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Antimicrobial Susceptibility of Food-Borne Pathogens Isolated from Frequently Consumed Cheeses in Bulgaria

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Abstract

Cheese is essential to the daily human diet. Rich in nutrients such as proteins and carbohydrates, it is the perfect environment for developing microorganisms. The study aimed to screen the antimicrobial resistance of pathogenic microorganisms isolated from imported Bulgarian cheeses. Randomly picked isolates of bacteria and fungi were cultured in selective and differential media. Isolates were identified using the Crystal Gram-positives system, catalase, PYR-test, and API 20 C AUX Candida. Antimicrobial susceptibility screening was performed against 19 antibiotics and three antifungal substances, according to EUCAST-2023 (The European Committee on Antimicrobial Susceptibility Testing). Isolated bacteria were identified as pathogenic *Enterococcus durans*, two strains of *Enterococcus faecalis* 1 and *2*, and *Staphylococcus simulans*. Isolated yeasts belong also to pathogenic *Candida krusei* – four strains, and *Candida glabrata* – one strain. Only one strain - *E. faecalis*-2 showed resistance to three antibiotics from the class of Fluoroquinolones (Ciprofloxacin, Levofloxacin, and Norfloxacin) while the other bacterial strains: *E. faecalis-1*, *E. durans,* and *S. simulans,* were susceptible to all tested antibiotics. The fungal isolates *C. krusei* and *C. glabrata* demonstrated susceptibility to all three tested antifungal drugs.

Keywords: cheese, food-borne pathogens, antibiotic susceptibility, antifungal susceptibility

Резюме

Сиренето е от важно значение за всекидневната човешка диета. Богато на хранителни вещества като протеини и въглехидрати, то е идеалната среда за развитие на микроорганизми. Изследването ни имаше за цел да изучи антимикробната чувствителнст на патогенни микроорганизми, изолирани от вносни и български сирена. Избрани изолати от бактерии и гъбички бяха култивирани върху селективна и диференциална хранителни среди. Изолатите бяха идентифицирани с помощта на системата Crystal Gram-positives, каталаза, PYR-тест и API 20 C AUX Candida. Беше извършен скрининг на изолираните от сирената микроорганизми за антимикробна чувствителност срещу 19 антибиотика и три противогъбични лекарства.Този скрининг беше осъществен съгласно EUCAST-2023 (Европейски комитет за тестване на антимикробната чувствителност). Изолираните бактерии бяха идентифицирани като патогените *Enterococcus durans*, два щама *Enterococcus faecalis* 1 и 2 и *Staphylococcus simulans*. Изолираните дрожди спадат към патогенните *Candida krusei* – четири щама и *Candida glabrata* – един щам. Само един щам - *E. faecalis*-2 показа резистентност към три антибиотици от класа на флуорохинолоните (ципрофлоксацин, левофлоксацин и норфлоксацин), докато другите бактериални щамове *E. faecalis*-1, *E. durans* и *S. simulans* бяха чувствителни към всички тествани антибиотици. Гъбичните изолати *C. krusei* и *C. glabrata* демонстрираха чувствителност и към трите тествани противогъбични лекарства.

Introduction

Cheese microbiota contains more than 500 species of Gram (+) and Gram (-) bacteria, yeasts, and molds identified in it so far (Giraffa, 2002; Almeida *et al*., 2014; Nunes *et al*., 2016; Bastam *et al*., 2021; Timonen *et al*., 2022). Recently, food-borne pathogens were determined in many cheeses from all over the world and produced from cow, ewe, and buffalo milk (de Oliveira *et al.,* 2017; Resende *et al*., 2018; Steinka, 2018; Rosario *et al*., 2021; Klempt *et al*., 2022; Pascu *et al*., 2022; Pas-

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quali *et al*., 2022; Polveiro *et al*., 2022; Szczuka *et al*., 2022;).

The most frequent food-borne bacteria found in cheese are *E. coli*, staphylococci, and enterococci (Jamet *et al.,* 2012; Camargo *et al*., 2021; Feuerstein *et al*., 2022; Polveiro *et al*., 2022). These bacteria cause human skin lesions, gastrointestinal inflammation, lesions of the internal organs, and renal failure. In addition to the presence of pathogenic microorganisms, cheese harbors antimicrobial-resistant bacteria and yeasts. Feeding animals with antibiotic-supplemented feed causes the emergence of antibiotic-resistant bacteria, which further enter the human gastrointestinal tract *via* dairy or meat products (Emes *et al.,* 2022). Antibiotic-resistant bacteria found in cheese were reported by Feuerstein (Feuerstein *et al*., 2022) and Timonen *et al*. (2022). Antifungal-resistant yeasts were described by Bintsis *et al.* (2021), and Emes *et al*. (2022). Besides, antibiotic resistance of enterococci was reported in a series of papers published by Giraffa (Giraffa, 2002), Jamet *et al.* (2012), Perin *et al*. (2014), Gaglio *et al*. (2016), Pasquali *et al*. (2022). Apart from their wide antibiotic resistance, enterococci were proven to infect immunocompromised patients (Chajecka-Wierzchowska *et al.,* 2017). Malti *et al.* (2015) even described that enterococci can cross the brain barrier and can be responsible for brain abscesses. *Staphylococcus aureus* has been manifested as a controversial food-borne pathogen due to its resistance to methicillin (MRSA). MRSA is associated with severe infections in humans and animals, such as bacteremia, wound infections, pyogenic lesions, and mastitis (Podkowik *et al.,* 2013; Sergelidis *et al.,* 2017). MRSA staphylococci were recently identified in cheese, as reported by Steinka (Steinka, 2018). Additionally, staphylococci isolated from traditional raw milk cheeses in Serbia were reported by Bulajic *et al.* (2017). The research group of Casaes Nunes (Nunes *et al*., 2016) documented enterotoxin production and the presence of antimicrobial-resistant staphylococci in Frescal cheese from Brazil, one of the most popular cheeses in this country. *Candida spp*. are frequent spoilers of fresh dairy products like cheese, cream, yogurt, and olives (Satchanska *et al.,* 2019). As reported by Bintsis (Bintsis, 2021), total raw milk yeast counts are generally in the range of 10–10^3 CFU/mL, and yeast genera commonly identified in raw milk include *Candida*, *Cryptococcus*, *Debaryomyces*, *Geotrichum*, *Kluyveromyces*, *Trichosporon*, *Pichia,* and *Rhodotorula*. The same author reported at least six *Candida* species found in cheese: *Candida catenulate*, *Candida etchellsii, Candida globosa, Candida lambica, Candida parapsilosis,* and *Candida zeylanoides*.

Our study aimed to screen the antimicrobial susceptibility of bacteria and yeast isolates cultured from frequently purchased imported and local Bulgarian cheeses.

Materials and Methods

Sample collection and processing

Samples were taken from seven types of high-end quality cheese purchased in big retail stores in Sofia, Bulgaria. Four kinds of cheese were imported from the EU, and three kinds of cheese were local products of Bulgaria (№1 - imported soft blue cow cheese, №2 - imported soft blue cow cheese, №3 - imported soft white goat cheese, №4 - imported soft white goat cheese, №5 – Bulgarian yellow cow cheese, №6 - Bulgarian yellow sheep cheese, and №7 – Bulgarian yellow buffalo cheese). Yellow hard cheese is popular in Bulgarian as “kashkaval”. All samples were analyzed before their expiry date. Samples were prepared as follows: 10g of each sample were aseptically cut and homogenized in 90 ml sterile Ringer’s solution. The solution contained NaCl 7.2g, $CaCl_2$ 0,17g, and KCl 0,37g dissolved into reagent-grade H_2O and brought to 1L. The solution pH was adjusted to 7,3-7,4. The obtained mixture was then heated to 44°C until complete fat dissolution. Afterward, serial dilutions (10^{-1} to 10^{-4}) were prepared, and 0.1ml of each dilution was spread on selective or differential agar.

Faecal Enterococci plating

KEAA (Kanamycin-Esculin-Azide agar) (Merck, Germany) was used to detect fecal enterococci. The medium was autoclaved under standard conditions. As the enterococci grow, they turn the white-colored agar black due to the metabolism of esculin. Enterococcal colonies grew as transparent to white colonies on it. Adding kanamycin to the medium inhibited the growth of other Gram (+) bacteria. The medium was autoclaved at 121°C/1 atm/20 min. After plating 0.1 ml of each dilution on agar, the Petri dishes were incubated for 48 h at 30°C. The experiment was conducted according to ISO/CD 21722.

Staphylococci plating

MSA (Manitol-Salt agar, 6.5% NaCl) (Merck, Germany) was applied to study the genus *Staphylococcus*. The medium was autoclaved at 121°C/1atm/20 min. On the orange-pink-colored agar, staphylococcal colonies grew in golden color, surrounded by a well-defined yellow halo. Inoculated Petri dishes were incubated for 48h at 30°C. The experiment was conducted according to ISO 6888-1:2021.

Fungi (*Candida* spp.) *plating*

Both conventional Candida (Hi-Media, India) and differential chromogenic agar (Hi-Media, India) were used to detect *Candida* spp. Colonies grew black-colored on white agar using conventional agar and white-to-rose-colored when using differential chromogenic agar. While the conventional agar was autoclaved, the chromogenic agar was prepared by boiling according to the manufacturer's instructions. Incubation of the inoculated plates lasted 7 days at 28°C. The experiment was conducted according to ISO 21527-1:2008.

Isolation and identification of pathogenic bacterial and fungal strains

Nine microbial isolates were selected to study their antibiotic and antifungal susceptibility. The random selection was based on the purity of microbial colonies and their morphological characteristics observed on selective and differential agar. The isolated microorganisms were further successfully cultivated on corresponding media and proven to be pure cultures. Identification was done using the Crystal Gram-positives system, the Catalase test, the PYR-test system, chromogenic media, and API 20 C AUX Candida. We used 19 antibiotics and 3 antifungal drugs currently applied in clinical practice, and listed in the EUCAST-2023 (European Committee on Antimicrobial Susceptibility Testing), with valid for 2023 concentrations. The randomly selected colonies were picked up from each plate for further cultivation and identification. Nine strains (4 bacterial and 5 fungal) were chosen for analysis, and were frozen in glycerol at -20°C and stored in the UPIZ "Educational and Research Laboratory of Biology"-MF-NBU's Microbial Collection, New Bulgarian University, Sofia, Bulgaria.

Antibiotic and antifungal susceptibility screening

Antibiotic susceptibility of the isolates was tested *via* the standard disc diffusion method, except for vancomycin and teicoplanin, as prescribed by EUCAST-2023. They were tested using the MIC method. One bacterial colony of each bacterial isolate was inoculated in 50ml liquid Nutrient Broth and cultivated overnight using an orbital shaker (BioSan, Latvia) at 25°C/140 rpm. Each overnight culture was diluted to $1x10^8$ CFU/ml and adjusted to 0.5 McFarland, corresponding to 0.150-0.160 turbidity measured at 600 nm on a Jenway 6315 spectrophotometer (Jenway, UK). Each inoculum of a bacterial isolate was consequently spread on Mueller-Hinton agar using a sterile swab. Antibiotics were applied as antimicrobial susceptibility test disks (Becton Dickinson, USA). Their concentrations corresponded to EUCAST-2023 Guidelines: ampicillin (AMP) – 2 µg, gentamicin (GEN 30) – 30 µg, gentamicin (GEN 10) – 10 µg, amikacin (AMK) – 30 µg, tobramycin (TOB) – 10 µg, cefoxitin (CX) – 30 µg, tetracycline (TCN) – 30 µg, tigecycline (TGC) – 15 µg, trimethoprim/sulfamethoxazole (TMP/SMX) – 25 µg, ciprofloxacin (CIP) – 5 µg, levofloxacin (LVF) – 5 µg, norfloxacin (NFX) – 10 µg, vancomycin (VAN) – 5 µg, teicoplanin (TEC) – 30 µg, linezolid(LNZ) – 10 µg, erythromycin (ERY) – 15 µg, clindamycin (CLI) – 2 µg, imipenem (IMP) – 10 µg, and nitrofurantoin (NIT) – 100 µg.

Following the procedure above, an antifungal susceptibility test was also conducted using the MIC Test Strip method. The following antifungals (HiMEDIA, India) were used: Amphotericin B, Fluconazole, and Micafungin.

All experiments were executed in triplicate.

Results and Discussion

Quality of dairy products regarding milk production, transportation, storage, and handling are under strict control in Europe. However, some of the abovementioned processes can be compromised, reducing the final product quality. The most commonly consumed cheeses in Bulgaria are soft and yellow – imported most from the EU or Bulgarian ones produced from cow, sheep, buffalo, and goat milk. The selection of studied cheeses was based on being frequently purchased by Bulgarians and represented cheeses of the upper-price segment. It should be mentioned that the diversity of cheese in Bulgaria is not as high as in France or Italy. The origin of the examined cheeses is presented in Table 1.

Table 1. Origin of the examined cheeses

№	Sample	Origin
1	Blue cow cheese	Imported from EU
2	Blue cow cheese	Imported from EU
3	White goat cheese	Imported from EU
4	White goat cheese	Imported from EU
5	Yellow cow cheese (Kashkaval)	Bulgarian
6	Yellow sheep cheese (Kashkaval)	Bulgarian
7	Yellow buffalo cheese (Kashkaval)	Bulgarian

Four out of the seven examined cheeses were imported (samples 1-4), while three (samples 5-7) were local Bulgarian products. The enumeration of the total number of viable bacteria, faecal enterococci, staphylococci, *Clostrdium perfringens*, *E. coli* and coliforms, yeasts, and molds was conducted according to the EUCAST-2023 Standard and is described in detail in a previously published paper (Satchanska *et al.,* 2024).

Isolation and identification of bacterial and yeast strains

Selected strains were isolated, cultivated on selective media (enterococci - on KEA agar, staphylococci – on MS agar, *Candida* - on Candida conventional and differential chromogenic agar), and further tested for their antibiotic resistance. Four out of nine isolates were identified as bacteria: *Enterococcus durans*, two strains of *Enterococcus faecalis*, and *S. simulans*. The remaining five isolates were identified as fungi – 4 strains of *Candida krusei* and one *Candida glabrata*. The isolated species and the sample they have been isolated from are listed in Table 2.

Table 2. Bacterial and fungal isolates obtained from cheese samples

№	Sample	Microbial isolate
4	White goat cheese, Imported EU	*E. durans*
		C. glabrata
5	Cow yellow cheese, Kashkaval, BG	*E. faecalis-1*
		E. faecalis-2
		S. simulans
		C. krusei - 1a
		C. krusei - 1b
		C. krusei - 1c
		C. krusei - 2

As seen from Table 2. seven out of nine strains (two strains of *E. faecalis*, one strain of *Staphylococus simulans,* and four strains of *C. krusei*) were isolated from sample № 5 - a Bulgarian cow yellow cheese, named kashakaval. One bactreial strain – *E. durans* and one fungal stran – *C. glabrata* were isolated from sample № 4 - an imported goat white cheese. Both samples (№ 4 and № 5) were microbially contaminated above the standards. Data are shown in Table 3. The results demonstrate that sample № 4 was heavily contaminated with faecal enterococci (however, for faecal enterococci do not yet exist a fixed standard) and *E. coli* and coliforms. It should be highlighted that *Clostridium perfringens* was detected only in this sample among the seven examined. Sample № 5 was contaminated with faecal enterococci and staphylococci.

Four bacterial strains – *E. faecalis*-1*, E. faecalis-2, E. durans,* and *S. simulans* were screened for their antibiotic susceptibility against 19 antibiotics belonging to different groups of antibiotics currently applied in clinics. Data depicted in Table 4 showed that one strain - *E. faecalis*-2, was resistant to all three fluoroquinolones tested - Ciprofloxacin, Levofloxacin, and Norfloxacin while strains *E. faecalis*-1, *E. durans,* and *S. simulans* demonstrated susceptibility to all tested antibiotics.

The antibiotics used in this experiment belong to currently used different classes of antibiotics as follows: aminoglycosides macrolides (inhibiting the bacterial protein synthesis), penicillins, cephalosporins, and glycopeptides (active against the bacterial cell wall synthesis), fluoroquinolones and sulphonamides (suppressing the bacterial nucleic acids synthesis). Five fungal strains - *C. krusei* 1a, *C. krusei* 1b, *C. krusei* 1c, C. *krusei* 2, and C. *glabrata* were studied for their susceptibility against currently used in clinical practice antifungal drugs. Table 5 shows that *C. krusei* isolates 1a, 1b, 1c, and 2 were sensitive to Amphotericin B and Micafungin but are intrinsically resistant to Fluconazole (-*). *C. glabrata* was found to be sensitive to all tested antifungals.

Recently, studies on the microbial quality of cheese were vastly performed due to the high potential of microbial contamination of such types of food. Extensive studies concerning the microbial quality of cheeses from all over the world were reported: Austria (Schornsteiner *et al.,* 2014), Brazil (Nunes *et al.,* 2016; Resende *et al.,* 2018; Camargo *et al.,* 2021), France (Jamet *et al.,* 2012), Germany (Klempt *et al*., 2022), Italy (Almeida *et al*., 2014), Mexico (Escobar-Zepeda *et al.,* 2016), Portugal (Costa *et al.,* 2022; Riquelme *et al.,* 2015),

Table 3. Enumeration of bacteria, yeasts, and molds in samples № 4 and № 5

№	Sample	Total number of bacteria	Faecal enterococci	Staphylococci	*E. coli*	*C. perfringens*	Yeasts and Molds
4	White goat cheese, Imported	$1.4x10^9$	$\geq 10^5$	0	$> 10^5$	$< 10^2$	*Penicillium camemberti*
5	Yellow cow cheese (kashkaval), Bulgaria	$6.9x10^5$	$2.8x10^5$	$\geq 10^5$	0	0	$5.0x10^2$ (yeasts)

Table 4. Antibiotic and antifungal susceptibility of the cheese isolates

№	Antibiotics	*E. faecalis 1*	*E. faecalis 2*	*E. durans*	*S. simulans*	Class
1.	AMP	S*	S	S	Not tested****	Penicillins
2.	GEN 30	S	S	S	Not tested	Aminoglycosides
3.	CIP	S	R**	S	S	Fluoroquinolones
4.	LVF	S	R	S	S	Fluoroquinolones
5.	NFX	S	R	S	S	Fluoroquinolones
6.	VAN	S	S	S	MIC-S***	Glycopeptides
7.	TEC	S	S	S	MIC-S	Glycopeptides
8.	TGC	S	S	S	S	Tetracyclines
9.	LNZ	S	S	S	S	Oxazolidinones
10.	CX	Not tested	Not tested	Not tested	S	Cephalosporins
11.	GEN 10	Not tested	Not tested	Not tested	S	Aminoglycosides
12.	AMK	Not tested	Not tested	Not tested	S	Aminoglycosides
13.	TOB	Not tested	Not tested	Not tested	S	Aminoglycosides
14.	ERY	Not tested	Not tested	Not tested	S	Macrolides
15.	CLI	Not tested	Not tested	Not tested	S	Lincosamides
16.	TCN	Not tested	Not tested	Not tested	S	Tetracyclines
17.	TMP/SMX	Not tested	Not tested	Not tested	S	Sulfonamides
18.	IMP	S	S	S	Not tested	Carbapenems
19.	NIT	S	S	S	S	Nitrofurans

Table 5. Antifungal susceptibility of the cheese isolates

Antifungals	*C. krusei* 1a	*C. krusei* 1b	*C. krusei* 1c	C. *krusei* 2	C. *glabrata*	Class
Amphotericin B	S	S	S	S	S	Polyenes
Fluconazole	- *	-	-	-	S	Triazoles
Micafungin	S	S	S	S	S	Echinocandins

* *C. krusei* is intrinsically resistant to Fluconazole.

Romania (Pascu *et al*., 2022), Spain (Nieto-Arribas *et al.,* 2011), Serbia (Bulajic *et al.,* 2017), Switzerland (Serrano *et al.,* 2018), Estonia (Timonen *et al*., 2022), Turkey (Kahraman-Ilıkkan *et al.,* 2022). Investigations are focused not only on the assessment of certain pathogenic microorganisms in cheese but also on their resistance to antibiotics and antifungals.

Enterococci

E. faecalis, *E. durans,* and *E. casseliflavus* have been reported by Gelsomino *et al.* (2002), Pasquali *et al*. (2022) and found in Cheddar cheese produced from cow milk, and Italian cow artisanal cheese. Besides *E. faecalis* and *E. caseliflavus,* Resende *et al.* (2018) found also *E. faecium* in Brazilian Minas soft cow cheese, and *E. durans* in artisanal cheese from the Western Balkans (Jahansepas *et al.,* 2022). Antibiotic resistance of enterococci was reported in a series of papers published by Giraffa (2002), Jamet *et al.* (2012), Perin *et al.* (2014), Gaglio *et al.* (2016), Pasquali *et al.* (2022). Apart from their wide antibiotic resistance, enterococci were proven to infect immunocompromised patients (Chajecka-Wierzchowska *et al.,* 2017). Malti *et al.* (2015) even described that enterococci can cross the brain barrier and can be responsible for brain abscesses. Our experiments showed that one out of three enterococcal strains showed resistance to three fluoroquinolones. This strain was isolated from Bulgarian yellow cow cheese – kashkaval.

Staphylococci

According to EC Standard 2073/2005, the limit for staphylococci in cheeses produced from raw milk is $1x10^4$-$1x10^5$ CFU/g. Serrano *et al.* (2018) reported $8.8x10^4$ CFU/g of staphylococci in seven Swiss cheeses. *S. aureus* has been manifested as a controversial food-borne pathogen due to its resistance to methicillin (MRSA). MRSA is associated with severe infections in humans, and animals, such as bacteremia, wound infections, pyogenic lesions, and mastitis (Podkowik *et al.,* 2013;

Sergelidis *et al.,* 2017). MRSA staphylococci were recently identified in cheese, as reported by Steinka (2018). Additionally, staphylococci isolated from traditional raw milk cheeses in Serbia were reported by Bulajic *et al.* (2017). The research group of Casaes Nunes (Nunes *et al*., 2016) documented enterotoxin production and the presence of antimicrobial-resistant staphylococci in Frescal cheese from Brazil, one of the most popular cheeses in this country.

During our experiments, one isolate of *S. simulans* was obtained from sample №5 (local cow yellow cheese, "kashkaval"). Recently, this bacterium has been considered as an emerging cutaneous pathogen. Generally, *S. simulans* is a well-established animal pathogen affecting cows, sheep, goats, and horses and can be easily transmitted to food of such origin. Besides, it is known as the causative agent of bovine mastitis. Bridget *et al.* (2016) described severe osteoarthritis and 5-month swelling of the right toe of a farmer due to *S. simulans*. Another report of *S. simulans* as a skin infectious agent and an authentic pathogenic agent of osteoarticular infections was described by Mallet *et al.* (2011). *S. simulans* grew in patients' synovial fluid and blood cultures in France. This bacterium was also recently described as strongly associated with endocarditis in broilers and humans. A putative reason for the high amount of staphylococci found in cheese sample № 5 (BG cow yellow cheese) is the collection of raw milk from cows affected by mastitis.

Yeasts and Molds

Yeasts are part of our normal microflora, and invasive infections only occur due to impaired immune function. They enter the bloodstream and cause fungemia (Arendrup, 2013). By contrast, molds are ubiquitous, and as their conidia are inhaled daily, infections generally occur in the airways. Four strains of *C. krusei* were isolated from Bulgarian yellow cow cheese "kashkaval" (sample № 5), while the fifth strain – *C. glabrata* was isolated from imported goat soft cheese (sample № 4). As Garnier *et al.* (2017) described, yeasts and molds can grow in various foods, including raw foods such as fruits, cereals, vegetables, meat, milk, and processed foods. *Candida* spp. are frequent spoilers of fresh dairy products like cheese, cream, yogurt, and olives (Satchanska *et al.,* 2019). As reported by Bintsis (2021), total raw milk yeast counts are generally in the range of 10–10^3 CFU/mL, and yeast genera commonly identified in raw milk include *Candida*, *Cryptococcus*, *Debaryomyces*, *Geotrichum*, *Kluyveromyces*, *Trichosporon*, *Pichia,* and *Rhodotorula*. The same author reported at least six *Candida* species found in cheese: *C. catenulate*, *C. etchellsii, C. glaebosa, C. lambica, C. parapsilosis,* and *C. zeylanoides*. Usually, heat treatment eliminates yeast contamination of milk. Garnier et al. (Garnier *et al.,* 2017) reported a disturbing finding that certain yeast species are highly resistant to pasteurization. The high number of yeasts frequently observed in cheese could be related to their ability to develop at low temperatures, ferment lactose, and assimilate organic acids, and, most importantly, they are resistant to high salt concentrations.

Conclusions

Antibiotic susceptibility screening demonstrated that the cheese samples were harboring bacteria susceptible to 19 antibiotics except for a strain *E. faecalis*-2, which was resistant to three antibiotics belonging to Fluoroquinolones. *Candida* isolates showed susceptibility to all three screened antifungals.

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