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Prevalence and Antimicrobial Resistance of *Staphylococcus aureus* in Some Types of Dairy Products in Baghdad Markets

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Authors' contributions

This work was carried out in collaboration among all authors. Author MHGK was responsible for the whole process, from conceptualization to lab work to authoring the paper. Authors AMT and SSA did all of the sample gathering, data analysis and paper editing. All authors read and approved the final manuscript.

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ABSTRACT

In both, the community and hospitals, methicillin -resistant *Staphylococcus aureus* (MRSA) seems to be a major problem. Because protein-rich foods are thought to be a rich reservoir for this bacterium, 55 samples were randomly collected from various outlets in Baghdad between November 2021 and January 2022 and analyzed using standard isolation protocols of food microbiology with some modification processing by the authors to determine the presence of multidrug-resistant *Staphylococcus aureus* in yogurt and ice cream retailed in our markets. There were 28 (50.9%) isolates of *S. aureus*, of which 12 (42.9%) were confirmed as MRSA, 8 (47.1%) were raw yogurt derivatives, and 4 (36.4%) were ice cream derivatives. As a result, it is suggested that MRSA is present in local food goods in Baghdad marketplaces, and it is advised that these products be monitored frequently to ensure the safety of the public.

Keywords: Baghdad markets; ice cream; Staphylococcus aureus; yogurt.

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1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus*, popularly known as MRSA, has the genetic make-up to survive being exposed to modern food processing equipment and human defenses. Therefore, By simulating the super pathogen's actions, we can lessen the threat of disease transmission to livestock used for human consumption along with human cases of food poisoning by adapting a hazard analysis and critical control points plan (HACCP strategy) regarding the environmental epidemiology of MRSA populations along the supply chain, from good management manufacturing practices to consumers using cutting-edge, rapid, and precise isolation tools [1,2]. This bacterium is well-known that may lead to clinical illness or food poisoning in both people and animals. As a result, there is rising worry in the fields of veterinary care and animal husbandry [3,4]. Staphylococcal food poisoning is often associated with extensive manual food handling, and unsanitary food preparation is a significant cause of infection [5,6]. Asymptomatic food handlers may harbor *S. aureus* and contaminate food while working with it [7,8]. The danger of illness from eating food tainted with MRSA has made this an urgent issue in public health and antibiotic resistance [9]. Toxin-producing strains of *Staphylococcus aureus*, called enterotoxigenic *Staphylococci*, are a major contributor to the spread of gastroenteritis [1]. Staphylococcal enterotoxins are considered a potential biological concern since they are stable in very hot conditions (100°C for 1 hour), may render individuals unable to function for several days, and can even last up to two weeks [10]. Reservoirs include ruminants, where it thrives on the skin and mucosa; from there, it may spread to other animals and humans via contaminated milk and dairy products; and finally, it can be found on the skin and mucosa of humans [6,11]. Researchers from all around the globe have looked at the presence and characteristics of MRSA in food items [12-14]. The purpose of this research was to compare the microbiological safety of a variety of dairy products sold in Baghdad's markets, both those made locally and those imported, to international food safety standards by testing for the presence of *S. aureus*, especially MRSA kind, in these goods.

2. MATERIALS AND METHODS

2.1 Sample Collection and Processing

From November 2021 through January 2022, a total of 55 samples have been collected at random from various districts along with marketplaces in Baghdad to examine the presence of *S. aureus* in several dairy products. Standard reference food microbiological methods [15-17], were used to evaluate and prepare the samples, after being slightly modified by the authors.

2.2 Bacterial Isolation and Growth Conditions

Procedures for bacterial isolation and identification were adapted from those used in food microbiology [18]. In accordance with what has been discussed, the identification was performed using conventional biochemical techniques [18]. Colonies that were positive for coagulase after 48 hours, were dark brown in color, shiny, convex, had entire margins with clear zones, and an opaque precipitation zone were picked and recultured at 35-37 o C for 24 hours, and then transferred to double-strength tryptone soya bean yeast extract broth (TSB-YE). The Electronic RapID TM Staph Plus Code Compendium Panel System (ERIC®) with Installation ERIC® CD along with Standard Color Differential Chart along with Online ATCC Codes (Remel, R8311009) seemed to be used for definitive species confirmation of S. aureus. Dry SPOT Staphytect Plus kits (Oxoid, DR0100M) were used to identify MRSA isolates by testing for the presence of certain virulence components that are only found in MRSA [19]. Before being kept in glycerin at -18°C, the MRSA isolates were further identified using the latex agglutination PBP2 test kit (Oxoid, DR0900A), for the detection of PBP2a in MRSA isolates [19].

2.3 Antibiotic Sensitivity Test

Methicillin (ME) 5 µg, cefoxitin (FOX) 30 µg, oxacillin (OX) 1 µg, gentamicin (GM) 10 µg, cephalexin (CL) 30 µg, erythromycin (E) 15 µg, tetracycline (T) 30 µg, and aztreonam (ATM) 30 µg (Oxoid, UK) were used based on Quinn *et al*. [20]. Bacterial isolates that thrived in Tryptone Soya Broth (Oxoid, CM0129, UK) supplemented with 0.6% Yeast Extract TSA-YE (TSB-YE) were grown for a 24 hour on Mueller-Hinton agar (Oxoid CM0337, UK) at 37 degrees Celsius. New pure bacterial cultures were inoculated into Nutrient broth (Oxoid CM000, UK) at a turbidity of 0.5 McFarland. Sterile cotton swabs have been used to inoculate Mueller-Hinton agar with bacteria from each solution. After incubating the plates at 37 degrees Celsius for 48 hours, the diameter of the inhibition zones was measured using calipers. This process began with a 5-minute drying period at 37 degrees Celsius before the antimicrobial discs were dispensed over the agar. The zone diameter breakpoints established by the Clinical and Laboratory Standards Institute were used to classify all isolates as sensitive, intermediate, or resistant [21].

2.4 Data Analyses

All the data that could be obtained was analyzed using MedCalc Software bvba version 18 (BE,USA https://www.medcalc.org/). To investigate the statistical significance of the difference in proportions, a chi-square test (χ^2) was performed using a 5% level of significance and two samples.

3. RESULTS AND DISCUSSION

Table 1 shows that out of 55 samples, 28 (50.9%) tested positive for *S. aureus*, with higher rates of positive raw yogurt along with ice cream samples (68% and 36.7%, respectively). The lowest incidence was found in imported ice cream (20%), while the greatest prevalence was found in raw yogurt (68%). In addition, 12 of the 28 *S. aureus* isolates seemed to be identified as MRSA (42.9%), with rates of isolation in raw yogurt along with ice cream being 47.1% along with 36.4%, respectively. Statistically, the type of sample has a significant influence ($p<0.05$) on the prevalence of *S. aureus* (χ^2 = 10.702, P = 0.0011), but it does not have a significant influence ($p>0.05$) on the occurrence of MRSA (χ^2 = 0.301, P = 0.5833).

The growth of multidrug-resistant bacteria, and parasites, along with adaptive tropism phenotypes, in the Iraqi ecology seems to be mostly attributable towards the country's poor or inadequate hygienic assessments (contamination and pollution), along with post-processing contamination within the food supply chain, particularly after 2003 [2,5,6,17,22-24]. According to our data (Table 1), we were able to successfully isolate 12 MRSA populations from various dairy products sold in Baghdad province's markets; A lack of sufficient sanitation throughout processing (dirty milk equipment, notably milk cans, asymptomatic maid milkers' carriers, flies, insects, filthy water, retailer) may have led to antibiotic-resistance in all of these isolates, especially Methicillin. These findings are important from a public health standpoint because they confirm the high tolerance of these isolates to high acidity, which explains the high isolation rates from yogurt, and they corroborate the findings of previous studies that implied this feature in *S. aureus* and provide a link to the resistance of this type of isolates to antibiotics, particularly those that cause dermatitis [25- 27]. However, *S. aureus* has several adhesion proteins on its surface, but it is not clear how these proteins work together to adhere to a given surface [28]. From a scientific and hygienic perspective, the isolation rates were higher than predicted based on similar research in neighboring countries; this may reflect a high level of contamination and the development of resistance in these pathogens due to the partial abuse of antibiotics in therapy or growth promoters, particularly in cows. These findings are unacceptable as restricted legislation in the United States, the United Kingdom, and Canada, particularly in the United States, where a zero-tolerance approach for MRSA in the production of edibles along with animal feed is in place, and when a ratio of isolation of more than 5% may indicate an unacceptable risk in enforcing banding regulations on goods from countries experiencing epidemics [29].

The antibiotic resistance profiles (ARP) of MRSA isolates seem to be studied, along with the results are shown in Fig. 1. The data showed that the frequency of resistance to ME (100%), OX (100%), FA (100%), E (90.9%), along with FOX (72.7%) was high in MRSA isolates, whereas resistance to T, GM, along with CL was as low as 45.5%. The resistance of all isolates to VAN (27.3%) and ATM (9.1%) was lower. Cefoxitin was the only antibiotic tested where resistance levels varied significantly by dairy source (p = 0.0094) (Table-2).

Penicillin's extensive use as a feed additive and growth booster for livestock may be to blame for the rise in beta-lactam resistance, whereas the use of spiramycin in livestock production may be to blame for the rise in resistance to macrolides [6,30-32]. Human and animal prophylactic and therapeutic usage, as well as use in livestock feed additives, have all contributed to the development of tetracycline-resistant microorganisms [6,30-32]. Antimicrobial-resistant bacteria, including *Enterococci* spp., seemed to be also found in several diet tasters [33], and their resistance to many medicines may account for MRSA's resistance to those same drugs [6]. There is some evidence that avoparcin use in agriculture has facilitated the spread of bacteria resistant to the antibiotic vancomycin (34). The decreased prevalence of resistance in MRSA isolates may explain why vancomycin was formerly thought to as a miracle medicine for treating *S. aureus* that had developed resistance to β-lactams (Table-2). The veterinary use of apramycin may contribute to the development of gentamicin resistance [34-36]. Therefore, prudent antibiotic usage may be the best way to lessen the impact of rising resistance to these drugs, many of which are essential for human survival.

Table 1. Prevalence of *S. aureus* and MRSA from raw yogurt and ice cream retailed in Iraqi markets

Sample's type	Source	No. of samples tested	n/N (%) *S. aureus*	No. of MRSA/ No. of *S.aureus* (%)
Raw yogurt	_	25	17 /25 (68%)	8/17 (47.1%)
Ice cream	Local	20	9 /20 (45%)	3/9 (33.3%)
	Imported	10	2 /10 (20%)	1/2 (50%)
	Total	30	11 /30 (36.7%)	4 /11 (36.4%)
Total	_	55	28 /55 (50.9%)	12/28 (42.9%)
P-value			0.0220	0.5833

Table 2. Data analysis of antibiotic sensitivity test for MRSA isolates based on type of samples

Antibiotics		Sample Sources			*p. value*
		Ice cream		Yogurt	
		Imported	**Local**	**Raw**	
Methicillin	Intermediate	0 (0%)	0 (0%)	0 (0%)	0.2143 NS
	Resistance	2 (18.2%)	2 (18.2%)	7 (63.6%)	
	Sensitive	0 (0%)	0 (0%)	0 (0%)	
Cefoxitin	Intermediate	0 (0%)	1 (9.1%)	0 (0%)	
	Resistance	1 (9.1%)	0 (0%)	7 (63.6%)	0.0094 S
	Sensitive	0 (0%)	1 (9.1%)	1 (9.1%)	
Oxicillin	Intermediate	0 (0%)	0 (0%)	0 (0%)	
	Resistance	2 (18.2%)	2 (18.2%)	7 (63.6%)	0.2143 NS
	Sensitive	0 (0%)	0 (0%)	0 (0%)	
Vancomycin	Intermediate	0 (0%)	0 (0%)	0 (0%)	
	Resistance	2 (18.2%)	0 (0%)	1 (9.1%)	0.5436 NS
	Sensitive	0 (0%)	2 (18.2%)	6 (54.5%)	
Fusidic acid	Intermediate	0 (0%)	0 (0%)	0 (0%)	
	Resistance	2 (18.2%)	3 (27.3%)	6 (54.5%)	0.6800 NS
	Sensitive	0 (0%)	0 (0%)	0 (0%)	
Erythromycin	Intermediate	0 (0%)	0 (0%)	1 (9.1%)	
	Resistance	1 (9.1%)	3 (27.3%)	6 (54.5%)	0.4075 NS
	Sensitive	0 (0%)	0 (0%)	0 (0%)	
Tetracycline	Intermediate	0 (0%)	0 (0%)	0 (0%)	
	Resistance	2 (18.2%)	0 (0%)	3 (27.3%)	0.6313 NS
	Sensitive	0 (0%)	3 (27.3%)	3 (27.3%)	
	Intermediate	0 (0%)	0 (0%)	1 (9.1%)	
Gentamicin	Resistance	1 (9.1%)	1 (9.1%)	3 (27.3%)	0.6313 NS
	Sensitive	1 (9.1%)	2 (18.2%)	2 (18.2%)	
Cephalexin	Intermediate	0 (0%)	0 (0%)	1 (9.1%)	
	Resistance	2 (18.2%)	0 (0%)	3 (27.3%)	0.6313 NS
	Sensitive	0 (0%)	3 (27.3%)	2 (18.2%)	
Aztreonam	Intermediate	1 (9.1%)	0 (0%)	1 (9.1%)	
	Resistance	0 (0%)	0 (0%)	1 (9.1%)	0.3171 NS
	Sensitive	1 (9.1%)	3 (27.3%)	4 (36.5%)	

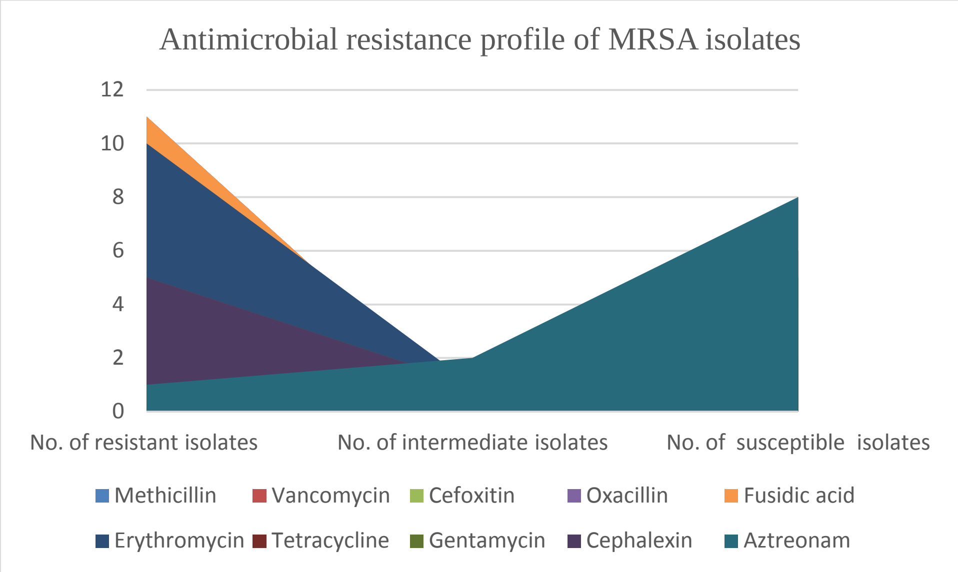

Fig. 1. Antimicrobial resiatance profile of MRSA isolated from some types of dairy products retailed in Baghdad markets

4. CONCLUSION

Based on our results, it has been reported that all isolates seemed to be very resistant toward methicillin as well as to most other chosen antibiotics; this represents the growth of resistance profiles to most antibiotics, notably lifesaving ones. This suggests that one of the most significant methods that should be taken to lessen the aggravation of this condition is to rationalize the use of antibiotics in both human and veterinary medicine.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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