Al-Muthanna J. For Agric Sci Print ISSN: 2226-4086 Online ISSN:2572-5149
Vol. 11 , Issue 1. 2024
https://muthjas.mu.edu.iq/ http://doi.org/10.52113/mjas04/11.1/4

A Comparison Study between Raw and Dried Milk Powder in Antimicrobial Susceptibility Pattern of *Staphylococcus aureus*.

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Received on 15/1/2024 Accepted on 29/2/2024 Published on 15/6/2024

Abstract

Milk is a rich source of nutrients that basically required by different neonatal species. At the same time; it can become microbial hazard to users when the principles of hygiene and purification are inferior. The purpose of the study was to explain the prevalence and the contamination rate of *Staphylococcus aureus* in raw and dried milk powder, differentiation of Methicillin Resistant *Staphylococcus aureus* (MRSA) from Methicillin Sensitive *Staphylococcus aureus* (MSSA) in Al- Muthanna Province; additionally profile of resistance for antibiotics. A total of forty milk samples were collected (during March 2021 to January 2022), included twenty samples of buffalo raw milk were obtained aseptically from the farms and kept in a sterile tubes which were directly transported into laboratory; whereas twenty dried milk powder samples were obtained from the local markets. The MRSA isolates were screened for susceptibility profile. The results clarified a significant difference between buffalo raw milk and dried milk powder at *P-value* < 0.05. Regarding to antimicrobial resistance the results confirmed vast majority of MRSA isolates from raw milk resistance for most antimicrobial especially Methicillin and Oxacillin percentage up 100% , while the resistance percentage up to (66.67%) and (33.33%) for Erythromycin and Amoxicillin respectively. Whereas, the highest rate of susceptibility was recorded in Rifampin antimicrobial which have effect on MRSA isolate reach to (100%). The study concluded the proper sanitary procedures, such as storage, handling, and transportation, are required to decrease the presence of *S. aureus* and MRSA in milk and their resistance to the majority of antibiotics.

Keywords: Raw Milk, Dried Milk Powder, MRSA, MSSA, Antimicrobial Resistance.

Introduction

Among the most prevalent public health problems in the world, is food borne disease. Food ordinary becomes a possible source of human infection because of contamination during collection, transportation, processing and production. It also acts as an important mean for the transmission of antimicrobial resistant (AMR) bacteria to humans via the food product and from livestock has been well recorded **(1)**.

Milk has abundant fundamental nutrients include vitamins, mineral, proteins, carbohydrates and lipids additionally; colostrum which boosts the immune system by antibodies. It is extremely used in diverse kinds and performs a critical part of the human nourishment **(2, 3, and 4).**

Besides the advantage and the nutritional values contained in it, milk is a suitable medium for growth of bacteria and the possibility of acting milk as a medium for transmitting of zoonotic diseases often occurs in cases **(5)**.

Since milk is a fantastic substrate for the growth of many pathogenic bacteria, it is a good vehicle for a wide variety of disease causative agents. Various forms of bacteria from the animal itself, human handlers, milking machines, contaminated vessels, polluted water, flies, dust,…etc. could be present in milk. From one animal to another, the amount of bacteria in the milk varies **(6).**

Staphylococcus aureus, especially MRSA, is the main contributing factor to mastitis, an udder tissue infection that causes irregularities in milk production in milk-producing ruminants like sheep, cattle, and goat that assume a major economic forfeiture to the dairy products **(3).**

Antimicrobial-resistant *S. aureus* which poses serious health hazards to consumers and is acknowledged by worldwide health organizations as one of the most significant health concerns of the century, may be the one affecting milk the most. Since there is little effective treatment for these kinds of bacteria in developing countries, methicillin-resistant *S. aureus* (MRSA) and other multidrug-resistant bacteria have emerged as a severe public health threat. The vast majority of the penicillin family's antibiotics, despite their thoroughness and continued widespread usage in veterinary and human medicine, are predisposing ineffective against of MRSA **(7)**.

The purpose of the study was to explain the prevalence and the contamination rate of *Staphylococcus aureus* in Raw and Powder Milk, differentiation of *Staphylococcus aureus* MRSA from MSSA.

Materials and Methods

Collection of Milk Sample

The study included a total of forty milk samples were collected (during March 2021 to January 2022) in Al-Muthanna Province, included twenty raw buffalo milk samples were obtained aseptically in the farms and kept in a sterile tubes which were directly transported to the laboratory; whereas twenty dried milk powder samples were obtained from the local markets.

Enrichment Culture

The collected milk samples were activated by inoculation one milliliter on Brain heart Infusion (Hi-media/India) broth then; after aerobically incubation of the tubes for overnight at 35-37^{o}C they were scrutinized for the absence or presence of bacterial growth **(8)**.

Isolation & Physical Characteristics of Bacteria

The positive culture of bacterial growth in the tubes were inoculated by streak plate Method onto Blood agar 5% (Micromedia/USA), Mannitol Salt agar (Hi-media/India), Nutrient agar (Hi-media/India), then aerobically incubating for the plates at 35-37^{o} C for 24 hours to distinguishes the general characteristics of microbial culture such as color, colony features, odor, and presence or absence hemolysis **(9).**

Identification and Selection of MRSA Strains

According to manufacturing company protocol, aggregate of 30.38 gm. were dissolved in 500 ml of Distilling Water (D.W.). Then, boiled to resolve the medium completely, without autoclaving and cooled to (45-50) °C. Sterile contents of 1 vial of MeReSa selective supplement or Cefoxitin supplement or both in combinations (as in protocol) were added aseptically for more selectivity as desired. After that, mixing medium was poured into sterile petri plates, and stored at (2-8) °C. The gram positive isolates were inoculated on Hi-Crome MRSA Agar Base medium modified and then aerobically incubated the plates at 35-37^{o} C for 24 hours to isolate of MRSA strains. The chromogenic mixture incorporated in the medium was specifically cleaved by *Staphylococcus aureus* to give green coloured colonies. Sodium pyruvate enhanced the growth of *Staphylococcus species*. This new medium was used for identification and selection of MRSA specifically, since it was designated for this purpose **(9).**

Antimicrobial Susceptibility Screening

The isolates of MRSA were screened for susceptibility profile to seven types of antimicrobial discs which provided by (Bioanalysis/Turkey). Via Disc Diffusion Method (Kirby-Bauer Method) on Mueller Hinton agar plate for screening their susceptibility; plates were incubated aerobically for 18-24hours at (35-37) °C then read and recorded the results according to **(10).**

Statistical Analysis

MedCalc Software Ltd. One-way Chi-sequared test. Version 22.009; 2023. Furthermore performing Microsoft Excel (2010) for the data like charts & percentage (%).

Ethical endorsement

The dried milk powder samples were collected randomly according to the instructions of the Iraqi Standard Criterion No.2/2270 in Sampling afferent by the Iraqi Central Organization for Standardization and Quality Control **(11**) Whereas the buffalo raw milk samples were collected after taking approval from the buffalo owners for sampling, all animal were treated ethically during the milk sample collected according to the instruction of Institutional Animal Care and Use Committee of Al Muthanna university.

Results & Discussion

According to the results of current study there is a significant difference in a means of number and percentages of *S. aureus* between raw and dried milk powder at *P-value* < 0.05, as shown in (**Table 1 and Figure 1**) the *S. aureus* isolates of raw milk were 10 (66.7%) from the total of 15 gram positive isolates while *S. aureus* isolates of powder milk were 2 (33.3%) from the total 6 gram positive isolates.

Although *S. aureus* is considered as a weak competitor to other bacterial groups such as

lactic acid bacteria that may be present in raw milk, its presence in high numbers with suitable storage conditions for its growth may give it the opportunity to multiply and increase its numbers at the expense of other bacterial groups, and the possibility of secretion intestinal poison (which has a direct effect on the health of the consumer in raw milk, may be due to contamination of the milk with *S. aureus* bacteria from human sources such as hands, nasal and oral cavities, boils and wounds, especially in the case of manual milking, or from animal sources such as the surface of the udder and nipples, in addition to possible presence of bacteria as a cause of animal mastitis, as indicated by **(12)**.

Table 1: Percentage (%) and Number of Gram positive bacteria and *S. aureus* Isolates from Raw buffalo and dried Milk Powder.

Type of Milk	Number of Sample		Gram Positive Isolates		*S. aureus* Isolates	
	No.	%	No.	%	No.	%
Raw Buffalo Milk	20	50 %	15	75 %	10	66.7 %*
Dried Milk Powder	20	50 %	6	30 %	2	33.3 %
Total	40				100%	
X^2			5.33			
P-value			0.02			

*: There is a significant difference in a means of number and Percentage of *S. aureus* between raw buffalo and dried milk powder at *P-value* < 0.05.

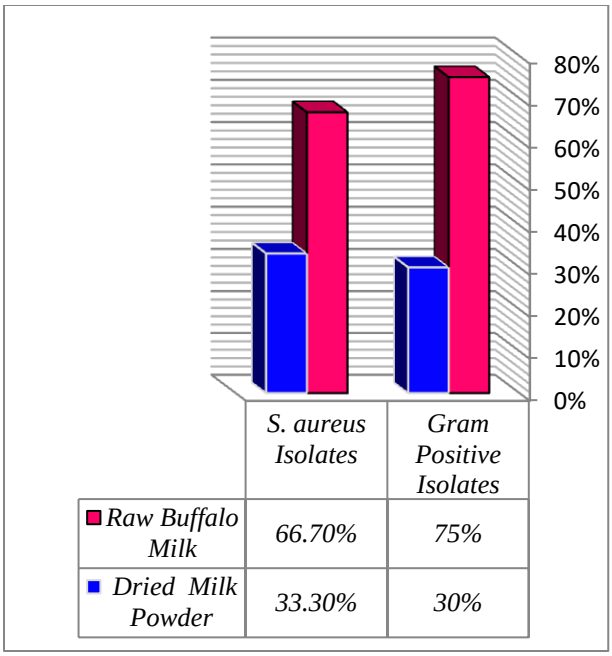

Figure 1: Percentage (%) of Gram Positive Bacteria, *S. aureus* Isolates from Raw and Dried Milk Powder.

The results as in **(Table 2 and Figure 2)** show the isolates of *S. aureus* from raw milk which resistance for methicillin (MRSA) that grow on Hi-Crome MRSA Agar Base Medium Modified reach up to 6 (60%) of the total 10 *S. aureus* on the contrary of *S. aureus* from dried milk powder which do not grow on Hi-Crome MRSA Agar Base Medium Modified while growth on MSA medium only indicate it's sensitive for Methicillin as in **(Figure 3)** The percentage of MRSA isolates in this study is compatible with the assertion of **(13)**. A variety of bacteria can cause mastitis, an inflammatory infection of the mammary glands, although *S. aureus* infections are a common cause (**14**).

At milking time, the use of contaminated water for washing the udder and teats could lead to moisten and dirty udder. Basic component of infection control and cross contamination can be avoided if using the container additionally; the hands of milker and milking pots are washed with suitable detergent and pure water (**15**).

Table 2: Percentage (%) and Number of MRSA & MSSA Isolates from Raw buffalo and Dried Milk Powder.

Type of Milk	*S. aureus*		MSSA*		MRSA **		**Total**	
	No.	%	No.	%	No.	%	No.	%
Raw Milk	10	66.7%	4	40 %	6	60 %	10	100%
Dried Milk Powder	2	33.3%	2	100 %	0	0 %	2	100%
Total	12					100%		
X^2			0.67		6.00			
P-value			0.41*		0.01**			

*: There is no a significant difference in a means of numbers and percentage of MSSA between buffalo raw milk and dried milk powder at *P-value* < 0.05

**: There is a significant difference in a means of numbers and Percentage of MRSA between raw buffalo milk and dried milk powder at *P-value* < 0.05.

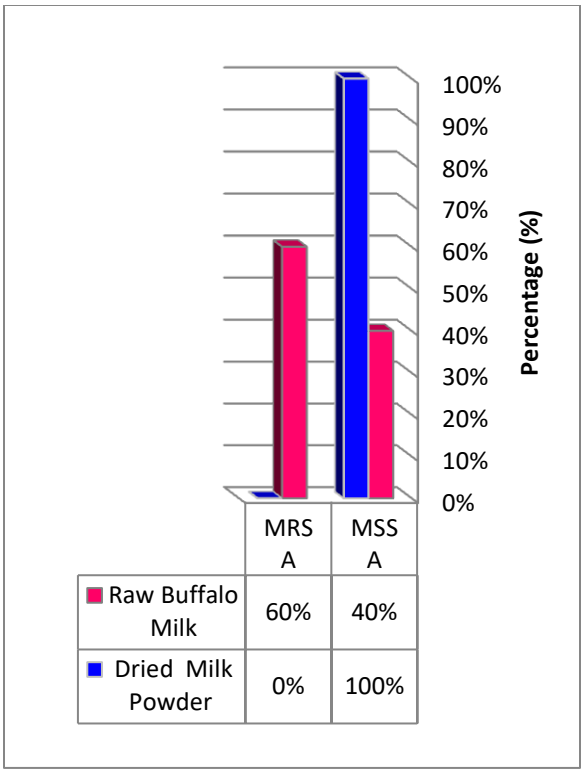

Figure 2: Percentage (%) of MRSA & MSSA Isolates from Raw Buffalo Milk and Dried Milk Powder.

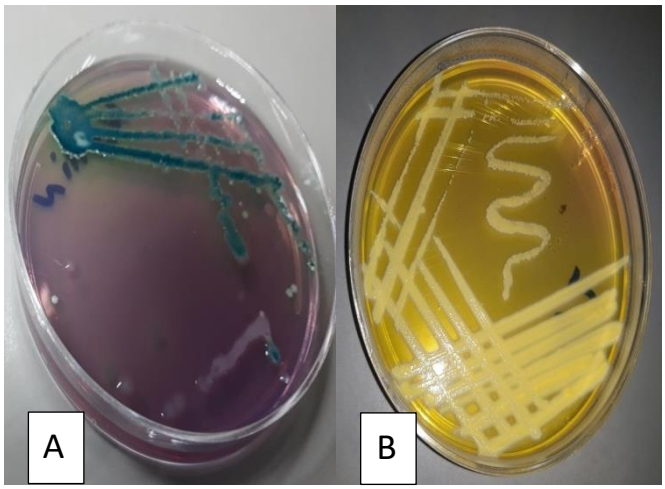

Figure 3: A. Isolates of MRSA on Hi-Crome MRSA Agar Base Medium Modified. B. Isolates of MSSA on MSAB Medium.

Concerning with antibiotics resistance of MRSA isolates; the results in **Figure 4, 5** and **Table 3.** show vast majority of MRSA isolates from raw milk resistance for most antimicrobial especially Methicillin and Oxacillin percentage up 100%, this result could be due to bacteria have the ability to generate endogenous resistance by decreasing their affinity for Penicillin – Binding Protein (PBP), and also the production of an altered penicillin-binding protein 2a (PBP2a) is mediated via mecC-gene or a mecA- gene. The altered PBP2a has a lesser affinity for β-lactam antimicrobials in relation with the normal PBP. Consequentlly, mecC/ mecA positive staphylococci are resistant to most β-lactam antibiotics **(16)**.

While the isolates varied in their resistance for Erythromycin & Amoxicillin at percentage up (66.67%) and (33.33%) respectively. However, compared to earlier investigations, the new study found substantially higher resistance from china **(17)** and from Nigeria **(18).**

The reason of resistance of MRSA towards Erythromycin & Amoxicillin could be due to a mutation in the target site, which leads to preventing it's attachment to the ribosomal segment 50S. In addition, antibiotics resistance may be due to the indiscriminate use of antimicrobial that used in animals treatment without consulting a veterinarian **(19)**.

Despite this, antimicrobial showed an effect on MRSA isolates up to (83.33%), they are broad spectrum antimicrobial and each of antimicrobial has effect on protein building process which attached Clindamycin antimicrobial to the ribosomal segment 50S. Whilst, Tetracycline antimicrobial attached to the ribosomal segment 30S, leads to prevention of protein synthesis in bacteria **(19) .**

While the highest rate of susceptibility was recorded for Rifampin antimicrobial which has effect on MRSA isolate reach up to (100%). Rifampin effect on the manufacture of nucleic acid of bacteria by its attachment with enzyme RNA polymerase enzyme leads to prevent of RNA polymerase enzyme in the bacteria (**20**).

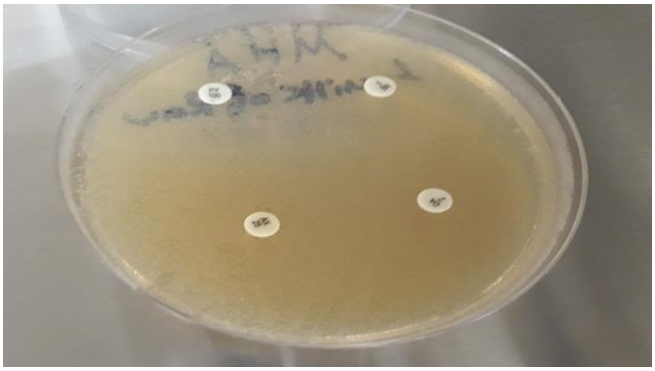

Figure 4: MRSA Isolates' Antimicrobial Susceptibility Pattern via Disc Diffusion Method.

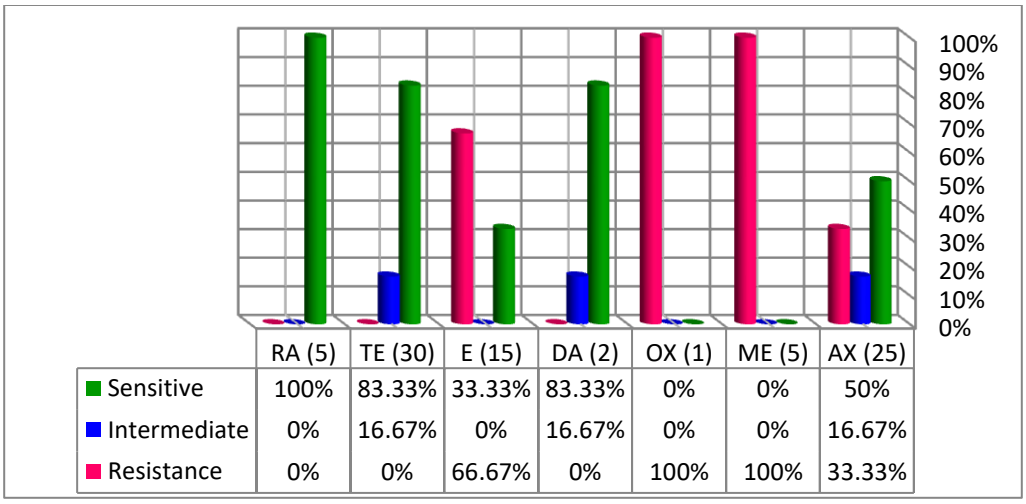

Figure 5: Percentage (%) of Antimicrobial Susceptibility Profile of MRSA Isolates.

Table 3: Percentage (%) and Number of Antimicrobial Susceptibility Profile of MRSA Isolates.

Antimicrobial Disc	Symbol	Concentration	Sensitive		Intermediate		Resistance	
			No.	%	No.	%	No.	%
Amoxicillin	AX	25	3	50%	1	16.67%	2	33.33%
Methicillin	ME	5	0	0%	0	0%	6	100%
Oxacillin	OX	1	0	0%	0	0%	6	100%
Clindamycin	DA	2	5	83.33%	1	16.67%	0	0%
Erythromycin	E	15	2	33.33%	0	0%	4	66.67%
Tetracycline	TE	30	5	83.33%	1	16.67%	0	0%
Rifampin	RA	5	6	100%	0	0%	0	0%

Conclusion

Strict farm management measures are required, in addition to proper sanitary procedures such as storage, handling, and transportation, to prevent *S. aureus* contamination. The presence of *S. aureus* and MRSA in milk is a cause for concern. Additionally, effective heat treatment followed by chilling can less the possibility of contamination with *S. aureus*. Monitoring of animals used in the production of food and improved food hygiene procedures can also help to lower the risk of microbial contamination. The findings of the current investigation showed that the raw milk recorded higher bacterial load of *S. aureus* especially MRSA than powder milk which is an allusion of unsuccessful milk management process. Moreover; MRSA isolates of raw milk were resistant to most antimicrobial especially Methicillin and Oxacillin percentage up 100%. Whereas, the highest rate of susceptibility was recorded in Rifampin antimicrobial which showed effect on MRSA isolate reach to (100%).

Acknowledgments

The author thanks the farms owners for their supports and help in providing sample collection throughout the study.

Conflict of Interest

There was no conflict of interest.

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