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Microbiological Quality and Presence of Foodborne Pathogens in Full-Fat Cow's Yellow Cheese in Bulgaria

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Abstract

Cheese plays a significant role in human nutrition, being one of the most popularready-to-eat foods produced worldwide. Cheese is rich in microbiota and more than 400 species of LAB, Gram (+) and Gram (-) bacteria, yeasts and molds have been identified in it so far. The rich nutritional content of cheese also favors the development of foodborne pathogenic bacteria. The aim of our study was to investigate the microbiota in commercially branded full-cream yellow cheese with a high market share as well as to search for pathogenic bacteria in it. An amount of different bacterial groups in 1 g of cheese was assessed using SPS agar (*Clostridium*), MCA agar (*Staphylococcus*), KEAA (fecal enterococci), YGS (yeasts), Candida agar (*Candida* spp.) and Nutrient broth (total number). Randomly picked isolates were subcultured on Columbia agar with 5% sheep blood; McConkey agar (BD, USA); Sabouraud and BBL CHROMagar Candida (BD, USA). Identification of isolates was carried out using Crystal (BD, USA) system; VITEK 2, bioMerieux, France, API 20 C AUX Candida (bioMerieux, France). Our results revealed that the total number of bacteria in the investigated sample was $6.9x10^5$. Cheese was negative for clostridia and coliforms. It was positive for yeasts but within the standard limit – $2x10^2$ CFU/g, including *Candida* spp. - $2x10^2$ CFU/g. Alarmingly, a high amount of fecal enterococci - $1.5x10^5$ CFU/g and staphylococci – $2x10^4$ CFU/g was detected. Bacteria isolated from the cheese sample were further identified as *Staphylococcus simulans, Enterococcus avium, Candida krusei* and *Cryptococcus neoformans*, and *S. simulans,* associated with endocarditis of chickens in humans. A putative reason for the staphylococcal contamination of cheese could be mastitis in the cows whose milk was processed. Another isolate, *E. avium,* was reported to be responsible for bacteremia and brain abscesses in humans and is known to be resistant to antibiotics. Yeasts, including *Candida* spp., complied with the standard but even at a small amount they could be harmful to immunocompromised consumers, children or pregnant women. Pathogens as *C. krusei* and *C. neoformans* were detected in the investigated sample.

Keywords: dairy products, food control, enterococci, staphylococci, *Candida*

Резюме

Кашкавалът е важен компонент от хранителната диета на човека и е един от най-популярните готови за консумация продукти. Той е богат на микрофлора като до момента в него са идентифицирани над 400 вида млечнокисели бактерии, Грам (+), Грам (–) бактерии, дрожди и плесени. Високата хранителна стойност на кашкавала обаче, също благоприятства развитието и на патогенни бактерии. Целта на нашето изследване е да се изучи микробиотата на фабрично произведен, пълномаслен кашкавал от краве мляко както и да се изследва наличието на патогенни микроорганизми. Изследваният кашкавал попада във високия ценови клас. Количеството на микроорганизми е определена в 1 грам продукт като са използвани различни хранителни среди: SPS агар (за *Clostridium*), MCA агар (за *Staphylococcus*), KEAA (за фекални ентерококи), YGS (за дрожди), Candida агар (за *Candida* spp.) и МПА (за общ брой аеробни бактерии). Случайно селектирани бактериални изолати са култивирани върху Columbia агар с 5% овнешка кръв, McConkey агар (BD, USA), Sabouraud и BBL CHROMagar

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Candida (BD, USA). Идентифицирането на изолатите е осъществено чрез апаратите Crystal (BD, USA); VITEK 2 и тестът API 20 C AUX Candida. Нашите резултати показват, че общият брой на бактериите в изследваната проба са $6.9x10^5$. В кашкавала не са открити клостридии и колиформи. В пробата установихме дрожди ($2x10^2$ CFU/гр), включително *Candida* spp. - $2x10^2$ CFU/g, но те не надвишават стандартните стойности. Обезпокоително, в кашкавала се съдържат високо количество фекални ентерококи - $1.5x10^5$ CFU/гр и стафилококи - $2x10^4$ CFU/гр. Изолираните от кашкавала бактерии са идентифицирани като *Staphylococcus simulans*, *Enterococcus avium*, *Candida krusei* and *Cryptococcus neoformans.* Вероятната причина за замърсяването със стафилококи е мастит на животните, от които е издоено млякото. *S. simulans* предизвиква ендоокартдити, а друг от изолатите - *E. avium* води до бактериемии и възпаление на мозъка. В литературата те са описани като резистентни на голям брой антибиотици. Броят на дрождите, в това число *Candida* spp., отговарят на стандартните стойности, но дори и в малко количество те могат да бъдат опасни за човека, особено за имунокомпрометирани пациенти, деца и бременни жени. В изследваната проба кашкавал също са открити патогени като *C. krusei* and *C. neoformans*.

Introduction

Nowadays, about 1.3 billion tons of food per year becomes spoiled or squandered worldwide, which represents about one third of all food (FAO). In Europe and North America, food waste per capita is 95-115 kg/year while in Africa and Asia it is only 6-11 kg/year. Especially severe, along with chemical and physical spoilage, is the microbiological spoilage. Bacteria and fungi are the main agents responsible for microbial spoilage and lead to numerous outbreaks (Garnier *et al*., 2017). Bacteria, yeasts and molds are able to grow in a wide variety of raw foods - cereals, fruits, vegetables, milk, meat as well as in processed ones. To avoid yeast, mold and bacteria development in dairy products, a broad spectrum of chemical preservatives are added, such as weak organic acids – sorbic acid, benzoic acid, propionic acid and their salts: potassium sorbate, calcium sorbate, sodium benzoate, potassium benzoate, calcium benzoate and sodium propionate as well as natamycin, a polyene antifungal antibiotic used in the food industry also as a preservative. (Garnier *et al*., 2017).

Cheese plays a significant role in human nutrition being one of the most popular ready-to-eat foods produced worldwide. The oldest cheese was found in the summer of 2018 in a tomb at Saqqara near Cairo (ktelegram.com). Researchers from the University of Catania, Italy, and from Cairo University, Egypt, estimated that it was produced between 1290 and 1213 B.C. or is 3.2 thousand years old. Besides its composition, dangerous bacteria were also detected. These bacteria were responsible in Ancient Egypt for the so-called "Mediterranean fever" affecting both animals and humans and causing skin breakage, lesions of the internal organs and renal failure.

Cheese is rich in microbiota – more than 400 species of LAB, Gram (+) and Gram (-) bacteria, yeasts and molds have been identified in it so far (Almeida *et al*., 2014)). The rich nutritional content of cheese also favors the development of foodborne pathogenic bacteria. Despite the technological progress in the food industry, bacterial and fungal contamination and spoilage of dairy products is the main threat for the dairy industry. The increased consumers' demand for less preservatives in food leads to the development of pathogenic bacteria, some of them harmful to immunocompromised consumers (transplanted, hemodialyzed persons with chronic diseases) pregnant women and children with an adverse effect on their health. Although numerous assessments of the microbiological quality of different kinds of cheese have been carried out in Europe and worldwide, in Bulgaria such microbiological analyses of cheese are still not profound enough.

Our study aimed to investigate the microbiota in commercial full-cream yellow cow cheese with a high market share and to isolate and identify bacteria in it, confirming or denying their pathogenicity.

Materials and Methods

Sampling

Cheese was purchased from a retail store and represents a cheese type located in the upper quality and price segment. It was moderately mellow, medium matured for about 45 days, according to the manufacturer's description. 10g of sample were aseptically cut and homogenized in 90 ml of Ringer's solution. The mixture was heated to 44°C in order to reach full fat dissolution. Further, serial dilutions (10^{-1} to 10^{-4}) were prepared and 0.1 ml of each dilution was spread on selective or differential media.

Total number of viable bacteria

The total number of viable bacteria was examined on Nutrient agar (Hi-Media, India and Oxoid, England). The medium was autoclaved at 121°C/1 atm/20min. Cultivation was carried out at 30°C for 48h. Enumeration was carried out applying the MPN method.

Number of Clostridium perfringens

C. perfringens was enumerated on SPS agar (Sulphite-Polymyxin-Sulphadiazine Agar, Hi-Media, India). The medium was autoclaved at 121°C/1 atm/20min. One ml inoculum of each dilution was poured out in the petri bottom dish and covered with 20 ml of SPS agar for deep seeding to create anaerobic conditions for clostridia. Incubation lasted 48h at 37°C. Enumeration was carried out using the MPN method.

Number of Staphylococci

MSA (Manitol-Salt agar, 6.5 % NaCl, Merck, Germany) was used to study the presence of members of the genus *Staphylococcus*. The medium was autoclaved at 121°C/1 atm/20min. On the orange-pink colored agar, staphylococcal colonies were with golden color surrounded by a well-defined yellow halo. Petri dishes were incubated for 48h at 30°C and enumerated by the MPN method.

Number of Enterococci

KEAA (Kanamycin-Esculin-Azide agar, Merck, Germany) was used for detection of faecal enterococci. The medium was autoclaved at standard conditions. Enterococci turned the white-colored agar black due to the metabolism of esculin. Enterococcal colonies grew as transparent to white colonies on it. Addition ofkanamycin to the medium ensured the growth inhibition of other Gram (+) bacteria. The medium was autoclaved at 121°C/1 atm/20 min. Incubation was carried out for 48h at 30°C. Count of enterococci was assessed using the MPN method.

Number of E. coli and Coliforms

VRBA agar (Violet Red Bile Agar) was applied to differentiate the number of *E. coli* and coliforms. Medium was autoclaved at 121°C/1 atm/20 min. Incubation lasted 48h at 30°C and the number of *E. coli* and coliforms was assessed using the MPN method.

Number of yeasts

The total number of yeasts and molds was analyzed on YGC agar (Yeast peptone-Glucose-Chloramphenicol agar). The medium was autoclaved at 121°C/1 atm/20 min. Incubation duration was 7 days at 28°C. Yeasts and molds were counted according to the MPN method.

Number of Candida spp.

Both Candida conventional (Hi-Media, India) and differential chromogenic agar (Hi-Media, India) were used for detecting *Candida* spp. Colonies grew black-colored on white agar using the conventional agar, and white-to-rose in color when using the differential chromogenic agar. While the first medium was autoclaved, the second one was prepared without autoclaving but by boiling according to the manufacturer's instructions. Incubation lasted 7 days at 28°C and the enumeration was according to the MPN method.

Isolation and characterization of bacterial strains from cheese

Randomly selected colonies were picked up from each plate for further cultivation andidentification. A total of 12 strains were collected and cultures were frozen in glycerol on appropriatemedia at -20 °C becoming part of the BioLab Microbial Collection, New Bulgarian University. The isolates were further sub-cultured on Columbia agar with 5% sheep blood; McConkey agar (BD, USA); Sabouraud and BBL CHROM agar Candida (BD, USA). Their identification was carried out using Crystal (BD, USA) system; VITEK 2 System (bioMerieux, France) and API 20 C AUX Candida (bioMerieux, France).

Results

Numerous investigations worldwide have reported bacterial contamination of different kinds of cheese as cow, ewe and buffalo cheese (Nieto-Aribas *et al*, 2011; Jamet *et al*., 2012; Levkov *et al*., 2014; Caeses *et al*., 2016; Bulajic *et al*., 2017; Watson *et al*., 2017; Serrano *et al*., 2018). Being rich in nutrients, the cheese is a medium highly favorable for bacterial germination. Our investigation on the microbiological quality of full cream cheese revealed that the total number of bacteria did not exceed the Standard values (Standard International Microbiological Criteria for Dairy Products, EC Directive 92/46/EEC: Cheeses made from raw milk and from thermized milk; Standard US FDA „Grade A" Pasteurized Milk Ordinance 2015 Revision). The total number of mesophilic aerobes (1) was $6.9x10^5$ CFU/g, data shown in Table 1. Analysis demonstrated that the studied cheese sample was negative for clostridia (2, Table 1) and *E. coli*&coliforms (5, Table 1). It conformed to the standard for yeasts and molds (6, Table 1) - $2x10^2$ CFU/g, including *Candida* spp. (7, Table 1) - $2x10^2$ CFU/g. Fecal enterococci (4, Table 1) were enumerated at quite significant amount - $1.5x10^5$

Table 1. Number of bacteria in full-cream cow cheese

№	Type of bacteria	Media	CFU/g	Compliance to Standard
1.	Total count	Nutrient Agar	$6.9x10^5$	Normal
2.	*C. perfringens*	SPS Agar	0	Normal
3.	Staphylococci	MS Agar	$>1.0x10^5$	Exceeds
4.	Enterococci	KEA Agar	$1.5x10^5$	n.a. ref. values
5.	*E. coli*&Coliforms	VRB Agar	0	Normal
6.	Yeasts and molds	YGS agar	<10	Normal
7.	*Candida* spp.	Candida chrome agar	<10	Normal

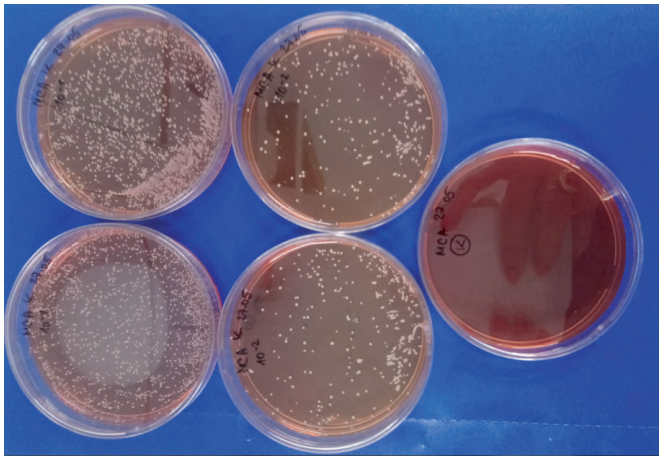

Fig. 1a Analysis of staphylococci in cow yellow cheese (1st vertical petri column - 10^{-1} dilution plating, 2nd vertical petri column - 10^{-2} dilution plating and 3rd vertical column - Control)

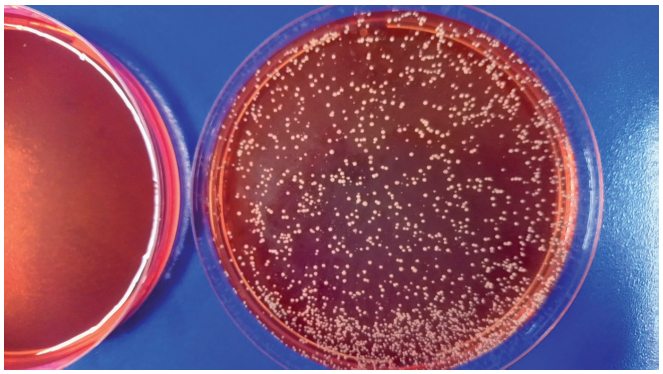

Fig. 1b Morphology of staphylococci from cow cheese (Left – Control, Right - colonies of staphylococci)

CFU/g and the staphylococci (3, Table 1) number was $2x10^4$ CFU/g, which comes close to the upper limit according to the Standard and even slightly exceeds it. Referent values for enterococci were not described in the Standard for the microbial quality of food (Standard US FDA „Grade A“ Pasteurized Milk Ordinance 2015 Revision)

Table 1 shows the summary results of the quantitative microbial analysis of the studied cheese sample.

As seen in Fig. 1a, staphylococci are present at a significantly high number showing the dilution $1x10^{-1}$ and $1x10^{-2}$. K stands for the Control (Fig. 1a and Fig. 1b). Figure 1 bshows typical staphylococcal morphology

Figure 2 shows Control (left) and *Candida* colonies on differential chromogenic agar (right). The black color of the right petri undoubtedly demonstrates the biochemical activity of *Candida* colonies (transparent to white in color on black

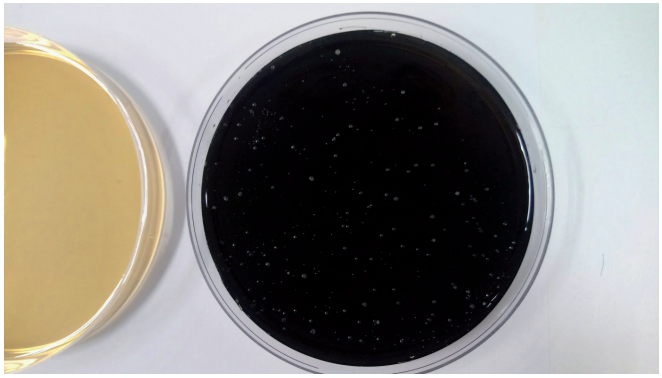

Fig. 2. Fecal enterococci of cow cheese, grown on KEAA (Left – Control, Right - enterococci turned the agar black due to esculin metabolism)

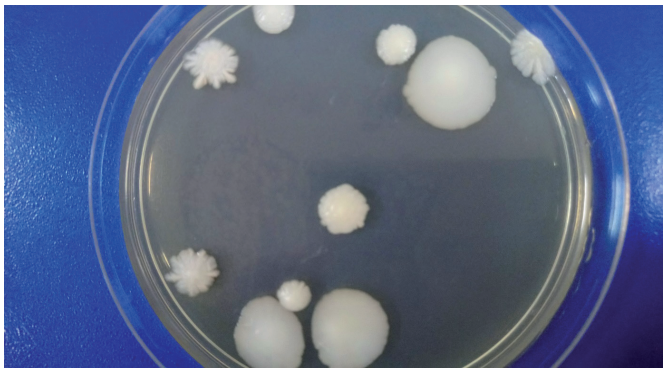

Fig. 3. Morphology of yeasts and molds in cow yellow cheese, grown on conventional YGC agar

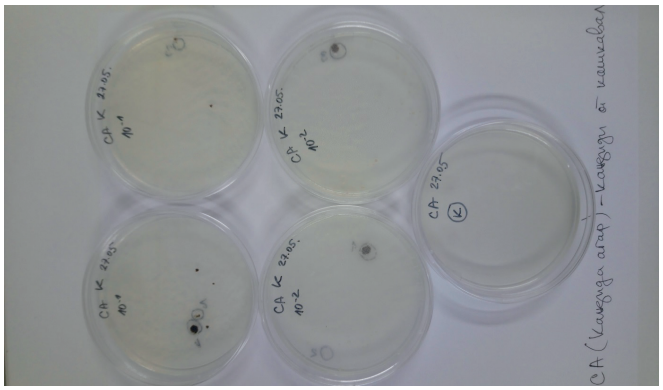

Fig. 4. Analysis of *Candida* spp. in cow cheese

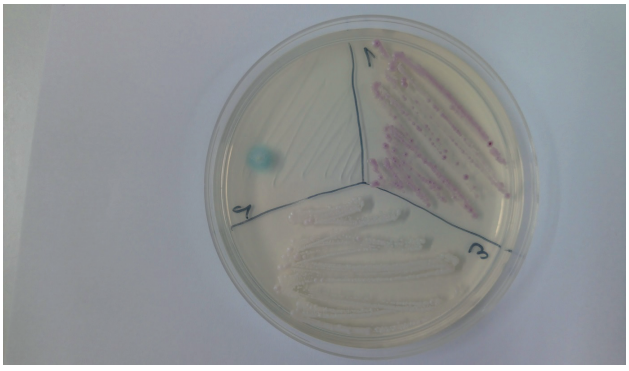

Fig. 5. Isolates of *Candida* spp. on Candida differential chrome agar further identified

agar) due to the conversion of esculin to esculetin.

Yeasts and molds in the investigated sample were of insignificant amount - >10 GFU/g as shown by the analysis on conventional agar. Morphology of single colonies is visualized in Fig. 3 appearing as white smooth-shaped colonies or rough, star-shaped ones.

Candida spp. were also represented at a very small amount - > 10 GFU/g and can be seen as black colonies growing on Candida differential chrome agar (Fig. 4).

In order to identify the bacteria detected in the cow cheese, 12 randomly selected bacterial isolates were further cultivated. Staphylococci, enterococci and Candida isolates were determined using specific methods aiming to confirm or deny their pathogenicity. Although the *Candida* number was within the Standard, isolates were also identified that could easily colonize as biofilm in the hosts and harm immuno-compromised consumers, children or pregnant women. Some of these isolates are displayed in Fig. 5.

After applying Crystal system; VITEK 2 System and API 20 C AUX Candida some of the selected isolates (4 out of 12) were identified as *Staphylococcus simulans*, *Enterococcus avium, Candida krusei* and *Cryptococcus neoformans*. Results are presented in Table 2.

Discussion

Cheese has been one of the main components of everyday human diet for millennia. The oldest sample was found in the summer of 2018 in an Egyptian tomb and was dated approximately three thousand years. Investigations on the microbial quality of varieties of cheese in France, Brazil, Switzerland, R. Macedonia, Czeck Republic, Serbia were recently reported (Nietto-Aribas *et al.*, 2011; Jamet *et al*., 2012; Levkov *et al*., 2014; Caeses *et al*., 2016; Bulajic *et al*., 2017; Serrano *et al*., 2018). In recent years, investigations are focused not only on the general assessment of certain bacteria in cheese but also on the characterization of foodborne pathogens in cheese and their resistance to antibiotics.

Revising the standards and literature, we found that the results about the microbial quality of cheese described in this paper comply with the European and International Standards and are consistent with the results obtained by other research groups.

Total count of bacteria in cheese

As recommended by the International Standards (Standard International Microbiological Criteria for Dairy Products, EC Directive 92/46/EEC: Cheeses made from raw milk and from thermized milk;Standard EC Reglament No 2073/2005 for Microbiological criteria of food, the upper limit of

total bacteria count in raw unpasteurized milk is 100 000 colonies per ml. Cheese, being produced from unpasteurized milk, is also abundant in bacteria, coming from the gastro-intestinal tract of animals as well as from the industrial equipment. As described in the Results section, the total mesophilic and aerobic microflora of cheese was enumerated at $6.9x10^5$ CFU/g (Table 1, (1). Similar results were described by Montel *et al*., 2014, who reported even a higher amount of bacteria – $8.2 x10^5$ CFU/g. Morales *et al*. (2009) have even announced a higher amount of mesophilic aerobes – larger than 10^6 CFU/g in a cheese sample from Brazil.

The presence of *C. perfringens* (Table 1, (2) in cheese is not routinely investigated during food analyses although it is one of the most common causative agents for food poisoning. *C. perfringens* cause tissue necrosis, bacteremia, emphysematous cholecystitis, and gas gangrene. The mechanism of intoxication with this pathogen involves the ingestion of 10^6-10^7 living cells per gram of food. The harmful effect of *C. perfringens* is mainly due to its enterotoxin, produced in the large human intestine during sporulation of the microorganism and released upon lysis of its sporangia (Encyclopedia on Food microbiology, 2014). Our study revealed no clostridia in the studied sample.

Amount of staphylococci

The number of staphylococci in the investigated cheese sample was enumerated at $>1x10^5$. According to EC standard 2073/2005, the limit of staphylococci in cheeses produced from raw milk is $1x10^4$-$1x10^5$ CFU/g. Serrano *et al*. (2018) reported an amount of staphylococci of $8.8x10^4$ CFU/g when examining the microbiological characteristics of seven Swiss cheeses. *S. aureus* has been manifested as a controversial foodborne pathogen due to its resistance to methicillin (MRSA). MRSA are associated with severe infections in humans and animals such as bacteriemia, wound infections, pyogenic lesions, and mastitis (Podkowik *et al*., 2013; Sergelidis and Angelidis, 2017). MRSA staphylococci were recently identified in cheese and reported by Steinka (2018). Additionally, a research groce of staphylococci isolated from traditional raw milk cheeses in Serbia. Similar data of Bulajic *et al*. (2017) has documented enterotoxin production and antimicrobial resistant staphylococci presence in cheese were also reported by the research group of Casaes Nunes (Nunes *et al*., 2016) concerning Frescal cheese from Brazil, one of most popular cheese in this country.

One isolate of the sample studied by us was identified and affiliated as *S. simulans*. 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 popular as a causative agent of bovine mastitis. Bridget *et al*. (2016) described severe osteoarthritis and 5 months swelling of the right toe of afarmer due to *S. simulans*. Another report of *S. simulans* as a skin infectious agent and an authentic pathogenic agent of osteoarticular infections crossing was described by Mallet *et al.* (2011). *S. simulans* grew in the sinovial liquid and in blood cultures in patients in France. It was recently described also as strongly associated with endocarditis both in broiler chicken and in humans as well (Stepien-Pysniak *et al*., 2017). A putative reason for the high amount of staphylococci found in the investigated cheese sample is the collection of raw milk from cows affected by mastitis.

Enterococci

The enterococci count in the investigated cheese sample was $1.5x10^5$ CFU/g. A variety of enterococcal strains contaminating cheese, such as *E. faecalis*, *E. caseliflavus*, *E. faecium* were reported by Resende *et al*. (2018). *E. faecalis* was found to be the most abundant - more than 50 % of all pathogens identified anddescribed by the authors. Antibiotic resistance of *Enterococci* was reported in a series of papers published by Jamet *et al*. (2012), Perin *et al*. (2014), Gaglio *et al*. (2016). Apart from their wide antibiotic resistance, enterococci were proven to infect immunocompromised patients (Chajecka-Wierzchowska *et al*., 2017). The authors elucidated the way in which foodborne enterococci enter the host immune system – *via* a plethora of virulence factors which promote colonization such as aggregation substances, collagen-binding protein-Ace, endocarditis specific antigen – EfaA, surface protein - Esp or such factors that affect tissues as citoliysin - Cyl, gelatinalse – GelE, hilaronidase – Hyl. Malti *et al*. (2015) even described that enterococci are capable of crossing the brain barrier and are responsible for brain abscesses. We consider the large number of enterococci and the *E. avium* isolated from the sample in this study as a cause of concern. *E. avium*, formerly called "Group Q streptococcus", is an apparent pathogen causing clinically significant bacteremia (Na *et al*., 2012). Interestingly, in this report most of the isolates from all patients hospitalized with bacteremia caused by *E. avium* were of gastrointestinal origin, which can

be considered an infection due to consumption of infected food.

E. coli and coliforms

Our study demonstrated less than 10 colonies of *E. coli* and coliforms counted at 48 h, which can be considered as free of *E. coli* cheese. Significantly higher amount of total coliforms in a similar kind of cheese was reported by Morales *et al*. (2009) - $1x10^3$ CFU/g in Brazilian cheeses, and of *E. coli* – between $1x10^2$ and $3.5x\ 10^6$ CFU/g. According to the EC standard, the limit for *E. coli* is less - $1x10^4$-$1x10^5$.

Yeasts & Candida

In our analysis, we demonstrated that of the yeast species, *C. crusei* and *C. neoformans* were detected in the analyzed cheese sample. Yeasts and molds are able to grow in a wide variety of foods including both raw (fruits, cereals, vegetables, meat and milk) and processed food as documented by Garnier *et al*. (2017). Besides *Candida spp*., *Debaryomyces*, *Kluyveromyces*, *Yarrowinia*, *Galactomyces* and *Saccharomyces* spp. are frequent spoilers of fresh dairy products (fresh cheese, cream and yogurt). Usually, heat treatment eliminates yeast contamination of milk. Garnier *et al.* (2017) described a disturbing finding that a certain yeast species is highly resistant to pasteurization. The frequently observed high number of yeasts in cheese could be related to their ability to develop at low temperature, ferment lactose and assimilate organic acids, and most importantly - they are resistant to high salt concentrations (Tofalo *et al*., 2014).

Yeasts are part of our normal micro-flora and invasive infections arise only when an impaired immune function occurs. Entering the bloodstream they cause fungaemia (Arendrup, 2013). On the contrary, molds are ubiquitous in nature and as their conidia are inhaled on a daily basis, infections generally occur in the airways. Anderupt (2013), also described the five most common types of human *candidiasis* caused by *Candida* species - *C. albicans, C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis* along with three rarely found species - *C. guilliemondii, C. lusitaniae* and *C. kefyr.* According to their virulence, the author grouped the above- mentioned species in descending order: I. *C. albicans* and *C. tropicalis,* II. *C. glabrata, C. lusitaniae* and *C. kefyr. III. C. krusej, C. parapsilosis* and *C. guilliemondii.* Although *C. krusei* has been classified as a species possessing not a high virulence, it is harmful to human health due to its resistance to a plethora of antibiotics as Anidulafungin, Caspofungin, Micafungin, Itraconasole, Posaconasole and Voriconalose, as reported by Pfaller *et al*. (2011). Jensen (2016), reported that antifungal resistance is a multifaceted clinical challenge and that most of the prevalent fungal infections were caused by *Candida* yeasts and *Aspergillus* molds. The author reported a high level of *C. krusei,* which is among the isolates of the studied sample) resistance to echinocandin due to homozigous mutation in the FKS1 gene leading to an amino acid substitution. Numerous reports describe that fungal resistance is still not fully illuminated and the problem is still not resolved globally.

C. neoformans (*Tremellomycetes* class) is an encapsulated yeast pathogen detected in the cheese sample in this investigation. Although not abundant, its presence can affect consumers, especially those with an immunocompromised system as well as children. Once it enters the host organism, its size increases (Prieto-Granada *et al*., 2010) and a thick gelatinous capsule develops rapidly. Serotypes A and B of this bacterium are usually isolated from pigeon droppings and cause diseases mainly in immunosupressed patients, while serotypes C and D were isolated from eucalyptus trees and more often affect patients with a normal immune system. *C. neoformans'* size is only 2 μm and makes its aerosolization very easy and alveolar deposition fast. The mucin-rich capsule of about 30 μm thickness (Zander and Farver, 2018) possesses an antiphagocytic effect and makes the bacterium very tough for assimilation. (Josef *et al*., 2008). *C. neoformans* grows for 4 weeks, disseminates widely and manifests as meningitis, parenchymal brain infection or pulmonary disease in pediatric patients with acquired immunodeficiency (Kathleen *et al*., 2008, Josef et al., 2008).

Conclusions

Our investigation demonstrated that the investigated yellow cheese complies with the national and international standards for microbiological quality except staphylococci and enterococci amounts, the latter two slightly exceeding the standard values. A putative reason for the staphylococcal contamination should be the mastitis of cows whose milk was processed. We also found that the total number of mesophilic bacteria in the sample corresponded to the referent values. The numbers of *E. coli* and coliforms as well as of yeasts and molds were within the standards. Among the bacterial isolates cultivated from the sample, pathogenic bacteria as *S. simulans, E. avium, C. crusei* and *C. neoformans* were identified. They could affect con-

sumers with impaired immune systems (transplanted, hemodialyzed persons), children and pregnant women as well as patients suffering from chronic diseases.

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