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Analysis of the Responses to Pasteurization Temperatures of Methicillin Resistant *Staphylococcus aureus* Derived from Milk Items

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aim: One of the leading causes of both community- and hospital-acquired infections is the superbug," multidrug-resistant *Staphylococcus aureus* (MRSA). Milk and dairy products seem to be highly vulnerable to a wide range of diseases. Therefore, we require processing procedures that both eliminate pathogens and lengthen the useful life of these products. The purpose of this study was to determine how susceptible MRSA isolates were to pasteurization temperatures after being recovered from dairy products.
Materials and Methods: For the purpose of pasteurization, ten MRSA isolates were exposed to both a 63°C LTLT (low temperature, long time) therapy for 30 minutes and a 72°C HTST (high temperature, short time) treatment for 16 seconds.

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Results: The findings indicated that all isolates were killed by a pasteurization-like treatment at low temperature for a long time (63°C for 30 minutes), but that particular adaptable isolates could survive a pasteurization-like treatment at high temperature for a short time (72°C for 16 seconds), which may point to a time- and temperature-dependent variation in the thermal tolerance mechanism.

Conclusion: The results showed that all isolates seemed extremely susceptible to damage from heat using LTLT pasteurization-like treatment (63°C for 30 minutes). Nevertheless, a particular number of these isolates succeeded in establishing colonies because they have quorum sensing structures that survive being handled by using heat damage at 72°C for 16 seconds. Pasteurized milk producers should be alerted to the fact that this microorganism has been found in contaminated milk since it compromises the product's safety and diminishes its value to consumers. As a result, it is strongly advised that manufacturers utilize varied temperatures for milk processing in order to remove the contaminating methicillin- and heat-resistant *S. aureus* (MHRSA).

Keywords: Milk products; Staphylococcus aureus; pasteurization treatment; survival rate.

1. INTRODUCTION

Staphylococcus aureus (S. aureus) seems to be a common bacterium that causes many diseases that are increasingly problematic in veterinary medicine and animal agriculture [1,2] due to its ability to cause clinical infections in humans and animals, as well as food poisoning everywhere, in animals and people. MRSA's meteoric surge as an antibiotic-resistant public health problem has been seen in both human and veterinary medicine [3]. It was first recognized as a nosocomial infection, and then as a community-linked contagion that spread throughout the community. Also, in recent years [4], a new strain of MRSA, designated non-typable (NT)-MRSA, has emerged as a potential health risk. It is a highly adaptable pathogen that may cause a broad variety of diseases in humans and animals, from mild skin infections to life-threatening ones like pneumonia and septicemia [4]. Foodborne illnesses are a major global public health problem, and staphylococcal food poisoning (SFP) is a leading source of these infections [5]. The vast majority of cases of foodborne illness may be traced back to carelessness in the kitchen. Taking precautions to avoid contamination before, during, and after food preparation is important [5]. Bacteria are responsible for two-thirds of all food-related illness outbreaks, and there are currently 250 different types of food-borne illnesses. The annual incidence of foodborne illness in the United States is 48 million, as reported by the Centers for Disease Control and Prevention (CDCs). In the United States alone, poisoned food has been responsible for at least 128,000 hospitalizations and the deaths of at least 3,000 people [6]. Enterotoxigenic *Staphylococci,* strains of *S. aureus* that have evolved and produced toxins, are a leading cause of gastroenteritis. Staphylococcal enterotoxins are a potential biological threat since they can incapacitate humans for multiple days to weeks and are stable in very hot conditions (100°C for 1 hour) [7]. A contagious mastitis pathogen, *S. aureus,* is spread often during milking from infected to uninfected cows [6]. Enterotoxigenic strains of *S. aureus* and other dangerous pathogens have been found regularly in a variety of foods, including milk, dairy products, and meats, especially touched foods [8-20]. Genetic adaptations have allowed MRSA to survive in the presence of modern food-processing equipment and the immune systems of their hosts. Mimicking the pathogen's behavior by shifting the HACCP strategy related to the environmental epidemiology of MRSA populations from good management manufacturing practices to consumers may reduce the risk of pathogen contamination of food-producing animals and human cases of food poisoning [21,22]. A food-borne bacterium like MRSA was targeted in a number of studies designed to ensure food safety [23-25].

Thermal processing is a crucial production step in the food industry. Major food processes, such canning, pasteurization, and sterilization, are necessary for preserving food because they kill harmful microorganisms. Traditional in-container thermal processing comprises hermetic canning, followed by heat treatment for a predetermined time and temperature, to kill off any potentially harmful bacteria present and extend the shelf life of the product with little loss in quality. Ultra-high temperature (UHT), low-temperature long-time (LTLT), and high-

temperature short-time (HTST) are all types of extreme heat treatments [26].

Pathogenic bacteria may be reduced or eliminated in both low- and high-moisture foods by the use of the heat treatment process known as pasteurization. Conventional thermal procedures like baking, roasting, and extruding, controlled condensation steam processes, and energy-based technologies like irradiation, radio frequency heating, and cold atmospheric plasma are used to pasteurize low-moisture foods [26]. Thermal pasteurization, dielectric heating, and microwaves, for example [27], are used for the microbial inactivation of high-moisture food such juices or pulp. Pasteurization is the process of heating food to an internal temperature of less than 100 degrees Celsius in order to kill all vegetative cells of bacteria, both harmful and harmless. Pasteurization is often used in conjunction with other preservation methods such as acidity, low water activity, and cold storage [26].

Although milk's bacteria count is reduced and its shelf life is extended during the pasteurization procedure. However, pasteurization kills all the good bacteria in milk, including the lactic acid bacilli that aid digestion and the immune system. It has been shown that pasteurized and powdered milk contain less nutrients compared to raw milk, suggesting that pasteurization may impact the nutritional components of milk.

The purpose of this research was to determine how susceptible MRSA isolates were to pasteurization temperatures after being recovered from dairy products.

2. MATERIALS AND METHODS

2.1 Methods of Bacterial Isolation and Culture

Ten MRSA isolates were taken from a previous investigation [2] that had been found in raw dairy products. Table 1 displays codes of the isolates and sample histories.

Methods for bacterial isolation and identification were adapted from those used in the field of food microbiology [2]. Biochemical methods similar to those already reported [2], were used for the identification. With the Electronic RapID TM Staph Plus Code Compendium Panel System (ERIC®) with Installation ERIC® CD and Standard Color Differential Chart and Online ATCC Codes (Remel, R8311009), biochemical tests seemed to be used to establish *S. aureus* as a distinct species. To detect specific virulence factors unique to MRSA [2], we used the dry SPOT Staphytect Plus kit (Oxoid, DR0100M) for their identification. Prior to being kept in glycerin at -18°C, the MRSA isolates were tested for their presence of PBP2a using a latex agglutination PBP2 test kit (Oxoid, DR0900A) [2].

2.2 Sample Preparation and Inoculation

Purchased at a local Baghdad province market, the imported ultra-heat-treated tetra-pack milk was inspected for free of

Table 1. Isolates sample codes and case history [28]

Isolate No.	Isolate Code	Case History of Sample
1*	CVMM 1	Milk from apparently healthy Cow.
2*	AGM 2	Caseated milk from mastitic Cow.
3	AGM 3	Caseated milk pooled from milk cans.
4	AFM 4	Milk pooled from milk cans.
5	AGC 5	Cheese made from raw milk from individual Cow.
6 *	AFC 6	Cheese made from raw milk from Cow with a history of food poisoning of milkmaid.
7	AFC 7	Cheese made from raw milk from milk cans.
8	AFC 8	Cheese made from raw milk from milk cans.
9	AFC 9	Cheese made from raw milk from milk cans.
10	AFC10	Cheese made from pooled raw milk from Cows.

CVMM = Isolated Milk from the College of Veterinary Medicine Field
AGM = Milk Isolate from the Abu-Ghraib Area
AGC = Cheese Isolate from the Abu-Ghraib Area
AFM = Al-Fudhaliyah Region Milk Isolate
AFC = Cheese Isolate from the Al-Fudhaliyah District
** The most significant and potent MRSA isolates from ten isolates*

Staphylococci on Baird-Parker agar before being divided up into 25-mL portions in sterile duplicate beakers (gradually 100- mL size). After being thawed at 4 degrees Celsius for a full day, all of the isolates were subcultured on Baird-Parker agar (Oxoid, CM1127) with no added enhancements and kept at 37 degrees Celsius for a full day. Each beaker had 10 newly isolated colonies put into it (induced contamination), shaken up with a vortex mixer, and then incubated at 35–37 degrees Celsius with non-infected UHT milk samples as a reference. One day later, we used the Miles and Misra method (surface-viable method) to count the number of bacteria in both the infected and uninfected samples by allowing five drops to land at the end of each dilution series on a plate of Baird-Parker agar (Oxoid, CM1127), letting it dry, and then incubating the plates at 37°C for 24 hours. The following formula was used to get the microbial load log titer:

CFU per mL =Average number of colonies for a dilution×50×dilution factor [29].

Then, infected and uninfected duplicate beakers were microwaved in two patches at 63°C for 30 minutes and 72°C for 16 seconds, with the microwave's heat monitored with a milk thermometer. After cooling at room temperature for 15 minutes, the treated samples were coated with Parafilms and stored in the fridge at 4°C for another night to see if any sublethally damaged isolates recovered. Samples were diluted decimally and counted using the Miles and Misra technique to assess whether or not the heat treatment killed the MRSA isolates or decreased the number of isolates present in the original infected samples [1].

2.3 Data Analyses

Statistical software, Statistical Package for the Social Sciences [30], has been used to perform analyses on the data, such as cross-tabulation and Chi-square testing, using the following formula:

$$X^2 = (O-E)^2/E$$

in which
O = Positive *S. aureus* was discovered in total samples (+ve).
E = Negative samples devoid of *S. aureus* expected from the initial total samples (-ve), using Steel and Torri's method of calculating Z-scores to identify statistically significant differences between the various study isolates [31].

3. RESULTS AND DISCUSSION

There has been extensive research over the past few years into the heat resistance of pathogenic bacterial strains. Pasteurization is thought to be effective against mesophilic bacteria, but these bacteria are growing more resistant as they evolve a heat shock response and learn to survive in harsh environments [32].

According to our findings, all of the isolates were killed by a pasteurization-like treatment at LTLT (63°C for 30 minutes) but survived a pasteurization-like treatment at HTST (72°C for 16 seconds) (Tables 2 and 3, Fig 1), indicating that various strains use a variety of strategies to survive high temperatures (including heat shock proteins and biofilm development), depending on the duration and temperature of the treatment. In the first experiment, *S. aureus* isolates were heated to 63 degrees Celsius for 30 minutes, which resulted in the cells' continuous stretching, dilation, and expansion due to the lack of time for the cells to resist the heat.

The cells' resistance to the shock was also weakened by the presence of Staphylohaemolysins [6]. According to the results of Jay et al. [33], the connection between milk-water and *S. aureus* cells will increase due to the thermal processing of milk, because milk contains 78% water, and the release of free active radicals (SH-groups) from milk casein will increase, leading to death via denaturing of the bacterial cells. Some thermally potent isolates can withstand 72°C for 16 seconds, which could be because they have the genetic potential to withstand sub-lethal temperatures or because they have the ability to rebuild housekeeping proteins and then survive power by modifying and changing their morphological characteristics into elongated sausage-chain cell walls as one thermally tolerant body (incomplete dissociation phenomenon) [6]. The heat-shock response is an essential defense mechanism for bacterial survival and adaptability to extreme temperatures. Certain regulatory proteins either make it easier or harder for the RNA polymerase enzyme to start the transcription process, which allows for precise control of heat-shock gene transcription [34]. Sub-lethal treatments may in fact stress the surviving cells, boosting their resistance to subsequent challenges with the same or different stresses, and

Table 2. The effects of long-term heat treatment at a low pasteurization temperature (63°C for 30 minutes) on the survivability of *S. aureus* isolates [28]

Isolates Codes	Before heat treatment	After heat treatment
	LSACB	LSACA
All	(9.7-10) CFU\ mL	NG

All = all isolates from 1-10; LSACB = log mean count of S. aureus after milk contamination and before heat treatment at 63°C for 30 minutes [(5-10) x 10^9 CFU/ mL or (9.7-10) CFU/ mL]; LSACA = log mean count of S. aureus in milk after heat treatment (at 63°C for 30 minutes) after incubation overnight inside a refrigerator at 4°C

Table 3. Heat treatment experiment at 72˚C for 16 seconds (High pasteurization temperature short time) [28]

Isolates Codes	LSACB CFU\ mL	LSACA CFU\ mL
CVMM 1	(9.7-10)^{A}	6.7^{E}
AGM 2	(9.7-10)^{A}	8.7^{B}
AGM 3	(9.7-10)^{A}	6.1^{F}
AFM 4	(9.7-10)^{A}	7.8^{D}
AGC 5	(9.7-10)^{A}	7.0^{E}
AFC 6	(9.7-10)^{A}	8.7^{B}
AFC 7	(9.7-10)^{A}	8.0^{C}
AFC 10	(9.7-10)^{A}	7.7^{D}
Others	(9.7-10)^{A}	NG^{G}

A, B, C,D,E,F,G: Indicate significant differences between isolates horizontally and vertically at a confidence interval (P0.05); LSACB = log mean count of S. aureus in milk after contamination and before heat treatment at 72°C for 16 seconds [(5-10) x 10^9 CFU mL or (9.7-10) CFU mL]; LSACA = log mean count of S. aureus in milk after heat treatment (at 72°C for 16 seconds)

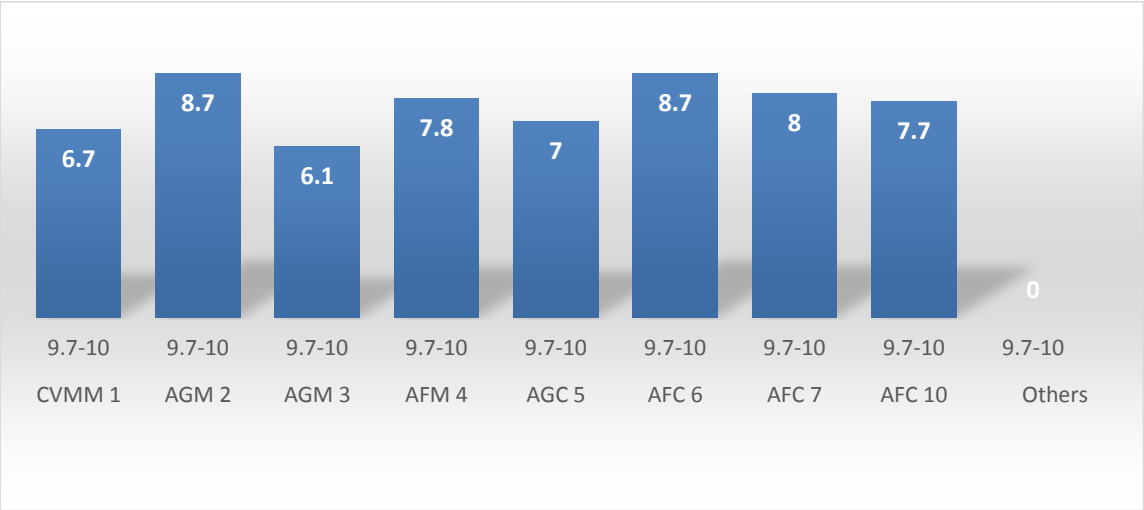

Fig. 1. Log mean count of *S. aureus* in milk after heat treatment CFU\ mL at 72˚C for 16 seconds

this phenomenon is becoming increasingly evident across an expanding range of bacteria. As a result, acclimating to extreme but non-lethal temperatures may enhance surviving cells' capacity to endure worse circumstances. Before reaching the consumer's table, the recently delivered food animal must travel a long distance. There are multiple separate phases, and the people engaged may or may not communicate with one another at each level. However, there are other potential threats to human life that depend on specific details. This means that primary production hygiene, immunization, logistical slaughter, cleaning, and disinfection methods should all be prioritized in areas with a history of pathogen prevalence.

More needs to be done to prevent the spread of disease through unchecked horizontal and vertical transmission. Additional molecular analysis of methicillin- and heat-resistant *S. aureus* (MHRSA) isolates, in particular AGM2 and AFC6, is required. Resistance to high heat treatment by MRSA has been indicated in several previous studies [32, 34–37]. Cleanliness in primary production, immunization, logistical slaughter, and disinfection procedures should all be used in areas where the frequency of infections has been significant. There needs to be more regulation of the lateral and vertical transmission of disease.

4. CONCLUSION

According to the results of this study, all of the isolates tested were extremely vulnerable to a pasteurization-like treatment (63°C for just 30 minutes) used in LTLT. However, some of these isolates showed stress-hardening phenomena (resistance to thermal shock processing at 72°C for just 16 seconds) because of quorum sensors. To guarantee compliance with food safety regulations and prevent foodborne disease outbreaks, these results urge further revision of the associated processing conditions. Since microorganisms rapidly evolve resistance to heat, it appears that a combination of actions is required.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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