j.Egypt.vet.med.Assoc 82, no 2-3. 175 – 188 (2022)

ISOLATION AND IDENTIFICATION OF MULTIDRUG-RESISTANT POTENTIAL PATHOGENS FROM RAW MILK AND SOME DAIRY PRODUCTS

By

Dina Ashraf [a], Ayah B. Abdel-Salam[b], Ombarak, R. A. [c] and Samir A[a]

[a] Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt.

[b] Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Egypt.

[c] Department of Food Hygiene and Control, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

ABSTRACT

Milk and milk products are vital food sources for humans. However, they could be a source of public health hazards if un-hygienically produced and handled. Among the most deleterious hazards that milk and milk products can transmit to the consumer are bacterial agents. Therefore, this study aimed to investigate the incidence of *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus Cereus* in raw cow's milk; Kariesh cheese and rice with milk (A dessert). A total of 112 samples (70 Raw Milk, 30 Kariesh Cheese and 12 Desserts) were examined. The incidence of *E. coli* was 82.85 %, 53.33 %, and 33.33 % in raw milk, Kariesh cheese, and rice with milk samples respectively. While the incidence of *S. aureus was* 17.14 %, 6.66%, and 0% in the same samples respectively. On the other hand, *B. cereus* incidence was 11.42 %, 6.66 and 66.66 % for same samples, respectively. Isolated potential pathogens (60 isolates of *E. coli,* 10 isolates of *S. aureus,* and 30 isolates of *B. cereus*) were subjected to antimicrobial susceptibility testing. The antibiogram of the isolates indicated that *E. coli* isolates were resistant to gentamycin (73.33%), followed by ampicillin (53.3%) and (16.66%) for cefotaxime. while all isolates were sensitive to tetracycline, ciprofloxacin, and trimethoprim - sulphamethoxazole by 100%. For *S. aureus*, all isolates were resistant to ampicillin (100%), followed by 60% resistance to each of tetracycline, cefotaxime, and gentamycin, while all isolates were sensitive by 100% for ciprofloxacin, amoxicillin-clavulanic acid, and trimethoprim-sulphamethoxazole. Regarding *B.cereus* all isolates showed resistance to ampicillin (100%), followed by amoxicillin (20%) and tetracycline (6.66%), while all isolates were sensitive

100% to vancomycin. multidrug resistance was conversed by 3 *S. aureus* isolates, 1 *E. coli* isolate and 1 *B. cereus* isolate. Conclusively, Our Findings denote the incidence of health hazards in raw milk and products produced from it such as Kariesh cheese; and the high incidence of antibiotic-resistant bacteria that can cause human illness and be difficult to be treated.

Keywords:

E. coli, S. aureus, B. cereus, Raw milk, Kariesh cheese, Rice with milk, Dessert, Multidrug resistance.

INTRODUCTION

Milk and dairy products represent one of the most elemental foods for all age categories because of their biological components,which are the basis for the elevation and maintenance of balanced nutrition**.** Milk is a multi-component blend, with its primary components of proteins, fat, lactose, vitamins, and minerals that are involved in the formation of a complex food **(Ľubica *et al.*, 2019).**

Milk and dairy products provide favorable environment for the growth of many microorganisms due to of their nutrient composition. Many studies have been performed to improve raw milk quality through reducing the risk of microbial contamination and increasing the chemical nutritional quality of dairy products. However, the high incidence of toxin producing pathogens makes consumption of raw milk or products prepared from it pose a serious risk to public health. Therefore, studies focused on the improvement of the dairy industry and the production of safe milk and products are highly considered recently.

several pathogenic bacterial species may contaminate milk and milk products. Most of these bacteria can produce toxins that cause food poisoning for human through ingesting either the toxins or other viable pathogenic bacteria together with its produced toxins **(Aneja *et al.*, 2002).** Bacterial pathogens in raw milk and dairy products made from it are *Mycobacterium bovis, Salmonella spp., Corynebacterium spp., Coxiella burnetii, Clostridium perfringens,* Brucella, *Staphylococcus* spp*., Bacillus Cereus, Yersinia enterocolitica, Campylobacter jejuni, Escherichia coli, Listeria spp*.,and coliforms.Among them *Staphylococcus aureus,Salmonella spp., Listeria monocytogenes*, *Bacillus Cereus* and *Escherichia coli* are the most common **(Olatunji *et al.*, 2009; Fadaei, 2014; Sima *et al.*, 2022).** According to public health agencies, these microbes are frequently linked to foodborne disorders and outbreaks. The presence of

these pathogenic bacteria in milk has become a major public health problem, particularly among individuals who continue to drink unpasteurized raw milk **(Claeys *et al.*, 2013).**

Bacterial contamination of milk may result from one or all of many factors**.** The milking process, utensils used for milking, and milking personnel are major routes of milk contamination. However, the presence of bacterial hazards in the milk may result from microorganisms that enter the udder through the teat canal **(Smith *et al.* 2007).** Other than unhygienic processing, milk and its products can be contaminated with harmful bacteria through mastitis, polluted air, or storage and transportation equipment **(Baylis, 2009).**

Total coliforms *E. coli*, and other enteric bacteria in Food, Water, Milk, and other dairy products are reliable indicators of fecal contamination. The presence of enteropathogenic and/or toxigenic microorganisms is indicated by the recovery of *E. coli* from food **(Soomro *et al.*, 2002).** Although most strains of *E. coli* live as commensals, many are opportunistic pathogens of humans and animals. Meanwhile, there are naturally pathogenic strains of *E. coli* that when ingested, cause gastrointestinal illness in humans and animals. The pathogenicity of *E. coli* strains can be due to the presence of one or more virulence factors such as invasiveness Factors, Heat-labile, and Heat-Stable Enterotoxins, and colonization factors **(Kaddu-Mulindw *et al.*, 2001).** *S. aureus*, a Gram-Positive, Coagulase-Positive ubiquitous bacterium, is considered one of the most common causes of disease in the world **(Pereira *et al.* 2009).** Milk and milk products are known to be a source of *S. aureus* contamination due to mastitis or milk handlers **(Bingol *et al.* 2012).** As milk and dairy products are good substrates for *S. aureus* with subsequent food poisoning. Therefore, reducing the level of such pathogens in milk and dairy products will lower the risk to consumers. Staphylococci are considered indicator microorganisms for hygiene; therefore, their presence may be referred to neglected hygienic measures applied during the production, processing, and distribution of milk and dairy products.

Coagulase-Positive *Staphylococcus aureus* (CPS) is considered the most important species of Staphylococci due to its pathogenicity and capability of enterotoxin production. Staphylococcal foodborne intoxication, in which major symptoms are vomiting and diarrhea occurs after ingestion of thermostable staphylococcal enterotoxins (SE) produced in food by enterotoxigenic strains of Coagulase-Positive staphylococci mainly *S. aureus* **(Ostyn *et al.*, 2010).** *Bacillus Cereus* is a foodborne pathogen and a common contaminant of various dairy products. The fast growth

rate of *B. cereus* has a bad impact on the shelf life of pasteurized milk. Four major toxins causing diarrhea, vomiting, and liver failure are produced by *B. cereus* **(Jessberger *et al.*, 2014).** Ingestion of more than $1x10^6$ organisms per gram of food or its toxins can cause illness **(FDA, 20099).** Symptoms Including Nausea, Diarrhea, and/or Vomiting, May occur between ½ hour and 24 hours after ingesting contaminated food. Symptoms rarely last more than 24 hours.

The presence of *B. cereus* spores in milk is unavoidable. This pathogen produces both toxins and spores capable of surviving pasteurization. *Bacillus Cereus* can grow at 5°C, Only slightly above refrigeration temperature. Therefore, even slight temperature abuse over a sixteen-day shelf life may render the product unsafe. Rapid detection of *B. cereus* in food is important to facilitate the application of quality control measures to eradicate *B. cereus* from food and improve diagnosis of food poisoning outbreaks **(Subhash *et al.*, 2019).**

Rice milk is a popular dairy dessert in egypt and in some european countries. Rice milk is prepared by cooking milk with rice and addition of sugar, cooled and served as dessert dish. It is desired among people of different ages due to its pleasant and satiating power.

The incidence of contamination of rice dessert with different pathogens is high due to unhygienic storage and distribution. *B. cereus* is one of the most commonly isolated pathogens from this product from raw Milk, Rice, Sugar and other powder additives **(Hegazi *et al.*, 2007).**

Inappropriate use of antibiotics is a common problem in human and veterinary Medicine, Which may result in the development of multidrug-resistant microorganisms. The antibiotic resistance in pathogenic bacteria is known as a big challenge for public health worldwide **(Sima *et al.*, 2022).** The emergence of Antibiotic-Resistant bacteria in both animals and humans requires urgent attention**.** The problem arises when antibiotic-resistant pathogens are transmitted from animals to humans in different ways including milk and dairy products leading to serious public health concerns**.** These concerns include infections that are difficult to treat and are associated with high mortality rates, extended hospital stays, and increased treatment costs **(Huijbers *et al.*, 2015).** The wide array of available dairy foods challenges food hygienists to find the best ways to prevent the entry of microorganisms,destroy those that do get in along with their enzymes, and prevent the growth and activities of those that escape processing treatments. Therefore, the purpose of this study was to figure out the incidence of some foodborne

pathogens and investigate their antibiotic resistance in raw milk and some dairy products due to their dangerous consequences as biological hazards on human health.

MATERIAL AND METHODS

Study Samples:

The study was conducted on a total of 112 samples; 70 samples of raw milk, 30 Kariesh cheese samples and 12 dessert samples (Rice with milk). Samples were randomly collected in their retail containers during the period from December 2020 to April 2021 at the consumer level from dairy shops, grocery stores, street vendors at different markets and dairy farms in cairo Giza governorates of egypt. The samples were collected and transported while cold in the insulated ice box, with minimum delay, to the laboratory.

Preparation of Samples:

Twenty-Five milliliters of raw milk or 25 grams of the other dairy products were prepared according to **(ISO 6887-5: 2020).**

1- Isolation and Identification of *E. coli*:

E. coli isolation was performed according to **ISO 16649-2 (2001) and identified** according to the methods recommended by **APHA (1992).**

2- Isolation and Identification of *S. aureus*:

Isolation of staphylococci was conducted as described by **(ISO 6888-1:1999).**

3- Isolation and Identification of *Bacillus cereus*:

Isol*ation* and *Identification* of *Bacillus Cereus* was *performed according* to **(ISO 7932:2004).**

4- Antimicrobial susceptibility testing of bacterial isolates:

Antimicrobial susceptibility patterns of bacterial isolates were determined on Mueller-Hinton agar (Oxoid,Hampshire, England) using the Kirby-Bauer disk diffusion according to **CLSI (2020)** guidelines.

E. coli isolates were tested against eight commercially available antimicrobial disks: ampicillin (AM, 10 µg), amoxicillin-clavulanic A. (AMC, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TE, 30 µg), and trimethoprim-sulphamethoxazole (SXT-25 µg).

For testing *S. aureus* isolates, eight antibiotics, that are commonly used in the field, were selected that were ampicillin (AM, 10 µg), amoxicillin-clavulanic A. (AMC, 30 µg), Cefotaxime (CTX, 30 µg),Ciprofloxacin(CIP,5 µg),Gentamicin(GM,10 µg),Tetracycline (TE, 30 µg), Trimethoprim-Sulphamethoxazole (SXT, 25 µg), Vancomycin (VA, 30 µg).

B. cereus isolates were tested against amoxicillin (AX, 25 µg), Ampicillin, Tetracycline, and Vancomycin (Oxoid, UK).

Bacterial inoculum was prepared by mixing pure colonies in sterile normal saline (0.85%) in eppendorf tubes with the turbidity adjusted to 0.5 McFarland standards equivalent to 1.0×10^8 CFU/ml. The prepared inoculum was uniformly streaked on Mueller-Hinton agar plates using sterile swabs then the antibiotic discs were applied**.**The plates were incubated at 37°C for 20^{hr}. and the diameters of the inhibition zones were measured, and the results were interpreted according to **CLSI (2012, 2020**) guidelines.

RESULTS

Table (1): Incidence of *E. coli* in examined samples.

Sample type	No. of samples	No. of positive samples (%)	No. of obtained isolates
Raw milk	**70**	**58 (82.85%)**	**60**
Kariesh Cheese	**30**	**16 (53.33%)**	**16**
Rice with milk (Desert)	**12**	**4 (33.33%)**	**6**
Total	**112**	**78 (69.64%)**	**82**

Table (2): Incidence of *S. aureus* in examined samples.

Sample type	No. of samples	No. of positive samples (%)	No. of obtained isolates
Raw milk	**70**	**12(17.14%)**	**16**
Cheese	**30**	**2 (6.66%)**	**6**
Rice with milk (Desert)	**12**	**0 (0%)**	**0**
Total	**112**	**14 (12.5%)**	**22**

Table (3): Incidence of *B. cereus* in examined samples.

Sample type	No. of samples	No. of positive samples (%)	No. of obtained isolates

Raw milk	**70**	**8 (11.42%)**	**14**
Kariesh Cheese	**30**	**2 (6.66%)**	**6**
Rice with milk (Desert)	**12**	**8 (66.66%)**	**24**
Total	**112**	**18 (16.07%)**	**44**

Table (4): Incidence of *E. coli, S. aureus,* and *B. cereus* in farm and market raw milk samples

Isolated Organisms	**Raw farm milk (N=42) No. of positive samples (%)**	**Raw market milk (N=28) No. of positive samples (%)**	**Total (N=70) No. of positive samples (%)**
E. coli	**34 (80.95)**	**24 (85.71)**	**58 (82.85)**
S. aureus	**9 (21.42)**	**3 (10.71)**	**12 (17.14)**
B. cereus	**3 (7.14)**	**5 (17.85)**	**8 (11.42)**

Table (5): Antibiotics sensitivity of *E. coli, S. aureus* and *B. cereus* isolated from examined samples.

Antimicrobial Agent*	***E. coli* No. (%) of isolates (N=60)**			***S. aureus* No. (%) of isolates (N=10)**			***B. cereus* No. (%) of isolates (N=30)**		
	R (%)	**I (%)**	**S (%)**	**R (%)**	**I (%)**	**S (%)**	**R (%)**	**I (%)**	**S (%)**
AM	**32 (53.3)**	**24 (40)**	**4 (6.66)**	**10 (100)**	**0 (0)**	**0 (0)**	**30 (100)**	**0 (0)**	**0 (0)**
AMC	**2 (3.33)**	**16 (26.66)**	**42 (70)**	**0 (0)**	**0 (0)**	**10 (100)**	**NA**	**NA**	**NA**
GM	**44 (73.33)**	**14 (23.33)**	**2 (3.33)**	**6 (60)**	**4 (40)**	**0 (0)**	**NA**	**NA**	**NA**
TE	**0 (0)**	**0 (0)**	**60 (100)**	**6 (60)**	**0 (0)**	**4 (40)**	**2 (6.66)**	**0 (0)**	**28 (93.33)**
CTX	**10 (16.66)**	**0 (0)**	**50 (83.33)**	**6 (60)**	**0 (0)**	**4 (40)**	**NA**	**NA**	**NA**
CAZ	**8 (13.33)**	**0 (0)**	**52 (86.66)**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
C	**0 (0)**	**10 (16.66)**	**50 (83.33)**	**0 (0)**	**2 (20)**	**8 (80)**	**NA**	**NA**	**NA**
CIP	**0 (0)**	**0 (0)**	**60 (100)**	**0 (0)**	**0 (0)**	**10 (100)**	**NA****	**NA**	**NA**
SXT	**0 (0)**	**0 (0)**	**60 (100)**	**0 (0)**	**0 (0)**	**10 (100)**	**NA**	**NA**	**NA**
AX	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**6 (20)**	**18 (60)**	**6 (20)**
VA	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**0 (0)**	**0 (0)**	**30 (100)**

***AM, ampicillin (10 µg); AMC, amoxicillin-Clavulanic A. (30 µg); AX, amoxicillin (25 µg); C, chloramphenicol (30 µg); CAZ, ceftazidime (30 µg); CIP, ciprofloxacin (5 µg); CTX, cefotaxime (30µg); GM, gentamycin (10 µg); SXT,trimethoprim- sulphamethoxazole (25 µg); TE, tetracycline (30 µg); VA, vancomycin (30 µg).**

**** NA, Not Applicable.**

Table (6): Multidrug-resistant (MDR) *E. coli, S. aureus,* and *B. cereus* isolated from examined samples.

Pathogen with MDR pattern	No. of strains with MDR pattern	MDR patterns*
E. coli	**1**	**AM, CTX, CAZ**
S. aureus	**2**	**AM, GM, TE**
	1	**AM, CTX, GM, TE**
B. cereus	**1**	**AM, AX, TE**

***AM, ampicillin (10 µg); AX, amoxicillin (25 µg); CAZ, ceftazidime (30 µg); CTX, cefotaxime (30 µg); GM, gentamycin (10 µg); TE, tetracycline (30 µg).**

DISCUSSION

Milk and its products are important sources of food for the human population all over the world. Despite their numerous health benefits, milk is an excellent medium for the growth of a large number of numerous bacteria that could represent human health hazards, such as *S. aureus,*

E. coli, and *B. cereus* which are involved in most foodborne illnesses. In addition to the danger of food poisoning, milk and its products may be potential sources of Multidrug-Resistant

(MDR)bacterial strains due to the misuse of antibacterial therapeutics in dairy farm management. When reaching human consumers, these MDR strains constitute a huge obstacle to the efficiency of the antibacterial therapeutics in human medicine. Also, MDR determinants could be transferred to other bacteria of clinical significance **(Ombarak *et al.*, 2018).**

The results of the current study highlighted that *E. coli* was the most prevalent among the investigated pathogens in the examined milk and cheese samples (Tables 1, 2, and 3). This could be attributed to the wide spreading of *E. coli* on the animal body and in the environment as the organism is a common fecal resident. Out of 112 tested samples, *E. coli* was isolated from 58 (82.85%), 16 (53.33%), and 4 (33.33%) raw milk, Kariesh Cheese, and

rice with milk samples, respectively with an overall isolation percentage of 78 (69.64%) (Table 1). The prevalence of *E. coli* in raw milk and its products is indicative of poor hygienic measures during milking, insufficient washing, and sterilization of dairy utensils, worker hands, and animal udders **(Sultana *et al.*, 2021).**

The high prevalence of *E. coli* in milk and cheese samples was previously reported by **Asmahan and Warda (2011) and Hussain *et al.* (2014)** who reported 63% and 50% prevalence rates of *E. coli*, respectively in raw milk in Sudan and Pakistan. On the other hand, the prevalence of *E. coli* was high in other reports but with lesser rates than ours as 26% by **(Sultana *et al.*, 2021),** 24% by **(Annalselvamalar *et al.*, 2018)** and 26.7% by **(Zeinhom and Abdel-Latef, 2014)**. This variation in the prevalence rates of *E. coli* could be attributed to differences in the origin of samples as well as the techniques used for the collection and transportation of samples or environmental conditions.The finding of **Sultana *et al.* (2021)** who isolated *E. coli* from yogurt samples supports the isolation of *E. coli* from rice with milk despite being boiled. On the other hand, *S. aureus* was isolated from raw milk at a higher rate (17.14%) than from Kariesh cheese (6.66%), while rice with milk samples were all negative. The overall incidence of *S. aureus* in the different types of samples was 12.5% (Table 2). These results may be attributed to the contamination of raw milk during production which could be easily controlled during dairy product processing either via high acidity percentage as in the case of Kariesh cheese or heat treatment as in the case of desserts. The high incidence of *S. aureus* in raw milk samples reported by **Zeinhom *et al*.(2021)** from Beni Suef, Egypt was attributed to environmental pollution, cross-contamination, and poor handling during transportation or in milk collection centers. In addition, the shedding of *S. aureus* from infected animals was also blamed. The Egyptian Organization of Standardization and Quality Control **(ES, 2005)** recommends that Kariesh cheese must be free from *S. aureus* or its toxins. However, in the current study, 6.7 % of samples were contaminated with the organism. **Zeinhom *et al.* (2021)** isolated *S. aureus* from Kariesh cheese in Egypt with a higher incidence rate (18%). This could be explained by the fact that Kariesh cheese is mostly homemade and manufactured with no prior heating or pasteurization of milk.

Looking at the incidence of *B. cereus*, it was clear that, the organism was more prevalent in the rice with milk than in other samples. (Table 3) showed that 8 samples were positive from the raw milk samples with incidence (11.42%), Only 2 samples from Kariesh cheese were

positive for *B. cereus* with an incidence percentage (6.66%) while the highest incidence for *B. cereus* was in rice with milk samples (66.66%). The extent of *B. cereus* contamination depends on the effectiveness of hygienic measures applied during the processing, handling, and distribution of milk products. The low prevalence of *B. cereus* in Kariesh cheese samples comes in agreement with previous reports that referred to the acidity of that kind of cheese **(Osama *et al.*, 2020).** On the contrary, the high prevalence of *B. cereus* in rice with milk samples could be due to the slight boiling during cooking which leads to the destruction of most of the vegetative bacterial species leaving the heat-resistant *B. cereus* spores to flourish after cooling.

The incidence of each bacterial species was compared between farm (n = 42) and market (n = 28) raw milk samples, to investigate different sources of contamination alongside the milk supply chain, in addition to studying the effect of time on milk quality from production till being sold in markets. *E. coli* were isolated with higher incidence from market milk samples than farm milk samples, with percentages of 85.71% and 80.95%, respectively. *E. coli* was isolated with a 4.76 % higher incidence from market milk samples than farm milk samples, which is considered as a non-significant difference between them. *B. cereus* was isolated with a higher incidence from market milk samples (17.85%) than in farm milk samples (7.14%). This may be attributed to the flourishing effect on *B. cereus* spores by decreasing other bacterial cells; in addition, different sources of contamination after milking may increase the level of bacteria especially spore-forming pathogens from the surrounding environment.On the contrary, *S. aureus* was isolated with a higher incidence from farm milk samples (21.42%) than in market milk samples (10.71%) indicating the good effect of cooling during storage and distribution on the *S. aureus* population (Table 4). Together with investigating the incidence rates, investigating the antibiotic resistance phenotypes of the pathogens isolated from food sources is crucial.

In our study, *E. coli* isolates showed high resistance rates against gentamicin (73.33%), ampicillin (53.3%), (16.66) for cefotaxime and were moderately sensitive to chloramphenicol, ceftazidime cefotaxime, and Amoxicillin-Clavulanic Acid, While (100%) sensitive to Tetracycline, Trimethoprim-Sulphamethoxazole, Ciprofloxacin. For *S. aureus*, all isolates were resistant to ampicillin and followed by tetracycline, cefotaxime, and gentamycin (60%). On the other hand, all the isolates were sensitive to ciprofloxacin, amoxicillin, clavulanic acid, and trimethoprim-sulphamethoxazole. Regarding *B. cereus* all isolates showed resistance to

ampicillin followed by amoxicillin (20%) and tetracycline (6.66%), all isolates were sensitive to vancomycin (Table 5). By definition, multidrug resistance (MDR) acquired non-susceptibility to at least one agent in three or more antimicrobial categories **(Magiorakos *et al.*, 2012)**. Results in (Table 6) illustrate the incidence of multidrug-resistant strains among the recovered isolates. The highest MDR rate was met with *S. aureus* of which 3 isolates out of 10 (30%) conferred resistance to three antimicrobials belonging to different categories with one of them even resistant to 4 agents.

On the other hand, only one out of 60 *E. coli* isolates (1.66%) and 1 out of 30 *B. cereus* isolates (3.33%), and all five isolates, isolated from raw milk, showed a multidrug resistance profile. Our findings coincide with some previous studies, in a study conducted by **Zeinhom *et al.* (2021)**, *S. aureus* isolates from raw milk and cheese were resistant to ampicillin (72%) and tetracycline (60%). Concerning *E. coli*, the MDR isolates prevalence (1.66%) detected in the present study is not as frightening as what was reported by **Ombarak *et al*. (2018)** who reported that 50% of *E. coli* isolates recovered from raw milk and cheese samples, in Egypt, were MDR. The absolute resistance of *B. cereus* to ampicillin agrees with the report of **Osama *et al*. (2020)** in Egypt. Detection of even one MDR isolate of *B. cereus*, as in the current study, is alarming as the bacterium is spore formers and able to reside in the environment for long periods carrying this transmissible criterion.

In conclusion, the safety of raw milk and milk products investigated in the current study is not as it should be. Sequentially, Such products represent a potential public health hazard.

Moreover, the existence of MDR strains exaggerates the problem and calls for attention to the urgent need for decisive rules and regulations to face the increasing misuse of antibacterial therapeutics in dairy herd management and emphasize the need for new natural antimicrobial therapeutic agents. Milk pasteurization is essential for the production of High-Quality and safe milk and dairy products that satisfies customers and national standards requirements.

Conflict of interest:

The authors declare that there is no conflict of interest.

REFERENCES

Aneja R.; Mathur B.; Chandan R.; and Banerjee A.; Technology of Indian Milk Product (2002): Handbook on Process Technology Modernization for Professionals, Entrepreneurs، and Scientists, Dairy India Yearbook, England.

Annalselvamalar, P.; Sekar, M.; Porter K.; Narayan, R.; Elango, A.; Kummar, M.; Sowmiya, M. and Devi, T. (2018): Screening of *E. coli* for its antimicrobial susceptibility pattern in milk and dairy products in Chennai, India. International journal of chemical studies, 6: 35-37.

APHA (American Public Health Association) (1992): Standard methods for the examination of dairy products 16th Ed., American public health Association, Washington.

Asmahan, A. A. and Warda, S. A. (2011): Incidence of *Escherichia coli* in raw cow's milk in khartoum state. British journal of dairy sciences, 2 (1): 23 -26.

Baylis, C. L. (2009): Raw milk and raw milk cheese as vehicles for infection by Verocytotoxin-Producing *Escherichia coli*. Int. J. Dairy Tech., 62: 293-307.

Bingol, E. B.; Cetin O.; Colak H. and Hampikyan H. (2012): Presence of enterotoxin and verotoxin in turkish cheeses sold in istanbul. Turkish J. Vet. Anim. Sci., 36: 424 - 432.

Claeys, W. L.; Cardoen, S.; Daube, G.; De Block, J.; Dewettinck, K., Dierick, K.; De Zutter, L.; Huyghebaert, A.; Imberchts, H.; Thiange, P.;Vandenplas, Y., and Herman, L. (2013): Raw or heated cow milk consumption: Review of risks and benefits. Food control, 31(1),251-262.

CLSI, (Clinical and Laboratory Standards Institute) (2012): Performance standards for antimicrobial disk susceptibility tests-approved standard, 11th ed. CLSI document M02-A11. Clinical and laboratory standards institute, wayne, PA.

CLSI, (Clinical and Laboratory Standards Institute) (**2020):** Performance standards for antimicrobial disk susceptibility tests-approved standard, 30th ed. CLSI document M100. Clinical and laboratory standards Institute, Wayne, PA.

ES. (Egyptian Standards) (2005): Egyptian organization of standardization and quality control. Issued by the ministry of industry and technological development. Egypt.

Fadaei A. (2014): Bacteriological quality of raw cow milk in Shahrekord, Iran, Veterinary World, 7 (4): 240 -243.

FDA, (Food and Drug Administration) (2009): Dairy Products - Microbial contaminants and alkaline phosphatase activity. FDA/ORA compliance policy Guide, Sec. 527/300 (Issued: 2009). Department of health and human Services, Public health service, Washington, DC.

Hegazi, N. A. ; El-Nawawy, M.A. and Hafez, A.H. (2007): Evaluation of rice dessert manufactured with different types of milk. J. Agric. Sci. Mansoura Univ., 32 (12): 10239 - 10243, 2007.

Huijbers, P.M.; Blaak, H.; De Jong, M.C.; Graat, E.A.; Vandenbroucke-Grauls, C.M. and De Roda Husman, A.M. (2015): Role of the environment in the transmission of antimicrobial

resistance to Humans: A review.Environmental science and technology, 49 (20), pp.11993-12004.

Hussain, A.; Ali, S.; Shakir, H.A.; Qazi, J.I.; Ali, N. and Ullah, N. (2014): Prevalence of *Escherichia coli* in milk and different milk products sold under market conditions at lahore. Scientific journal of animal science, 3 (1): 22-26.

ISO, (International Organization For Standardization) (16649-2:2001): Microbiology of food and animal feeding STuff - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* - Part 2: Colony-Count technique at 44°C using 5 - Bromo - 4 -Chloro-3-Indoyl β-D-Glucuronide.

ISO (International Organization for Standardization) (6887-5:2020): Microbiology of food chain preparation of test Samples, Initial Suspension, and decimal dilutions for microbiological Examination - Part 5: Specific rules for the preparation of milk and milk products.

ISO (International Organization for Standardization) (6888-1:1999): Microbiology of food and animal feeding Stuff - Horizontal method for the enumeration of Coagulase-Positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium.

ISO (International Organization for Standardization) (7932:2004): Microbiology of food and animal feeding Stuff - Horizontal method for the enumeration of presumptive *Bacillus Cereus* - Colony - Count technique at 30°C

Jessberger, N.; Dietrich, R.; Bock, S.; Didier, A.; Märtlbauer, E. (2014): *Bacillus Cereus* enterotoxins act as major virulence factors and exhibit distinct cytotoxicity to different human cell lines. *Toxicon* 77, 49-57

Kaddu-Mulindw, D. H.; Aisu, T.; Gleier, K.; Zimmermann, S. and Beutin, L. (2001): Occurrence of shiga Toxin-Producing *Escherichia coli* in fecal samples from children with diarrhea and from healthy zebu cattle in uganda. Int J food microbiol 66, 95 -101.

Ľubica, K.; Kristína, P. and Zdenka, K. (2019): The importance of milk and dairy products **Magiorakos, A. P.; A. Srinivasan, R. B.; Carey, Y.; Carmeli, M. E.; Falagas, C. G.; Giske, S.; Harbarth, J. F.; Hindler, G.; Kahlmeter, B.; Olsson-Liljequist, D. L.; Paterson, L. B.; Rice, J.; Stelling, M. J.; Struelens, A.; Vatopoulos, J. T.; Weber and Monnet, D. L. (2012):** Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin microbiol infect; 18 (3): 268-281.

Olatunji, E.; Ahmed, I.; and Ijah, U. (2009): Evaluation of microbial qualities of skimmed milk (Nono) in nasarawa State, Nigeria, in proceedings of the 14th annual conference of the journal of food quality 7 paediatrics. Association of nigeria. (ASAN) Lautech, Ogbomoso, Nigeria.

Ombarak, R. A.; Hinenoya, A.; Elbagory, A.M. and Yamasak, S. (2018): Prevalence and molecular characterization of antimicrobial resistance in *escherichia* coli isolated from raw milk and raw milk cheese in egypt. Journal of food protection. 81(2): 226-232.

Osama, R. Marwa, A.; Amir, A.; Maha, A. (2020): Prevalence and antimicrobial resistance of *Bacillus Cereus* in milk and dairy Products. Mansoura veterinary medical journal 21: 11 - 18.

Ostyn, A.; De Buyser, M. L.; Guillier, F.; Groult, J.; Felix, B.; Salah, S.; Delmas, G. and Hennekinne, J. A. (2010): First evidence of a food poisoning outbreak due to staphylococcal enterotoxin type E, France, 2009. Euro surveillance, 1; 15 (13):19528.

Pereira, V.; Lopes, C.; Castro, A.; Silva, J.; Gibbs, P.; Teixeira, P. (2009): Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. Food microbiol. 26: 278-282.

Sima, H.; Mir-Hassan, M.; Sahar, N. G.; Seyed, A. K.; Abolfazl, H. and Zahra, B. (2022): High prevalence of antibiotic resistance in pathogenic foodborne bacteria isolated from bovine milk. Scientific Reports, 12, 3878.

Smith, K.; Peter, K.; Daniela, H.; and Melchior, S. (2007): Foodborne pathogenic microorganisms and natural toxins. Food safety and applied nutrition, 10: 119-150.

Soomro, A.H.; Arain; Khaskheli, M. and Bhutto, B. (2002): Isolation of *Escherichia* coli from raw milk and milk products in relation to public health sold under market conditions at tandojam. pakistan journal of nutrition, 1 (3), 151-152.

Subhash, C. M.; Abhishek, G.; Shekhawat, S. S.; Bincy, J.; Hitesh, K. and Nirmal, K. (2019): Isolation and identification of *Bacillus Cereus* from milk and milk products in Udaipur, Rajasthan, India. Int. J. Curr. Microbiol. App. Sci. (2019) 8 (9): 2783-2787.

Sultana, T.; Rabbi, B.R.; Sarker, M.S.; Islam, Begum, M.I.A.; and Hossain, K.M.M. (2021): Prevalence and antibiotic resistance patterns of *Escherichia* coli isolated from milk and milk products. J. Bio-Sci. 29 (2): 81-91.

Zeinhom, M.M.A, and Abdel-Latef, G.K. (2014): Public health risk of some milk-borne pathogens. Beni-Suef University Journal of Basic and Applied Sciences, 3: 209-215.

Zeinhom M.M.A and Abed A.H. (2021): prevalence, characterization, and control of *staphylococcus aureus* Isolated from raw milk and Egyptian Soft Cheese**.** Journal of Veterinary Medical Research 27 (2): 152-160.