. Resistance gene research

To search for resistance genes, Polymerase Chain Reaction (PCR), with adaptation in reagent concentrations, was employed to amplify specific gene regions. For this, the reactions were adapted to a final volume of 12.5 μL per microtube, containing 100 ng DNA template, primers (10pmol each), and 6.25 μL of Go-TaqGreen Master Mix (Promega®).

Staphylococcus spp. isolates were submitted to gene search for the *blaZ*, *mecA*, *mecC*, *tet(L)*, *tet(M)*, *tet-38*, *msrA*, *norA*, *norB*, *norC*, *vanA,* and *vanB* genes. For bacteria of the *Enterococcus* spp. the following genes were searched *blaZ*, *tet(L)*, *tet(M)*, *msrA*, *norA*, *norB*, *norC*, *vanA,* and *vanB*. *M. caseolyticus* isolates were submitted to PCR to search for the genes *mecA* and *mecC* (Table 2).

Then, 10 μL of the reaction were subjected to electrophoresis for 40 min at 100 V in a 1.5% agarose gel stained with BlueGreen, visualized and photographed in a photo documenter under ultraviolet light. As a positive control were used reference strains to detect specific regions. As a negative control, DNA-Free Water (QIAGEN, Hilden, Germany) was used.

2.6. Statistical analysis

The results of microbiological analysis, polymerase chain reaction and disc diffusion technique were expressed in absolute and relative frequencies [23]. The Epi Info (TM) 3.5.2 program (Centers for Disease Control and Prevention-CDC, Atlanta-USA) was used to perform the statistical calculations.

3. Results

3.1. Microorganisms isolated in the study

In the microbiological analysis, the growth of microorganisms was obtained in 94.06% (111/118) of the analyzed samples. A total of 111 isolates were obtained and 31 species of *Staphylococcus* spp. (62.20%; 69/111), *Enterococcus* spp. (11.60%; 13/111), *M. caseolyticus* (10%; 11/111), *Bacillus* spp. (3.60%; 4/111), *Enterobacter* spp. (3.60%; 4/111), *Aureobasidium pullulans* (1.80%; 2/111), *Corynebacterium camporealensis* (1.80%; 2/111), *Issatchenkia occidentalis* (1.80%; 2/111), *Kocuria kristinae* (1.80%; 2/111), *Aerococcus viridans* (0.90%; 1/111) and *Filifactor villosus* (0.90%; 1/111). The bacterium *S. aureus* had the highest frequency in the artisanal goat coalho cheese production chain (Table 3).

3.2. Microorganisms isolated from human

Regarding the humans, milkers presented a *S. aureus* frequency of 23.07% (3/13) in the hands and 38.46% (5/13) in the nasal cavities; on the other hand, cheese handlers presented a frequency of 15.38% (2/13) in the hands and 46.15% (6/13) in the nasal cavities. *S. epidermidis* had a frequency similar to *S. aureus* in the hands of milkers and handlers and, in the nasal cavities, were detected the frequencies of 46.15% (6/13) in milkers and 38.46% (5/13) in handlers. In addition, the occurrence of several species of bacteria that, until now, had not been reported in the production chain of artisanal goat cheeses was detected (Table 4).

3.3. Phenotypic and genotypic profile of resistance

Regarding resistance genes, *Staphylococcus* spp. from production environments (25 isolates) and humans (44 isolates) presented the following frequencies (Fig. 2). The *mecC* and *tet-38* genes were not detected.

Enterococcus spp. in humans and production environments presented, respectively, the following frequencies 33.33% (1/3) *tet(M)* in

Table 2
Genes, oligonucleotide sequences and size of amplified fragments.

Gene	Sequence (5' – 3')	Fragment Size (pb)	References
blaZ	F- AAGAGATTTGCCTATGCTTCR- GCTTGACCACTTTTATCAGC	517	Sawant et al. [13]
mecA	2 W- TGGTATGTGGAAGTTAGATTGGGAT2X-CTAATCTCATATGTGTTCCTGTATTGGC	155	Nakagawa et al. [14]
mecC	1A- CATTAAAATCAGAGCGAGGC1B- TGGCTGAACCCATTTTTGAT	188	Paterson et al. [15]
tet(L)	F- TCGTTAGCGTGCTGTCATTCR- GTATCCCACCAATGTAGCCG	267	Ng et al. [16]
tet(M)	GTG GAC AAA GGT ACA ACG AGCGG TAA AGT TCG TCA CAC AC	406	Ng et al. [16]
norA	F- TGCAATTTCATATGATCAATCCCR- AGATTGCAATTCATGCTAAATATT	150	Truong-Bolduc et al. [17]
norB	F- ATAAGGTAAGATAACTAGCAR- ATCTCTATTTGCCTCCCTATA	150	Truong-Bolduc et al. [18]
norC	F- ATAAATACCTGAAGCAACGCCAACR- AAATGGTTCTAAGCGACCAA	200	Truong-Bolduc et al. [18]
tet-38	F- TTCAGTTTGGTTATAGACAAR- CGTAGAAATAAATCCACCTG	200	Truong-Bolduc et al. [19]
vanA	F- GGGAAAACGACAATTGCR- GTACAATGCGGCCGTTA	732	Dutka-Malen et al. [20]
vanB	F- GTGACAAACCGGAGGCGAGGAR- CCGCCATCCTCCTGCAAAAAA	430	Clark et al. [21]
msrA	F- TCCAATCATTGCACAAAATCR- AATTCCCTCTATTTGGTGGT	890	Martineau et al. [22]

Table 3
Frequency of isolated microorganisms with their origin and detection by property (humans and production environment).

Microorganisms	Origin by property	Frequency
Aerococcus viridans	MH (4)	(0.90%; 1/111)
Aureobasidium pullulans	HH (7); CMT (7)	(1.80%; 2/111)
Bacillus megaterium	HH (9); HNC (9)	(1.80%; 2/111)
Bacillus mojavensis	MB (11)	(0.90%; 1/111)
Bacillus subtilis	MB (11)	(0.90%; 1/111)
Corynebacterium camporealensis	MFS (6); CMT (6)	(1.80%; 2/111)
Enterobacter asburiae	CMT (5)	(0.90%; 1/111)
Enterobacter cloacae	MB (9); CM (6)	(1.80%; 2/111)
Enterobacter kobei	CMT (9)	(0.90%; 1/111)
Enterococcus durans	CM (9); CMT (9)	(1.80%; 2/111)
Enterococcus faecalis	MB (1); CMT (10); HH (10, 11)	(3.60%; 4/111)
Enterococcus faecium	MB (3); CM (3; 10); MFS (11)	(3.60%; 4/111)
Enterococcus gallinarum	CM (3)	(0.90%; 1/111)
Enterococcus hirae	MH (9)	(0.90%; 1/111)
Enterococcus sulfureus	CMT (3)	(0.90%; 1/111)
Filifactor villosus	MH (1)	(0.90%; 1/111)
Issatchenkia occidentalis	MH (2); CMT (2)	(1.80%; 2/111)
Kocuria kristinae	MB (6)	(1.80%; 2/111)
Macrococcus caseolyticus	MB (2, 3, 4, 7, 11); MH (3); CM (1, 10); CMT (11); HH (1, 3)	(9.90%; 11/111)
Staphylococcus aureus	MB (5, 7); MFS (4, 7); MH (5, 8); MNC (3, 4, 5); CM (4, 7, 8); CMT (4, 7, 8); HH (7, 8); HNC (2, 3, 4, 5, 8, 11)	(23.40%; 26/111)
Staphylococcus capitis	MFS (1); CM (1); CMT (6, 1); HNC (10)	(4.51%; 5/111)
Staphylococcus carnosus	MNC (2)	(0.90%; 1/111)
Staphylococcus cohnii ssp *urealyticus*	MB (1); MH (1)	(1.80%; 2/111)
Staphylococcus epidermidis	MB (1, 10); MH (7, 8, 9); MNC (1, 6, 7, 9, 10, 11); CM (5); CMT (5); HH (6, 7, 8, 10); HNC (1, 6, 7, 8, 10)	(22.50%; 25/111)
Staphylococcus gallinarum	MB (8)	(0.90%; 1/111)
Staphylococcus haemolyticus	MH (6); HH (5)	(1.80%; 2/111)
Staphylococcus hominis	HH (10)	(0.90%; 1/111)
Staphylococcus piscifermentans	CM (10)	(0.90%; 1/111)
Staphylococcus sciuri	MH (11)	(0.90%; 1/111)
Staphylococcus simulans	MH (11)	(0.90%; 1/111)
Staphylococcus warneri	MFS (6, 10); HNC (8)	(2.70%; 3/111)
Total	**111**	**100%**

MB (milking bucket); MFS (milk filtration sieve); MH (milker hands); MNC (milker nasal cavity); CM (cheese molds); CMT (cheese making table); HH (handler hands) and HNC (handler nasal cavity).

E. faecalis; 60% (6/10) *tet(M)*, 10% (1/10) *tet(L)*, 10% (1/10) *norB* and 20% (2/10) *vanB*. The *blaZ*, *msrA*, *norA*, *norC,* and *vanA* genes were not detected. For *M. caseolyticus,* there was no detection of any of the searched genes.

As for phenotypic resistance to antimicrobials, *Staphylococcus* spp. presented the following frequencies, 46.37% (32/69) for penicillin G, 10.14% (7/69) for amoxicillin associated with clavulanic acid, 10.14% (7/69) for cefoxitin and oxacillin, 20.28% (14/69) for tetracycline, 10.14% (7/69) for enrofloxacin, 10.14% (7/69) for erythromycin, and 4.34% (3/69) for vancomycin. No isolate was resistant to linezolid. The isolates of *Enterococcus* spp. presented the following frequencies: 61.53% (8/13) for gentamicin, 38.46% (5/13) for tetracycline, 61.53% (8/13) for neomycin, 30.76% (4/13) for rifampicin, 30.76% (4/13) for enrofloxacin, 23.07% (3/13) for erythromycin, and 15.38% (2/13) for vancomycin. No *Enterococcus* spp. was resistant to penicillin G or linezolid. *M. caseolyticus* isolates showed sensitivity to all tested antimicrobials.

4. Discussion

The detection of microorganisms in all stages of cheese production can be attributed to improper hygienic-sanitary conditions in the production chain. The microbiological safety of artisanal cheeses made with raw goat milk is most associated with the sanitary conditions applied in obtaining the milk and in the stages of cheese manufacturing, hygienic and sanitary failures compromise food safety [24].

The high frequency of *Staphylococcus* spp. it is an indication of compromised microbiological safety of cheeses and inadequate hygienic-sanitary practices in the production chain of goat milk and its derivatives [25]. The species of microorganisms involved in the contamination of the obtaining and elaboration stages of this type are not yet elucidated in Brazil. Due to restricted identification methods, only *S. aureus* has been identified as a contaminating species [9,10]; the other species usually are described as *non-aureus Staphylococcus* [26]. However, using MALDI-TOF we detected the occurrence of other species such as *S. capitis*, *S. carnosus*, *S. cohnii ssp urealyticus*, *S. epidermidis*, *S. gallinarum*, *S. haemolyticus*, *S. hominis*, *S. piscifermentans*, *S. sciuri*, *S. simulans,* and *S. warneri*, which so far had not been reported in this production chain.

In addition to these, six species of *Enterococcus* spp. were identified in the different stages of manufacturing on properties 1, 3, 9, 10, and 11 (Table 3), reinforcing possible sanitary failures and lack of good manufacturing practices application, since the presence of *Enterococcus* spp. in a cheese production environment, it is usually attributed to the lack of hygiene of the people involved in the cheese manufacturing and/or the inadequate health management of the herd [27].

The presence of *M. caseolyticus* in properties 1, 2, 3, 4, 7, 10, and 11 (Table 3) denotes probable health issues in the herds. Although *M. caseolyticus* is not considered a human pathogen, they are known as relevant infectious pathogens in veterinary medicine [28].

Several pathogenic microorganisms detected here have not been reported yet in the cheese-making environment, as is the case of *C. camporealensis* identified in farm 6 (Table 3). *C. camporealensis* has been reported in the literature as responsible for causing subclinical mastitis in sheep [29,30]. It is suggested that *C. camporealensis* is also capable of infecting goats and being carried by milk, as they were isolated in stages of obtaining milk and making cheese. Non-pathogenic environmental contaminating microorganism, such as *B. megaterium*, were also detected [31]; the occurrence of the species *B. mojavensis* and *B. subtilis* in milking buckets on the property 11 is important, as they can produce toxins resistant to high temperatures and cause food poisoning outbreaks [32].

K. kristinae (Property 6), is a pathogenic bacterium responsible for invasive infections in various tissues in humans of any age [33]. *A. viridans* (Property 4), is responsible for infections in humans. It is involved in arthritis, septicemia, endocarditis, and meningitis [34].

Table 4
Frequency of microorganisms isolated from human beings involved in obtaining milk and making goat coalho cheese.

Properties	Milkers	MH	MNC	Handlers of cheeses	HH	HNC
1	1	*F. villosus*	*S. epidermidis*	1	*M. caseolyticus*	*S. epidermidis*
	2	*S. cohnii ssp urealyticus*	*S. epidermidis*			
2	3	*I. occidentalis*	*S. aureus*	2	XXX	*S. aureus*
3	4	*M. caseolyticus*	*S. aureus*	3	*M. caseolyticus*	*S. aureus*
4	5	*A. viridans*	*S. aureus*	4	XXX	*S. aureus*
5	6	*S. aureus*	*S. aureus*	5	*S. haemolyticus*	*S. aureus*
	7	*S. aureus*	*S. aureus*			
6	8	*S. haemolyticus*	*S. epidermidis*	6	*S. epidermidis*	*S. epidermidis*
7	9	*S. epidermidis*	XXX	7	*S. aureus*	*S. epidermidis*
8	10	*S. aureus* and *S. epidermidis*	*S. warneri*	8	*S. aureus*	*S. aureus*
				9	*S. epidermidis*	*S. epidermidis*
9	11	*E. hirae* and *S. epidermidis*	*S. epidermidis*	10	*B. megaterium*	*B. megaterium*
10	12	XXX	*S. epidermidis*	11	*E. faecalis*	*S. epidermidis*
				12	*S. hominis*	*S. capitis*
11	13	*S. sciuri*	*S. epidermidis*	13	*E. faecalis*	*S. aureus*

MH (milker hands); MNC (milker nasal cavity); HH (handler hands); HNC (handler nasal cavity) and XXX (there was no microorganism growth).

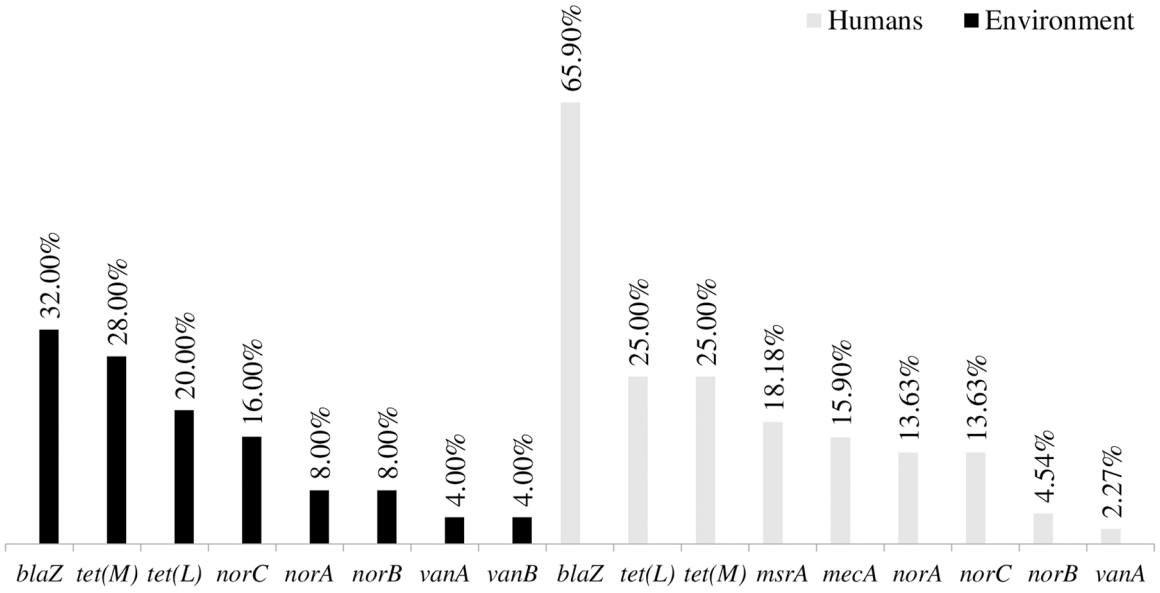


Fig. 2. Frequency distribution of resistance genes in goat cheese and human beings (milkmen and handlers) production environment.

F. villosus is a pathogenic bacterium responsible for periodontal diseases [35].

In the hands of the milker and cheese pressing table (Property 2), yeasts of the species *I. occidentalis* were isolated, which is an important probiotic yeast used in treatments for infections caused by pathogenic yeasts resistant to antifungal agents [36]. *A. pullulans* (Property 7) was also detected, considered a saprophytic fungus that can become an opportunistic pathogen, causing fungemia and compromising the lungs [37].

S. aureus was the most frequent bacteria in our study. Several studies have reported *S. aureus* as the most frequent pathogen in milk and cheese processing environments. Jakobsen et al. [38] analyzed nine farms producing goat cheese in Norway and described a frequency of 98.8%. In the United States, the frequency of *S. aureus* was 67% in 21 properties [39].

In humans, we also detected a high frequency of bacteria of the *Staphylococcus* genus (Table 4) which, even though they constitute the microbiota of the nostrils, the surface of the hands and skin, are used as an indicator of personal hygiene.

The isolation of *E. hirae* in milker 11 and *E. faecalis* in handlers 11 and 13 (Table 4) is also noteworthy. *E. hirae* and *E. faecalis* are considered relevant microorganisms in the microbiota of some artisanal cheeses made with goat milk [4,40]. However, the presence of *Enterococcus* spp. in the elaboration of artisanal cheeses can be considered an indicator of fecal contamination and/or lack of hygiene [41].

Antimicrobial resistance is considered one of the greatest threats to human and animal health. For many years, studies on antimicrobial resistance and the spread of resistant infectious agents have primarily focused on isolates from clinical samples. However, research reveals differentiated dynamics in the food production chain, being considered an important transmission route of antimicrobial-resistant bacteria [42,43].

In *Staphylococcus* spp., the *blaZ* gene was the most frequent; this gene is responsible for conferring phenotypic resistance to β-lactams with unstable rings through the action of β-lactamases [44]. Phenotypically, 46.37% (32/69) of *Staphylococcus* spp. exhibited resistance to penicillin G. Similar results were obtained by Santos et al. [26] in the same Brazilian state for *Staphylococcus* spp. Resistance to β-lactams is attributed to decades of incorrect use in human and veterinary medicine [45].

Regarding amoxicillin associated with clavulanic acid, despite not being used in the treatment of caprine mastitis, it is one of the most indicated antimicrobials in human medicine due to its broad spectrum and potential to fight microorganisms that produce β-lactamases [46]. This potential was reflected in the data obtained, as only 10.14% (7/69) of *Staphylococcus* spp. were resistant to amoxicillin associated with clavulanic acid. The association of β-lactams and clavulanic acid is used

as a therapeutic strategy to enhance the effect of β-lactams and inactivate possible penicillinases [47]. Even with this strategy, *Staphylococcus* spp. carrying *mecA* gene are still able to resist, being resistant to practically all β-lactams (except the latest generation cephalosporins) [48]. *Staphylococcus* spp. *mecA* gene carriers are considered one of the most important pathogens in the food production environment. Obaidat et al. [6] analyzed 26 samples of goat milk collected in cooling tanks in Jordan, and 11.5% (3/26) had *S. aureus* carrying the *mecA* gene. A recent study of *mecA* carrier *Staphylococcus* spp. in China detected a frequency of 16.1% (9/56) [49].

In our study, all *Staphylococcus* spp. *mecA* gene carriers were from humans, four were from the *S. epidermidis* species (MO 9 and 10; FNO 11; FNM 1), and three *S. aureus* (FNO 4 and 5; FNM 5) (Table 4). All were phenotypically resistant to cefoxitin and oxacillin. Humans with methicillin-resistant *Staphylococcus* can cause food contamination if good manufacturing practices are not rigorously applied [50].

In addition to these genes, were detected genotypic (*tet(M)* and *tet (L)*) and phenotypic resistance mechanisms to tetracyclines in *Staphylococcus* spp. and *Enterococcus* spp. The decades of widespread tetracyclines use [16], underdose, environmental contamination [51], and horizontal transference of resistance genes contributed to these increased frequencies over time [52]. *Enterococcus* spp. carriers of the *tet (L)* and *tet(M)* genes were identified in various production stages (Table 3).

Other genes may also be related to resistance to tetracyclines, such as *tet-38*, which chromosomally encodes an efflux pump in *S. aureus*, conferring resistance to this class and certain unsaturated fatty acids [53], however, the acquisition of this gene is associated with resistance to tetracyclines and the ability of the microorganism to survive or not in a given environment [16,53].

The presence of antimicrobial-resistant microorganisms in the production of artisanal cheeses generates an economic loss, as well as, a risk to human and animal health. Strains previously described in hospital infections are now also reported in the food production environment, such as *Staphylococcus* spp. and *Enterococcus* spp. carrying the *msrA* gene [54,55].

Staphylococcus spp. and *Enterococcus* spp. carrying the *msrA* gene are, usually, resistant to macrolides, lincosamides, and streptogramins and are responsible for nosocomial infections of difficult treatment due to the broad resistance spectrum [54]. The presence of *Staphylococcus* spp. *msrA* carriers in artisanal cheese production chains in Brazil is a warning, as it reveals a relevant source of dissemination of this agent to humans. The spread of multiresistant microorganisms in different environments, including food production, may be associated with the inappropriate use and excessive prescription of antibiotics in human and veterinary medicine [45].

The class of fluoroquinolones is widely used in human medicine and the treatment of caprine mastitis [56]; this use induces a selective pressure and the emergence of resistance genes (*norA*, *norB,* and/or *norC*) that confer resistance to quinolones and fluoroquinolones [18]. The frequent use of quinolones and fluoroquinolones caused global dissemination of mutations and resistance mechanisms acquisitions that boosted genotypic and phenotypic resistance processes in several species of *Staphylococcus* spp. and *Enterococcus* spp., and the emergence of several genes from the *nor* class [57].

Despite the selective pressure exerted by antimicrobials, the genetic compatibility of bacteria from different genera is a critical factor for the spread of antimicrobial resistance worldwide. The *vanA* and *van*B genes are examples of this spread. For years, these genes were thought exclusive to human microorganisms, however, genetic compatibility allowed different species of microorganisms to transfer these resistance mechanisms to other species that, in addition to infecting humans, can contaminate different environments [58].

The occurrence of microorganisms carrying the *vanA* and *vanB* genes with phenotypical resistance to vancomycin in the artisanal goat cheese production chain is unprecedented. The *vanA* gene was detected in an isolate of *S. hominis,* from the hands of a handler, and in one *S. cohnii ssp. urealyticus* isolated from a milking bucket. Three isolates carried the *vanB* gene (two *Enterococcus durans* and one *S. warneri*) (Table 3).

The presence of vancomycin-resistant microorganisms in the production environment and/or cheeses can be a severe health risk for humans. According to Russo et al. [59], the importance of vancomycin-resistant *Enterococcus* spp. is related to its ability to infect humans and to transfer genes encoding vancomycin resistance. The genetic analysis determined that horizontal in vivo transfer of vancomycin resistance from *E. faecalis* to *S. aureus* generated the vancomycin-resistant *S. aureus* (VRSA) isolate from Michigan. The acquisition of the *vanA* gene in the Michigan VRSA isolate occurred via interspecies transfer by the transposon Tn1546, gene carrier, of the vancomycin-resistant co-isolate *E. faecalis* [60]. After this transfer, VRSA started to synthesize D-alanyl-D-lactate instead of D-alanyl-D-alanine [61].

In our results, were identified isolates of different *Staphylococcus* spp. species and vancomycin-resistant *Enterococcus durans*, which suggest that these isolates are probably of human origin and that they are possibly infecting humans and animals, in addition to contaminating the manufacturing utensils and/or artisanal goat coalho cheese.

The use of other antimicrobials of the aminoglycoside class (gentamicin and neomycin) and rifamycins (rifampicin) were applied to isolates of *Enterococcus* spp. due to its frequent use in veterinary and human medicine [62]. These classes are composed of important antimicrobials, but incorrect use promoted the emergence of resistance [63], mainly observed in aminoglycosides, where 61.53% of *Enterococcus* spp. were resistant.

In addition to these antimicrobials, linezolid was also tested and, despite the existence of resistant bacteria [64], all isolates evaluated were sensitive, and linezolid was efficient *in vitro* even against isolates of *Enterococcus durans* resistant to vancomycin and *Staphylococcus* spp. resistant to methicillin and vancomycin, of this study.

5. Conclusion

This study identified emerging multiresistant pathogens at different stages of the production chain of artisanal coalho cheese made with goat milk and the importance that people involved in obtaining goat milk and manufacturing artisanal coalho cheese exert in the dissemination of these agents through inadequate hygienic practices, which consequently puts human and animal health at risk.

Ethical clearance

All experimental procedures performed were in accordance with the principles adopted by the Human Ethics Committee of the University of Pernambuco, CAAE license: 94618718.8.0000.5207.

Funding

The study was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for financing the project (Process N° 409107/2018-2).

Author Contributions

BBA and SCT performed the sample collections; BBA, SCT, RPO and MCC were responsible for the microbiological analysis; RGC and MAJ were responsible for performing the MALDI-TOF technique; BBA carried out the writing of the manuscript; JWPJ and RAM were responsible for coordinating, guiding and revising the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for financing the project (Process N° 409107/2018-2) and to the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco for the doctoral scholarship (IBPG-0467-5.05/17).

References

[1] B.A. Kamimura, M. Magnani, W.A. Luciano, F.B. Campagnollo, T.C. Pimentel, V. O. Alvarenga, B.O. Pelegrino, A.G. Cruz, A.S. Sant'ana, Brazilian artisanal cheeses: an overview of their characteristics, main types and regulatory aspects, Compreh. Rev. Food Sci. Food Saf. 18 (2019) 1636–1657, https://doi.org/10.1111/1541-4337.12486.

[2] R.C.R.E. Queiroga, B.M. Santos, A.M.P. Gomes, M.J. Monteiro, S.M. Teixeira, E. L. Souza, C.J.D. Pereira, M.M.E. Pintado, Nutritional, textural and sensory properties of coalho cheese made of goats', cows' milk and their mixture, Lebensm. Wiss. Technol. 50 (2013) 538–544, https://doi.org/10.1016/j.lwt.2012.08.011.

[3] L. Sacarrão-Birrento, A.M.De Almeida, The Portuguese Serrana goat breed: a review, Trop. Anim. Health Prod. 53 (2021) 114, https://doi.org/10.1007/s11250-020-02553-7.

[4] A.M. Martín-Platero, E. Valdivia, M. Maqueda, M. Martínez-Bueno, Characterization and safety evaluation of enterococci isolated from Spanish goats' milk cheeses, Int. J. Food Microbiol. 132 (2009) 24–32, https://doi.org/10.1016/j.ijfoodmicro.2009.03.010.

[5] D. Schoder, D. Melzner, A. Schmalwieser, A. Zangana, P. Winter, M. Wagner, Important vectors for *Listeria monocytogenes* transmission at farm dairies manufacturing fresh sheep and goat cheese from raw milk, J. Food Prot. 74 (2011) 919–924, https://doi.org/10.4315/0362-028X.JFP-10-534.

[6] M.M. Obaidat, A.E. Bani Salman, A.A. Roess, High prevalence and antimicrobial resistance of *mecA Staphylococcus aureus* in dairy cattle, sheep, and goat bulk tank milk in Jordan, Trop. Anim. Health Prod. 50 (2017) 405–412, https://doi.org/10.1007/s11250-017-1449-7.

[7] E. Robinson, M. Travanut, L. Fabre, S. Larréché, L. Ramelli, L. Pascal, A. Guinard, N. Vincent, C. Calba, L. Meurice, M.A. Le Thien, E. Fourgere, G. Jones, N. Fournet, A. Smith-Palmer, D. Brown, S. Le Hello, M.P. La Gandara, F.X. Weill, N.J. Silva, Outbreak of *Salmonella* Newport associated with internationally distributed raw goats' milk cheese, France, 2018, Epidemiol. Infect. 148 (2020) 1–8, https://doi.org/10.1017/S0950268820000904.

[8] C.M.C.G. De Leon, F.G.C. Sousa, M.M.S. Saraiva, P.E.N. Givisiez, N.M.V. Silva, R.F. C. Vieira, C.J.B. Oliveira, Equipment contact surfaces as sources of *Staphylococcus* carrying enterotoxin-encoding genes in goat milk dairy plants, Int. Dairy J. 111 (2020), 104827, https://doi.org/10.1016/j.idairyj.2020.104827.

[9] R.C.S. Castro, A.P.D. Oliveira, E.A.R. Souza, T.M.A. Correia, V.S. Souza, F.S. Dias, Lactic acid bacteria as biological control of *Staphylococcus aureus* in coalho goat cheese, Food Technol. Biotechnol. 56 (2018) 431–440, https://doi.org/10.17113/ftb.56.03.18.5736.

[10] A.P.D. Oliveira, M.M. Costa, D.M. Nogueira, F.S. Dias, Characterisation of *Staphylococcus aureus* strains from milk and goat cheese and evaluation of their inhibition by gallic acid, nisin and velame of the Brazilian caatinga, Int. J. Dairy Technol. 70 (2019) 1–12, https://doi.org/10.1111/1471-0307.12673.

[11] M. Wolters, H. Rohdea, T. Maier, C. Belmar-Campos, G. Frankea, S. Scherpea, M. Aepfelbacher, M. Christner, MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant *Staphylococcus aureus* lineages, Int. J. Med. Microbiol. 301 (2011) 64–68, https://doi.org/10.1016/j.ijmm.2010.06.002.

[12] CLSI - Clinical and Laboratory Standards Institute, 2017. Performance Standards for Antimicrobial Susceptibility Testing, 27th ed. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA (CLSI supplement M100 (ISBN 1–56238-804–5 [Print]; ISBN 1–56238-805–3 [Electronic])).

[13] A.A. Sawant, B.E. Gillespie, S.P. Oliver, Antimicrobial susceptibility of coagulase-negative *Staphylococcus* species isolated from bovine milk, Vet. Microbiol. 134 (2009) 73–81, https://doi.org/10.1016/j.vetmic.2008.09.006.

[14] S. Nakagawa, I. Taneike, D. Mimura, N. Iwakura, T. Nakayama, T. Emura, M. Kitatsuji, A. Fujimoto, T. Yamamoto, Gene sequences and specific detection for Panton-Valentineleukocidin, Biochem. Biophys. Res. Commun. 328 (2005) 995–1002, https://doi.org/10.1016/j.bbrc.2005.01.054.

[15] G.K. Paterson, A.R. Larsen, A. Robb, G.E. Edwards, T.W. Pennycott, G. Foster, D. Mot, K. Hermans, K. Baert, S.J. Peacock, J. Parkhill, R.N. Zadoks, M.A. Holmes, The newly described *mecA* homologue, *mecALGA251*, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species, J. Antimicrob.Chemother. 67 (2012) 2809–2813, https://doi.org/10.1093/jac/dks329.

[16] L.K.I. Ng, M.A. Martin, M. Mulvey, Multiplex PCR for the detection of tetracycline resistant genes, Mol. Cell. Probes 15 (2001) 209–215, https://doi.org/10.1006/mcpr.2001.0363.

[17] Q.C. Truong-Bolduc, X. Zhang, D.C. Hooper, Characterization of *NorR* Protein, a Multifunctional Regulator of *norA* Expression in *Staphylococcus aureus*, J. Bacteriol. 185 (2003) 3127–3138, https://doi.org/10.1128/JB.185.10.3127-3138.2003.

[18] Q.C. Truong-Bolduc, J. Strahilevitz, D.C. Hooper, *NorC*, a new efflux pump regulated by *MgrA* of *Staphylococcus aureus*, Antimicrob. Agents Chemother. 50 (2006) 1104–1107, https://doi.org/10.1128/AAC.50.3.1104-1107.2006.

[19] Q.C. Truong-Bolduc, P.M. Dunman, J. Strahilevitz, S.J. Projan, D.C. Hooper, *MgrA* Is a Multiple Regulator of Two New Efflux Pumps in *Staphylococcus aureus*, J. Bacteriol. 187 (2005) 2395–2405, https://doi.org/10.1128/JB.187.7.2395-2405.2005.

[20] S. Dutka-Malen, S. Evens, P. Couvarlin, Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant Enterococci by PCR, J. Clinic. Microbiol. 33 (1995) 24–27, https://doi.org/10.1128/jcm.33.1.24-27.1995.

[21] N.C. Clark, R.C. Cooksey, B.C. Hill, J.M. Swenson, F.C. Tenover, Characterization of glycopeptide-resistant enterococci from U.S. hospitals, Antimicrob. Agents Chemother. 97 (1993) 2311–2317, https://doi.org/10.1128/AAC.37.11.2311.

[22] F. Martineau, N. Lansac, C. Me, P.H. Roy, M. Ouellette, M.G. Bergeron, Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*, Antimicrob. Agents Chemother. 44 (2000) 231–238, https://doi.org/10.1128/AAC.44.2.231-238.2000.

[23] A. Field. Descobrindo A Estatística Usando O SPSS, Second ed.,, Artmed, Porto Alegre, 2009, p. 688.

[24] D. Tadjine, S. Boudalia, A. Bousbia, Y. Gueroui, G. Symeon, L.M. Boudechiche, A. Tadjine, M. Chemmam, Milk heat treatment affects microbial characteristics of cows' and goats' "Jben" traditional fresh cheeses, Food Sci. Technol. 41 (2020) 1–8, https://doi.org/10.1590/fst.00620.

[25] X. Xing, Y. Zhang, Q. Wu, X. Wang, W. Ge, C. Wu, Prevalence and characterization of *Staphylococcus aureus* isolated from goat milk power processing plants, Food Control 59 (2016) 644–650, https://doi.org/10.1016/j.foodcont.2015.06.042.

[26] A.S. Santos, D.C.V. Lima, A.C.A. Abad, P.R.F. Oliveira, J.G. Silva, G.S. Moura, A.T. F. Silva, V.S. Amorim, M.M. Costa, R.A. Mota, Staphylococci isolates from buffalo, goat and sheep mastitis in the Northeast region of Brazil, J. Dairy Res. 87 (2020) 290–294, https://doi.org/10.1017/S0022029920000771.

[27] A.C. Camargo, E.A. Costa, A. Fusieger, R.D. Freitas, L.A. Nero, A.F.D. Carvalho, Microbial shifts through the ripening of the "Entre Serras" Minas artisanal cheese monitored by high-throughput sequencing, Food Res. Int. 139 (2021) 1–10, https://doi.org/10.1016/j.foodres.2020.109803.

[28] G.L.P.A. Ramos, H.C. Vigoder, J.S. Nascimento, Technological applications of macrococcus caseolyticus and its impact on food safety, Curr. Microbiol. 78 (2021) 11–16, https://doi.org/10.1007/s00284-020-02281-z.

[29] J.F. Fernández-Garayzába, M.D. Collins, R.A. Hutson, I. Gonzalez, E. Fernández, L. Domínguez, *Corynebacterium camporealensis* sp. nov., associated with subclinical mastitis in sheep, Int. J. System. Bacteriol. 48 (1998) 463–468, https://doi.org/10.1099/00207713-48-2-463.

[30] C. Rückert, A. Albersmeier, A. Winkler, A. Tauch, Complete genome sequence of *Corynebacterium camporealensis* DSM 44610, isolated from the milk of a manchega sheep with subclinical mastitis, Genome Announc. 3 (2015) 1–2, https://doi.org/10.1128/genomeA.00572-15.

[31] M. Eppinger, B. Bunk, M.A. Johns, J.N. Edirisinghe, K.K. Kutumbaka, S.S. K. Koenig, H.H. Creasy, M.J. Rosovitz, D.R. Riley, S. Daugherty, M. Martin, L.D. H. Elbourne, I. Paulsen, R. Biedendieck, C. Braun, S. Grayburn, S. Dhingra, V. Lukyanchuk, B. Ball, R. Ul-Qamar, J. Seibel, E. Bremer, D. Jahn, J. Ravel, P. S. Vary, Genome Sequences of the Biotechnologically Important Bacillus megaterium Strains QM B1551 and DSM319, J. Bacteriol. 193 (2011) 4199–4213, https://doi.org/10.1128/JB.00449-11.

[32] C. Apetroaie-Constantin, R. Mikkola, M.A. Andersson, V. Teplova, I. Suominen, T. Johansson, S. Salkinoja-Salonen, *Bacillus subtilis* and *B. mojavensis* strains connected to food poisoning produce the heat stable toxin amylosin, J. Appl. Microbiol. 106 (2009) 1976–1985, https://doi.org/10.1111/j.1365-2672.2009.04167.x.

[33] R.S.Ž. Zarić, A.V. Pejčić, S.M. Janković, M.J. Kostić, M.N. Milosavljević, M. J. Milosavljević, V.D. Opančina, Antimicrobial treatment of *Kocuria kristinae* invasive infections: systematic review, J. Chemother. 31 (2019) 109–119, https://doi.org/10.1080/1120009X.2018.1542551.

[34] I. Ezechukwu, M. Singal, M. Igbinosa O., *Aerococcus Viridans*: case report, microbiology, and literature review, A. J. Case Rep. 20 (2019) 697–700, https://doi.org/10.12659/AJCR.914866.

[35] P. Bathini, S. Foucras, I. Dupanloup, H. Imeri, A. Perna, J.L. Berruex, M.A. Doucey, J.M. Annoni, L.A. Alberi, Classifying dementia progression using microbial profiling of saliva, Diagnos. Assess. Prognos. 12 (2020) 1–5, https://doi.org/10.1002/dad2.12000.

[36] L. Kunyeit, N.K. Kurrey, K.A. Anu-Appaiah, R.P. Rao, Probiotic yeasts inhibit virulence of non-albicans Candida Species, mBio 10 (2019) e02307–e02319, https://doi.org/10.1128/mBio.02307-19.

[37] S.R. Mehta, S. Johns, P. Stark, J. Fierera, Successful treatment of *Aureobasidium pullulans* central catheter-related fungemia and septic pulmonary emboli, IDCases 10 (2017) 65–67, https://doi.org/10.1016/j.idcr.2017.08.017.

[38] R.A. Jakobsen, R. Heggebø, E.L. Sunde, M. Skjervheim, *Staphylococcus aureus* and *Listeria monocytogenes* in Norwegian raw milk cheese production, Food Microbiol. 28 (2011) 492–496, https://doi.org/10.1016/j.fm.2010.10.017.

[39] D.J. D'Amico, C.W. Donnelly, Microbiological quality of raw milk used for small-scale artisan cheese production in Vermont: effect of farm characteristics and practices, J. Dairy Sci. 93 (2010) 134–147, https://doi.org/10.3168/jds.2009-2426.

[40] G. Suzzi, M. Caruso, F. Gardini, A. Lombardi, L. Vannini, M.E. Guerzoni, C. Andrighetto, M.T. Lanorte, A survey of the enterococci isolated from an artisanal Italian goat's cheese (semicotto caprino), J. Appl.Microbiol. 89 (2000) 267–274, https://doi.org/10.1046/j.1365-2672.2000.01120.x.

[41] R. Gelsomino, M. Vancanneyt, M. Cogan, S. Condon, J. Swings, Source of enterococci in a farmhouse raw-milk cheese, Appl. Environ. Microbiol. 68 (2002) 3560–3565, https://doi.org/10.1128/AEM.68.7.3560-3565.2002.
[42] W. Chajęcka-Wierzchowska, A. Zadernowska, B. Nalepa, M. Sierpińska, L. Łaniewskatrokenheim, Coagulase-negative staphylococci (CoNS) isolated from readyto-eat food of animal origin-phenotypic and genotypic antibiotic resistance, Food Microbiol. 46 (2015) 222–226, https://doi.org/10.1016/j.fm.2014.08.001.
[43] L.F. Ribeiro, R.A. Sato, A.S. Pollo, G.A.M. Rossi, L.A. Amaral, Occurrence of methicillin-resistant *Staphylococcus* spp. on Brazilian dairy farms that produce unpasteurized cheese, Toxins 12 (2020) 1–10, https://doi.org/10.3390/toxins12120779.
[44] H.Z. Zhang, C.J. Hackbarth, K.M. Chansky, H.F. Chambers, A proteolytic transmembrane signaling pathway and resistance to beta-lactams in staphylococci, Science 291 (2001) 1962–1965, https://doi.org/10.1126/science.1055144.
[45] R.R. Watkins, R.A. Bonomo, Overview: the ongoing threat of antimicrobial resistance, Infect. Dis. Clin. N. Am. 34 (2020) 649–658, https://doi.org/10.1016/j.idc.2020.04.002.
[46] S.W. Lee, Y. Choi, K. Jang, S.H. Yoon, J.-Y. Chung, Multiple-dose pharmacokinetics and safety of amoxicillin/clavulanate in healthy elderly subjects, Int. J. Clin. Pharmacol. Therapeut. 58 (2020) 634–641, https://doi.org/10.5414/CP203773.
[47] S.M. Drawz, R.A. Bonomo, Three decades of β-lactamase inhibitors, Clin. Microbiol. Rev. 23 (2010) 160–201, https://doi.org/10.1128/CMR.00037-09.
[48] S.J. Peacock, G.K. Paterson, Mechanisms of methicillin resistance in *Staphylococcus aureus*, Annu. Rev. Biochem. 84 (2015) 577–601, https://doi.org/10.1146/annurev-biochem-060614-034516.
[49] H. Cai, X. Kou, H. Ji, X. Wang, H. Wang, Y. Zhang, S. Lu, B. Li, J. Dong, Q. Wang, J. Zhou, D. Hu, Prevalence and characteristics of *Staphylococcus aureus* isolated from Kazak cheese in Xinjiang, China, Food Control 123 (2020), 107759, https://doi.org/10.1016/j.foodcont.2020.107759.
[50] A.G.M. Gonzalez, L.M.P. Marques, M.S.A. Gomes, J.C.C. Beltrão, M.G. Pinheiro, L. M.R. Esper, G.R. Paula, L.A. Teixeira, F. Aguiar-Alves, Methicillin-resistant *Staphylococcus aureus* in minas frescal cheese: evaluation of classic enterotoxin genes, antimicrobial resistance and clonal diversity, FEMS Microbiol. Lett. 364 (2017) 1–7, https://doi.org/10.1093/femsle/fnx232.
[51] J.B. Regitano, R.M.P. Leal, Comportamento e impacto de antibióticos usados na produção animal brasileira, Revista Brasileira de Ciências do Solo 34 (2010) 601–616, https://doi.org/10.1590/S0100-06832010000300002.
[52] A. Akya, R. Chegenelorestani, J. Shavaisi-Zadeh, A. Bozorgomid, Antimicrobial resistance of *Staphylococcus aureus* isolated from hospital wastewater in Kermanshah, Iran, Risk Manag. Healthc. Policy 13 (2020) 1035–1042, https://doi.org/10.2147/RMHP.S261311.
[53] Q.C. Truong-Bolduc, G.R. Bolduc, H. Medeiros, J.M. Vyas, Y. Wang, D.C. Hooper, Role of the *Tet38* efflux pump in *Staphylococcus aureus* internalization and survival in epithelial cells, Infect. Immun. 83 (2015) 4362–4372, https://doi.org/10.1128/IAI.00723-15.
[54] C. Zhao, A. Hartke, M. La Sorda, B. Posteraro, J.-M. Laplace, Y. Auffray, M. Sanguinetti, Role of methionine sulfoxide reductases A and B of *Enterococcus faecalis* in oxidative stress and virulence, Infect. Immun. 78 (2010) 3889–3897, https://doi.org/10.1128/IAI.00165-10.
[55] K. Prabhu, S. Rao, V. Rao, Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples, J. Lab. Phys. 3 (2011) 25–27, https://doi.org/10.4103/0255-0857.62488.
[56] M.C. Lima, M. Barros, T.M. Scatamburlo, R.C. Polveiro, L.K. Castro, S.H. S. Guimarães, S.L. Costa, M.M. Costa, M.A.S. Moreira, Profiles of *Staphyloccocus aureus* isolated from goat persistent mastitis before and after treatment with enrofloxacin, BMC Microbiol. 20 (2020) 1–12, https://doi.org/10.1186/s12866-020-01793-9.
[57] D.C. Hooper, G.A. Jacoby, Mechanisms of drug resistance: quinolone resistance, Ann. N. Y. Acad. Sci. 1354 (2015) 12–31, https://doi.org/10.1111/nyas.12830.
[58] G. Werner, B. Neumann, R.E. Weber, M. Kresken, C. Wendt, J.K. Bender, Thirty years of VRE in Germany – "expect the unexpected": the view from the national reference centre for staphylococci and enterococci, Drug Resist. Updates 53 (2020) 1–12, https://doi.org/10.1016/j.drup.2020.100732.
[59] N. Russo, C. Caggia, A. Pino, T.M. Coque, S. Arioli, C.L. Randazzo, *Enterococcus* spp. in Ragusano PDO and Pecorino Siciliano cheese types: a snapshot of their antibiotic resistance distribution, Food Chem. Toxicol. 120 (2018) 277–286, https://doi.org/10.1016/j.fct.2018.07.023.
[60] L.M. Weigel, D.B. Clewell, S.R. Gill, N.C. Clark, L.K. Mcdougal, S.E. Flannagan, J. F. Kolonay, J. Shetty, G.E. Killgore, F.C. Tenover, Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*, Science 302 (2003) 1569–1571, https://doi.org/10.1126/science.1090956.
[61] F.D. Lowy, Antimicrobial resistance: the example of *Staphylococcus aureus*, J. Clin. Invest. 111 (2003) 1265–1273, https://doi.org/10.1172/JCI18535.
[62] H. Langoni, A. Salina, G.C. Oliveira, N.B. Junqueira, B.D. Menozzi, S.F. Joaquim, Considerações sobre o tratamento das mastites, Pesquisa Veterinária Brasileira 37 (2017) 1261–1269, https://doi.org/10.1590/S0100-736X2017001100011.
[63] I.J. Onakpoya, Antituberculosis and antileprosy medications, Side Eff. Drugs Annu. 42 (2020) 299–308, https://doi.org/10.1016/bs.seda.2020.07.012.
[64] S. Fioriti, G. Morroni, S.N. Coccitto, A. Brenciani, A. Antonelli, V. Di Pilato, I. Baccani, S. Pollini, L. Cucco, A. Morelli, M. Paniccià, C.F. Magistrali, G. M. Rossolini, E. Giovanetti, Detection of oxazolidinone resistance genes and characterization of genetic environments in enterococci of Swine origin, Italy, Microorganisms 8 (2020) 1–19, https://doi.org/10.3390/microorganisms8122021.