EXPLORING PATHOGENIC BACTERIA IN CHEESE: INSIGHTS FROM MICROBIAL ISOLATION STUDIES

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This study investigates the presence of pathogenic bacteria in various types of cheeses, focusing on Escherichia coli, Staphylococcus aureus, Salmonella spp., and Listeria monocytogenes. Cheese samples were subjected to microbial isolation and identification techniques, revealing the prevalence of these pathogens. Escherichia coli and Staphylococcus aureus were commonly detected, while Salmonella spp. and Listeria monocytogenes were less frequently found. These findings underscore the importance of robust food safety measures in cheese production and highlight the need for continued vigilance in monitoring and controlling pathogenic bacteria to ensure consumer safety.

Keywords: food pathogens, *Escherichia coli, Staphylococcus aureus, Salmonella spp., Listeria monocytogenes*.

1. Introduction

Cheese, cherished for its rich flavors and diverse textures, occupies a revered place in culinary traditions worldwide. Yet, amid the delight of savoring its creamy goodness lies a crucial concern: the presence of pathogenic bacteria that can jeopardize consumer health. In recent years, heightened awareness of food safety risks has prompted rigorous scientific inquiry into the microbial landscape of cheese, with a particular focus on the detection and identification of pathogenic microorganisms [1, 2]. The detection of pathogenic bacteria in cheese is not merely an academic pursuit; it is a vital endeavor aimed at safeguarding the well-being of consumers. Each year, foodborne illnesses linked to contaminated cheese underscore the importance of proactive measures to mitigate these risks. In response, researchers have endeavored to develop robust methodologies for the reliable detection and isolation of pathogenic] bacteria, enabling swift action to prevent outbreaks and protect public health [3].

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Within the complex world of cheese microbiology lie lurking potential threats to consumer health, represented by the quartet of *Escherichia coli (E. coli)*, *Staphylococcus aureus (S. aureus)*, *Salmonella*, and *Listeria*. These pathogenic bacteria, though often invisible to the naked eye, wield the power to instigate foodborne illnesses, prompting a meticulous exploration into their presence and prevalence within various cheese types. Contamination of food products, including dairy products like cheese, with pathogenic *Escherichia coli* is a significant concern for food safety authorities worldwide. While most strains of *E. coli* are harmless, certain pathogenic strains, such as *E. coli* O157:H7, can cause severe foodborne illnesses, including diarrhea, abdominal cramps, and in severe cases, kidney failure [4, 5]. While often considered a commensal organism, *S. aureus* can produce toxins that cause food poisoning when present in contaminated food products. Foodborne illness caused by *S. aureus* typically manifests with symptoms such as nausea, vomiting, abdominal cramps, and diarrhea, and can occur rapidly after consuming contaminated food, including cheese [6, 7]. Transmission of *Salmonella* occurs primarily through the consumption of contaminated food, particularly raw or undercooked animal products. Symptoms of salmonellosis include diarrhea, fever, abdominal cramps, and vomiting, with severe cases potentially leading to dehydration and hospitalization [8]. Unlike many other foodborne pathogens, *Listeria* can grow at refrigeration temperatures, making it a significant concern for food safety. Consumption of foods contaminated with *Listeria monocytogenes* can lead to listeriosis, a serious illness characterized by fever, muscle aches, nausea, and, in severe cases, meningitis or septicemia, particularly in vulnerable populations such as pregnant women, newborns, and immunocompromised individuals [9, 10].

CHROMagar is a type of chromogenic culture medium designed to differentiate and identify microbial species based on the colors of the colonies they form. It contains chromogenic substrates that release colored compounds when specific enzymatic reactions occur. These reactions are characteristic of certain microbial species, allowing for their identification [11]. They are standardized methods for using chromogenic culture media like CHROMagar. These methods are often described in guidelines and standards set by various organizations such as the Clinical and Laboratory Standards Institute (CLSI), the International Organization for Standardization (ISO), and national public health agencies. The standardization ensures that the results are reliable, reproducible, and comparable across different laboratories. When using these standardized methods, it's important for laboratories to follow the specific procedures and incubation conditions outlined in the standards to ensure the accuracy and reliability of the results. This includes proper sample preparation, inoculation, incubation temperatures, and times, as well as interpreting the results based on the color and morphology of the colonies that develop on the media [12].

The ISO 16649-1 series provides guidelines for the enumeration of *E. coli* in food and animal feeding stuff using chromogenic coliform agar, which can be applied to CHROMagar or similar media [13]. The ISO 6888-1 series outlines methods for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) in products intended for human consumption or feeding of animals. These methods can be adapted for use with CHROMagar *Staph aureus* [14]. For *Listeria monocytogenes*, the ISO 11290-1 series specifies the method for detection in food and animal feed. This series includes guidelines for using chromogenic *Listeria* agar, such as CHROMagar *Listeria*, for both the detection and enumeration of *Listeria* [15]. The ISO 6579 series provides a standard method for the detection of *Salmonella* spp. in food and animal feeding stuff. While this standard primarily focuses on non-chromogenic media, many laboratories incorporate chromogenic media like CHROMagar Salmonella into their testing protocols to enhance detection and specificity [16].

In this article, we delve into the realm of cheese microbiology through the lens of consumer safety. We explore the methodologies employed to detect pathogenic bacteria in various types of cheeses, from soft and luscious Roquefort's to aged and tangy cheese. By understanding the nuances of microbial detection in cheese, we empower consumers, producers, and regulatory agencies alike to uphold the highest standards of food safety and enjoy cheese with confidence.

2. Materials and methods

2.1. Equipment and Reagents

To identify the bacteria, present in different types of cheese, we use chromogenic culture media. Chromogenic media represent a valuable tool in the diagnosis and monitoring of pathogenic bacteria in both the medical field and the food industry, contributing to public health protection and food safety assurance.

For the determination of bacterial cell counts of *Escherichia coli* species, CHROMAgar *E. coli* culture medium was utilized. Also, for the determination of *Staphylococcus aureus* bacteria, CHROMagar *Staphylococcus* culture medium was employed. To determine the bacterial cell counts of *Listeria* species, CHROMAgar *Listeria* base medium was used. The CHROMAgar *Salmonella* Plus base medium was utilized for quantifying the bacterial cell counts of the Listeria monocytogenes species.

We prepared all the culture media according to technical documentation of the utilized culture medium. By employing CHROMAgar chromogenic culture medium, we aimed to streamline the process of pathogenic bacteria detection in cheese samples, leveraging its selective properties to enhance the sensitivity and specificity of microbial analysis. For homogenization, the Blender bag with lateral filter homogenizer was used (MASTICATOR - Lab Paddle Blenders Iul Instruments).

2.2. Sample Collection and Preparation

Twenty cheese samples were chosen primarily from markets in various regions of the country (Bucharest, Arad, Sinaia, Făgărașul Nou, etc.) and from Chișinău, Republic of Moldova. Those from supermarkets were specifically selected as unpackaged, with only one exception to observe if there was any difference. A type of Roquefort cheese with 50% price reduction was chosen, especially since it is made from raw milk and is easily populated with microorganisms. Additionally, various types of packaged cheese were chosen, but from households (in Făgărașul Nou) to test if they met hygiene conditions.

The samples were systematically collected using aseptic techniques to avoid contamination and ensure the integrity of the samples. Each sample was obtained from the core of the product using sterile instruments, minimizing contact with the air and external surfaces. The collected samples were immediately placed in sterile, sealable containers to maintain their microbiological state. Upon collection, the samples were securely sealed and labeled with the date, time, and specific details of the sample's origin. They were then transported to the laboratory in insulated coolers with ice packs to maintain a consistent temperature of approximately 4 °C. This temperature control was critical to prevent the proliferation of microorganisms during transit. After arrival at the laboratory, samples were assessed for their integrity and then stored at 2-8 °C because an immediate analysis is not feasible. The storage conditions were closely monitored to ensure that the temperature remained within the desired range, thus preventing any alteration in the microbial content of the samples. The analysis of the samples was conducted within 24 hours of collection to minimize any potential changes in the microbial flora. This prompt analysis was crucial to obtain an accurate representation of the microbiological state of the samples at the time of collection. 10 g of cheese sample were weighed in sterile bags, over which 90 mL of sterile water was added. The samples were homogenized for 60-90 seconds (depending on the texture of the cheese) before further processing. Using a sterile automatic pipette with sterile tips, 1 mL of the diluted cheese samples was pipetted into sterile Petri dishes. Subsequently, sterilized, and cooled culture chromogenic medium was poured into the Petri dishes, and the contents were homogenized through circular movements.

The Petri dishes were allowed to solidify, and then all Petri were placed in a thermostat at 37 °C for 3 days. All samples were performed in triplicate. Plates exhibiting coloration corresponding to the respective culture medium contained pathogenic microorganisms.

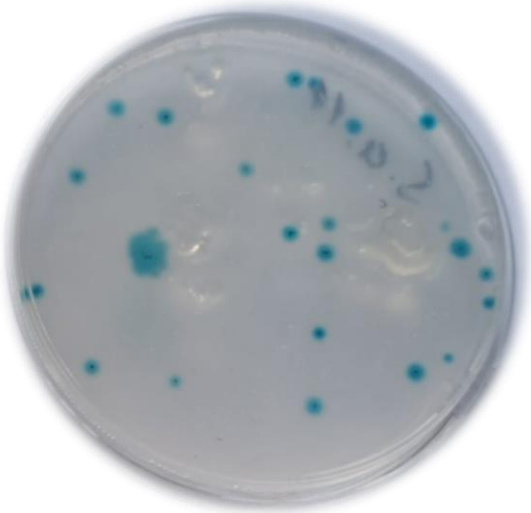
3. Results and discussion

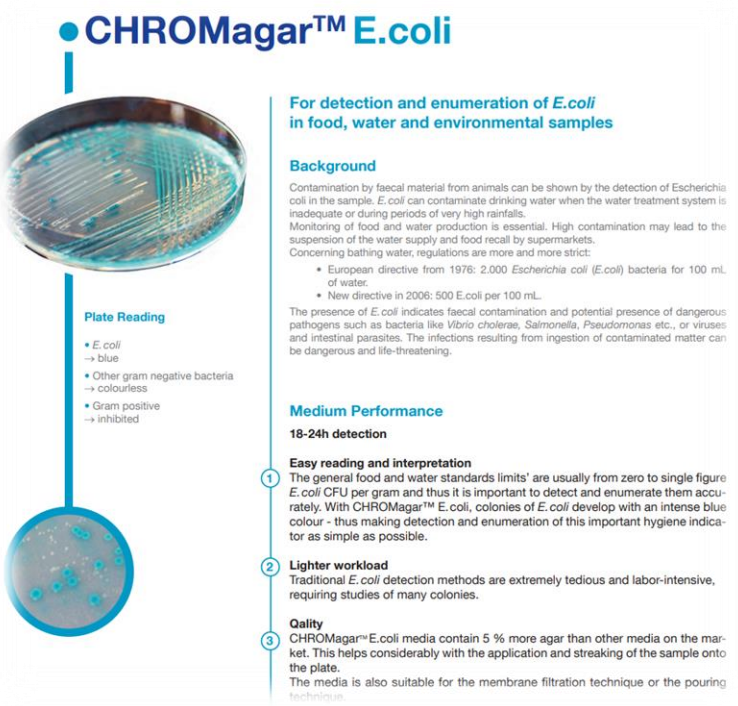
The utilization of CHROMAgar chromogenic culture medium in our study facilitated the rapid and specific identification of pathogenic bacteria within cheese samples, enhancing the accuracy and efficiency of microbial detection (see 2.1. section). The adoption of CHROMAgar chromogenic culture medium allowed for the clear visualization (Table 1) of pathogenic bacterial colonies in cheese samples, enabling precise identification and enumeration of target organisms.

In Table 1, the presence of pathogenic bacteria in certain cheese samples is depicted using CHROMagar chromogenic media.

Table 1

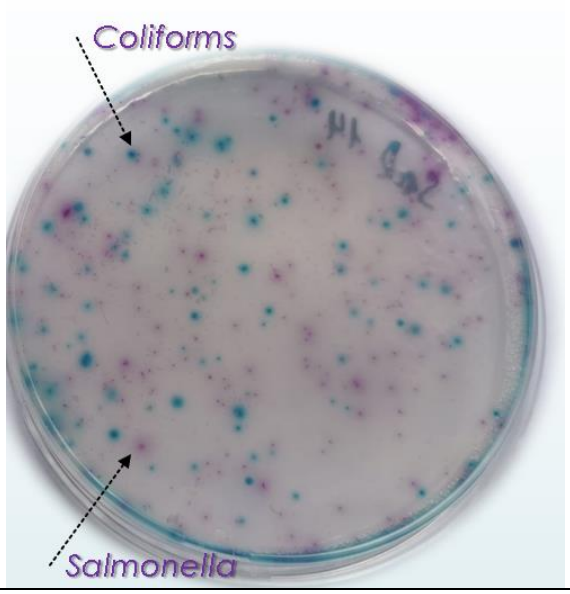
Detection of some pathogenic bacteria within the cheese sample

Code sample	Image of the cheese sample under examination
17	
	The color indication of bacteria presence as described in the technical documentation of the utilized culture medium



14 **Image of the cheese sample under examination**

14



The color indication of bacteria presence as described in the technical documentation of the utilized culture medium

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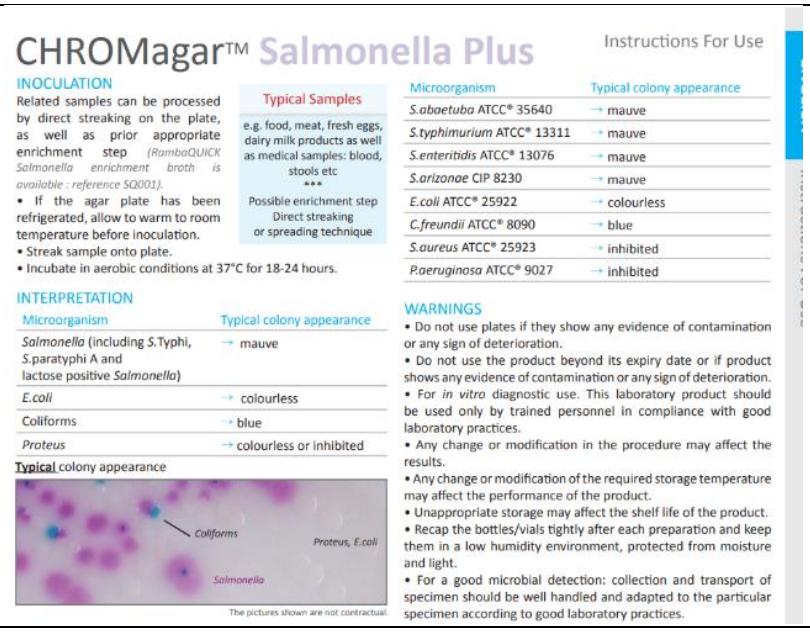
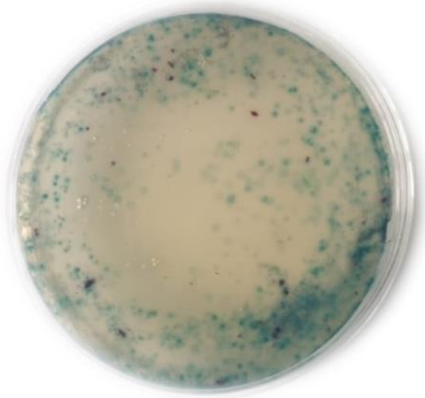
<tr><td></td><td> 
</td></tr>
<tr><td></td><td>Image of the cheese sample under examination</td></tr>
<tr><td>1</td><td>  </td></tr>
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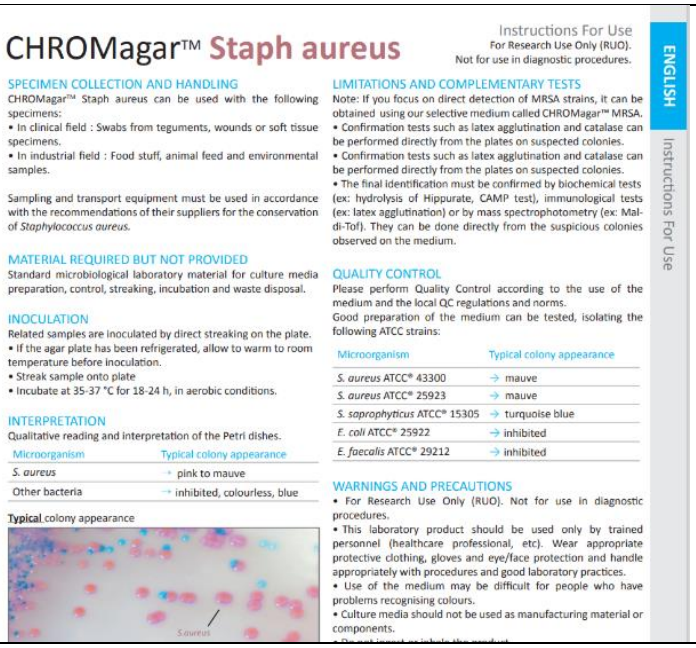

CHROMagar™ Staph aureus

Instructions For Use
For Research Use Only (RUO).
Not for use in diagnostic procedures.

ENGLISH

Instructions For Use

SPECIMEN COLLECTION AND HANDLING

CHROMagar™ Staph aureus can be used with the following specimens:
• In clinical field : Swabs from teguments, wounds or soft tissue specimens.
• In industrial field : Food stuff, animal feed and environmental samples.

Sampling and transport equipment must be used in accordance with the recommendations of their suppliers for the conservation of *Staphylococcus aureus*.

MATERIAL REQUIRED BUT NOT PROVIDED

Standard microbiological laboratory material for culture media preparation, control, streaking, incubation and waste disposal.

INOCULATION

Related samples are inoculated by direct streaking on the plate.
• If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.
• Streak sample onto plate
• Incubate at 35-37 °C for 18-24 h, in aerobic conditions.

INTERPRETATION

Qualitative reading and interpretation of the Petri dishes.

Microorganism	Typical colony appearance
S. aureus	→ pink to mauve
Other bacteria	→ inhibited, colourless, blue

Typical colony appearance

LIMITATIONS AND COMPLEMENTARY TESTS

Note: If you focus on direct detection of MRSA strains, it can be obtained using our selective medium called CHROMagar™ MRSA.
• Confirmation tests such as latex agglutination and catalase can be performed directly from the plates on suspected colonies.
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• The final identification must be confirmed by biochemical tests (ex: hydrolysis of Hippurate, CAMP test), immunological tests (ex: latex agglutination) or by mass spectrophotometry (ex: Mal-di-Tof). They can be done directly from the suspicious colonies observed on the medium.

QUALITY CONTROL

Please perform Quality Control according to the use of the medium and the local QC regulations and norms.
Good preparation of the medium can be tested, isolating the following ATCC strains:

Microorganism	Typical colony appearance
S. aureus ATCC® 43300	→ mauve
S. aureus ATCC® 25923	→ mauve
S. saprophyticus ATCC® 15305	→ turquoise blue
E. coli ATCC® 25922	→ inhibited
E. faecalis ATCC® 29212	→ inhibited

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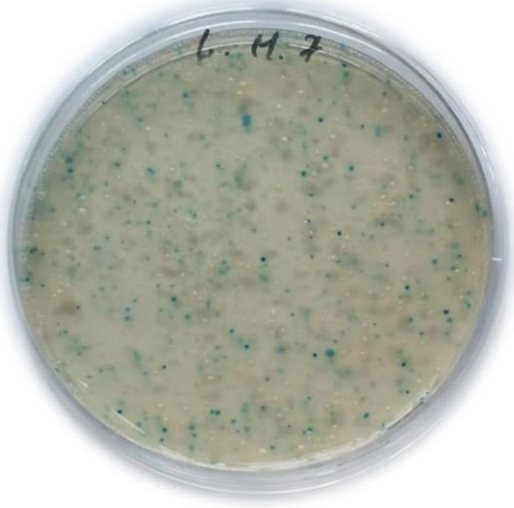
Image of the cheese sample under examination



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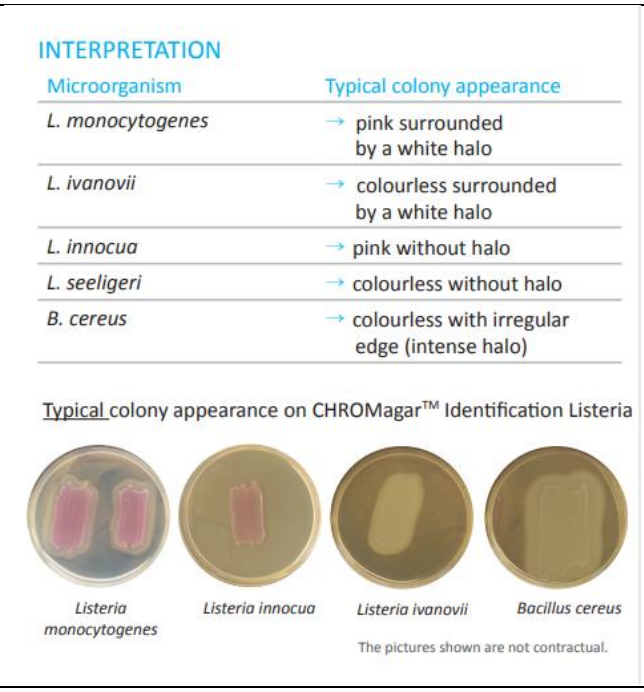
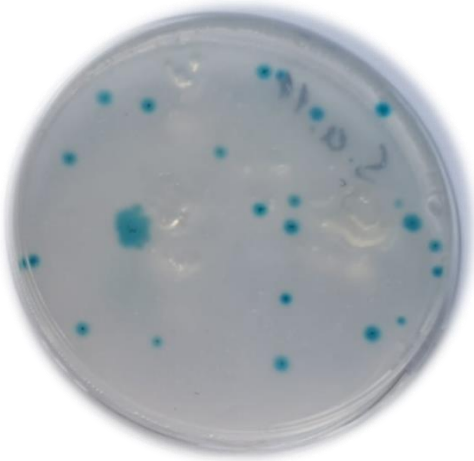
<tr><td>7</td><td> 
</td></tr>
<tr><td>Code sample</td><td>Image of the cheese sample under examination</td></tr>
<tr><td rowspan="2">17</td><td>  </td></tr>
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	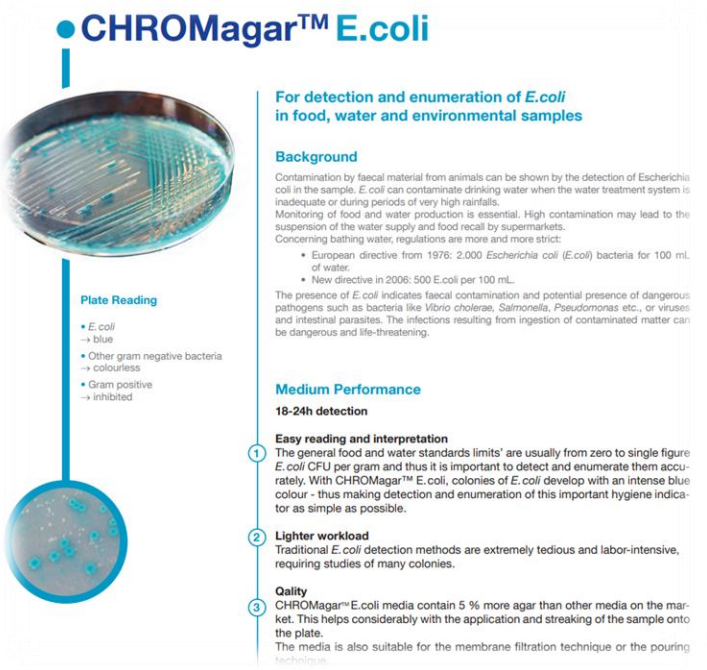
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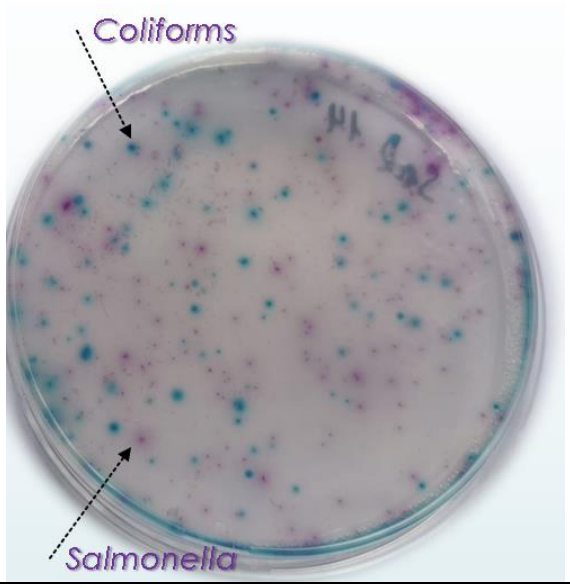
14	
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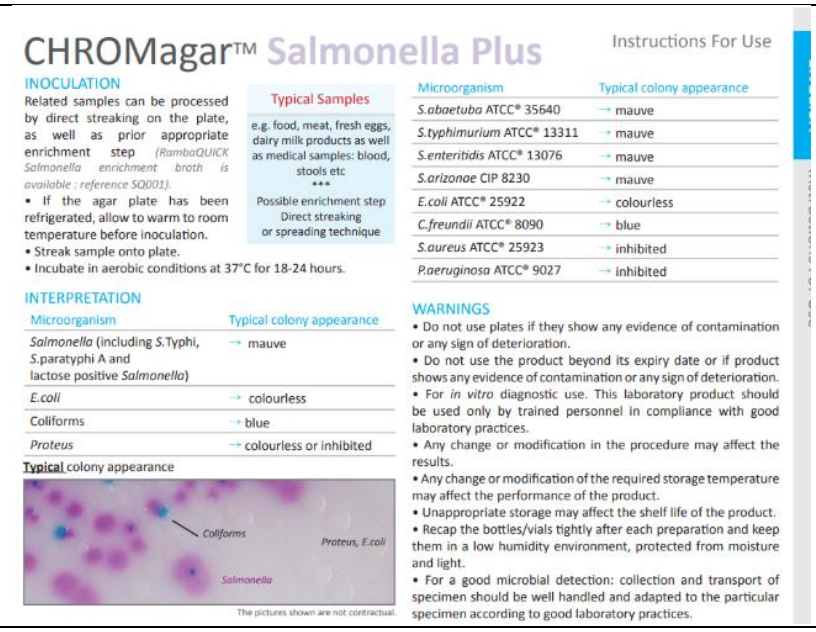
<tr><td></td><td> 
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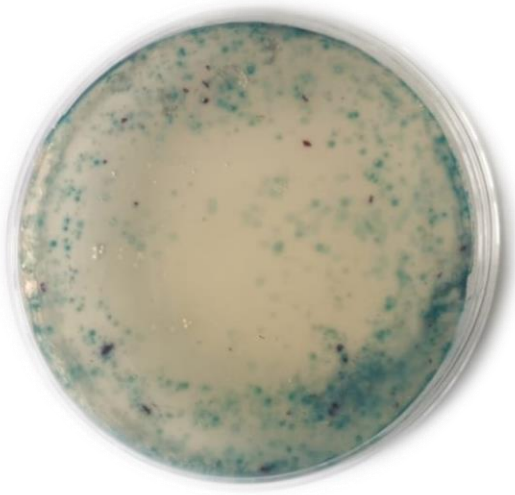
<tr><td>1</td><td>  </td></tr>
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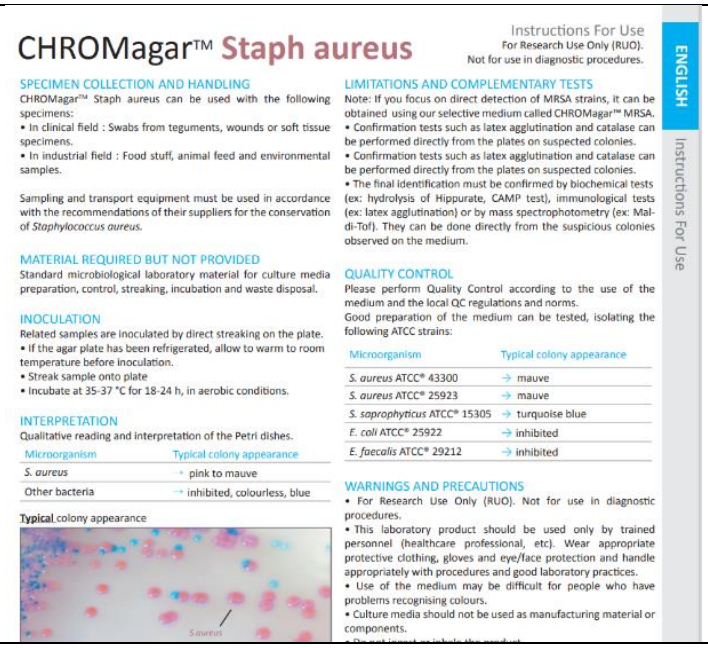

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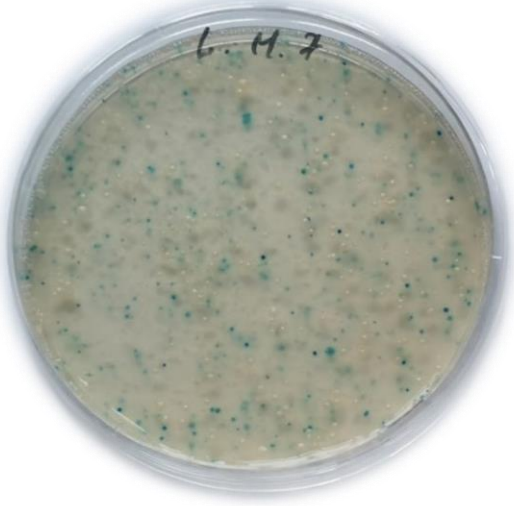

7 **The color indication of bacteria presence as described in the technical documentation of the utilized culture medium**

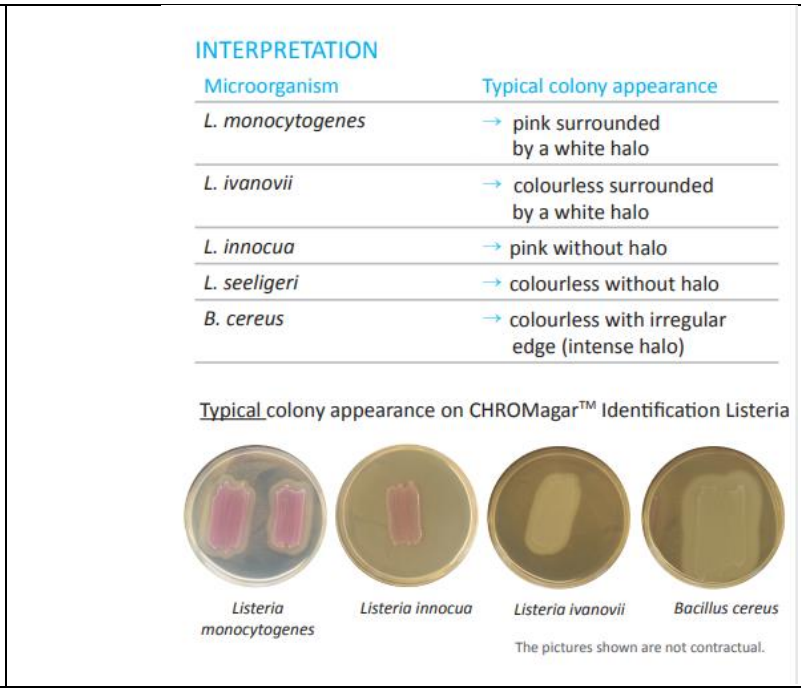

Table 2 shows the detection of pathogenic bacteria within the cheese sample.

CHROMagar *E. coli* can produce blue colonies if the medium is designed to detect a different enzyme or biochemical characteristic specific to *E. coli*, such as β-galactosidase activity, which is common target in chromogenic media for *E. coli* identification [20].

For *Salmonella*, CHROMagar utilizes substrates that are metabolized by enzymes produced by *Salmonella* species. This metabolic activity results in the development of colored colonies (purple), which stand out against other bacteria that do not produce the same enzymatic reactions. This selective coloring aids in the rapid identification and differentiation of *Salmonella* from other enteric bacteria [17].

In the case of *Listeria* on CHROMagar, the appearance of pink colonies with a white halo is indicative of *Listeria* species. The pink color typically results from the enzymatic activity of *Listeria* spp. that cleaves chromogenic substrates in the agar. The white halo that sometimes surrounds the colonies can be due to the bacterial colony's natural morphology or a specific reaction with the agar medium, which helps in distinguishing *Listeria* from other bacteria [18].

For *S. aureus*, CHROMagar is formulated to detect the presence of enzymes like phosphatase. *S. aureus* colonies on this medium will typically appear as mauve or pink colonies due to the enzymatic cleavage of the chromogenic substrate. This

color differentiation helps distinguish *S. aureus* from other staphylococci and microbial flora [19].

The results highlight the importance of strict hygiene measures throughout the cheese production and distribution process to ensure consumer safety. "Telemea" Cow's Milk Cheese (packaged, Supermarket, Bucharest) and Aged Cow's Milk Cheese (packaged, Chisinau Supermarket) by absence of detected pathogenic bacteria suggests better hygiene standards in commercially packaged cheese compared to unpackaged varieties.

Table 2

Detection of pathogenic bacteria within the cheese sample

Type of cheese/Code sample	**Detection of pathogenic bacteria within the cheese sample**			
	E. coli	***S. aureus***	***Listeria***	***Salmonella***
Roquefort cheese (1)		√	√	√
aged sheep's milk cheese (unpackaged, Chisinau market) (2)			√	
fresh cow's milk cheese (unpackaged, Chisinau market) (3)				√
aged cow's milk cheese (unpackaged, Chisinau market) (4)				√
aged cow's milk cheese (packaged, Chisinau supermarket) (5)				
"telemea" goat's milk cheese (unpackaged, "Obor" market, Bucharest) (6)	√		√	
"telemea" cow's milk cheese (unpackaged, "Obor" market, Bucharest) (7)			√	
cottage cheese (unpackaged, "Obor" market, Bucharest) (8)				√
"telemea" salted cow's milk cheese (packaged, "Obor" market, Bucharest), type1 (9)	√			√
"telemea" salted cow's milk cheese (packaged, "Obor" market, Bucharest), type 2 (10)	√		√	√
salty telemea cheese from cow's and sheep's milk ("Obor" market, Bucharest) (11)		√	√	
"telemea" goat's milk cheese (packaged, Amilact company, Făgărașul Nou) (12)	√			√
smoked cow's milk cheese (packaged, Dâmboviţa County) (13)			√	

smoked cow's milk cheese (unpackaged, Sinaia area) (14)			√	√
"telemea" cow's milk cheese (unpackaged, Arad County) (15)				√
lightly salted goat's milk curd (packaged, Amilact company, Făgăraşul Nou) (16)	√		√	
"telemea" sheep's milk cheese (packaged, Amilact company, Făgăraşul Nou) (17)	√		√	
non-smoked cow's milk cheese (unpackaged, Sinaia area) (18)			√	
Burduf cheese (packaged, Sinaia area) (19)	√	√	√	√
"telemea" cow's milk cheese (packaged, supermarket, Bucharest) (20)				

The presence of all tested pathogenic bacteria in Burduf cheese indicates significant contamination issues, raising concerns about safety and quality. The presence of *E. coli*, *S. aureus*, and *Listeria* in Roquefort Cheese, combined with its 50% price reduction, underscores the potential health risks associated with purchasing perishable products at reduced prices. This caution is particularly relevant considering that this type of cheese is made from raw milk rather than pasteurized. Therefore, it is not advisable to purchase perishable items at half price, as they may pose significant health hazards. Detection of pathogenic bacteria in some unpackaged types of cheese highlights potential risks associated with fresh, unpackaged cheese sold in markets. Detection of pathogenic bacteria in cottage cheese underscores the importance of proper handling and storage practices for fresh dairy products. **T**he packaged aged cow's cheese from Chisinau and the sample of salty cow 'telemea' from the Supermarket in Bucharest, were the only samples that did not exhibit bacterial contamination. Considering these findings, it is advisable to purchase packaged cheese for safety reasons.

It can be inferred that hygiene standards are not adhered to in marketplaces, as well as by certain producers who fail to comply with these regulations. Considering these findings, it is imperative to exercise caution when purchasing perishable food items at discounted prices, as our health may be compromised.

6. Conclusions

Out of the 20 samples examined, pathogenic bacteria were detected in 18 samples, along with additional colonies highlighted by staining that did not belong to the targeted pathogen category. Among these were coliform bacteria. The only samples that were not contaminated with the tested pathogenic bacteria were: packaged aged cow's cheese from Chisinau and the sample of salty cow "telemea" (the salt worked as an antibacterial agent) from Supermarket, Bucharest. Further investigation into the specific factors contributing to contamination in certain cheese types is warranted for effective mitigation strategies. We can conclude that

hygiene conditions are not respected in the tested markets, as well as among producers who also do not respect these rules. Therefore, consumers are advised against prioritizing cost-saving measures over food safety considerations.

Acknowledgments

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