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# EFFECT OF NISIN AND CINNAMON ON THE GROWTH AND SURVIVAL OF ENTEROTOXIGENIC METHICILLIN-RESISTANT STAPH. AUREUS IN STERILE MILK AND ICE CREAM

(With 4 Figures)

By

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## تأثير النيسين والقرفة على نمو وبقاء ميكروب المكور العنقودى الذهبى المفرز للسموم والمقاوم للميثيسيللين فى اللبن المعقم والآيس كريم

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تم دراسة تأثير تركيزات مختلفة من النيسين (١٠٠، ٢٠٠وحدة/مل) على نمو الميكروب المكور العنقودى الذهبى المفرز للسموم والمقاوم للميثيسيللين المحقون فى عينات اللبن المعقمة معمليا والمضاف إليها (١×١٠$^{٧}$/مل) من الميكروب والذى تم عزله مسبقاً من عينات لبن الخام حيث حفظت العينات عند درجات حرارة الغرفة (٢٠±٢°م) والثلاجة (٤±٢°م) وقد تم فحص العينات بعد الحقن مباشرة وكل اثنتى عشرة ساعة. وقد أسفرت الدراسة على أن تركيز النيسين ودرجة الحرارة هما العاملان المؤثران على بقاء هذا الميكروب ، ولم يتم عزل الميكروب بعد ٧٢ ساعة فى التركيز (٢٠٠ وحدة/مل) بينما لم يتم عزله بعد ٩٦ ساعة فى التركيز (١٠٠وحدة/مل) من التخزين فى درجة حرارة الغرفة. وعلى الجانب الآخر لم يتم عزل الميكروب بعد ٤٨و٢٤ ساعة من التخزين فى درجة حرارة الثلاجة وعند تركيزات (١٠٠، ٢٠٠وحدة/مل) على الترتيب. وقد أشتملت الدراسة أيضا على دراسة تأثير القرفة بنسب ٠,٣و ٠,٦% على الميكروب المكور العنقودى الذهبى المقاوم للميثيسيللين والمفرز للسموم فى عينات الآيس الكريم المصنع معمليا والتى تم حفظها تحت درجات تجميد -٤±٢ و -١٨±٢°م، وقد تم فحص العينات مباشرة بعد التصنيع واليوم الأول والثانى والثالث ثم أسبوعياً لمدة ٤ أسابيع لعد ميكروب المكور العنقودى الذهبى. ولخصت الدراسة إلى أن درجة حرارة التجميد -١٨±٢°م مع التركيز ٠,٦% كان لهما دور كبير فى تثبيط نمو الميكروب حيث أنه لم يتم عزله بعد أسبوع واحد من التخزين.

## SUMMARY

The effect of nisin on the survival of enterotoxingenic methicillin-resistant *Staph. aureus* (MRSA) was evaluated using different concentrations of nisin (0.00, 100 and 200 IU/ml) in a laboratory

prepared sterile milk and inoculated with the previously isolated and identified MRSA to yield a concentration of $1x10^7$ cfu/ml. The inoculated sterile milks were kept at room temperature (20±2°C) and refrigerator temperature (4±2°C). MRSA counts were determined using Oxacillin Resistance Screen Agar Base (ORSAB) supplement with two antibiotics-oxacillin at (2 mg/L) and polymyxin B (50.000 IU/L). ORSAB and pH value were determined every twelve hours. MRSA strains couldn't be detected after 96 and 72 h in the samples of sterile milk containing nisin in concentrations of 100 and 200 IU/ml, while, in the control sample the MRSA survived till the end of the 96 h of storage at room temperature. Moreover, MRSA failed to be detected after 48 and 24 h in the samples of sterile milk containing nisin in concentrations of 100 and 200 IU/ml and storaed at refrigerator temperature, respectively. Ice cream was prepared at the laboratory to study the effect of ground cinnamon in concentrations of 0.3 and 0.6% on the growth and survival of enterotoxingenic MRSA a concentration of $2x10^7$ cfu/ml at freezing (-4±2°C) and deep freezing (-18±2°C) temperatures. The obtained results showed that the advantage of using 0.6% is better than using of 0.3% of ground cinnamon.

***Key words:*** *Enterotoxigenic MRSA, milk, nisin, ice cream, cinnamon.*

## INTRODUCTION

MRSA infections are of the global health issues due to the severity of the illnesses that may occur. The roles and the sources of food contamination are still unclear since only few reports on the presence and possible origin of MRSA in foods are available. In his analysis of specimens from food-producing animals, including milk, Lee (2003) found 15 strains harbouring the *mecA* gene and most of the MRSA isolates were from milk. He concluded that contaminated foods of animal origin may represent a source of MRSA infection for humans. Kitai *et al.* (2005) suggested that food handlers were the source of contamination. This finding emphasized the role of humans as an important reservoir of MRSA. In the two foodborne acquired MRSA outbreaks, food handlers carrying the epidemic strains in their nostrils were identified to be the source of contamination of the incriminated food (Kluytmans *et al.*, 1995 and Jones *et al.*, 2002).

Food additive is a substance or mixture of substances, other than the basic food stuff, which are present in food as a result of any aspect of production, processing, storage, or packaging. Consequently,

biopreservatives and natural preservatives are needed which posses antimicrobial activity and cause no problems to the handlers and consumers. In this respect, nisin and cinnamon were tested for their inhibitory activity towards the growth of some microorganisms and used as food additives.

Nisin is a natural antimicrobial peptide of 34 amino acids produced by *Lactococcus lactis subsp. lactis* (Carr *et al.*, 2002). The peptide is suggested to be effective against a wide range of Gram positive bacteria including *Staph. aureus* (Narasimhan *et al.*, 1988 and Choi *et al.*, 2000). In the U.S., nisin was confirmed to be "generally recognized as safe" in 1988 (F.D.A., 1988). Because of its high antibacterial activity and nontoxicity for humans, nisin has already been employed as food preservative for a long time and is licensed by 48 countries around the world (Deegan *et al.*, 2006).

Cinnamon has been used since ancient times both as a culinary spice and for medicinal and other purposes. The medical properties of cinnamon were utilized by ancient Egyptians. Its mild anti-inflammatory, anti-spasmodic, and anti-clotting properties are believed to be due to its content of cinnamaldehyde. Cinnamon extracts have also inhibited the growth of cultured tumor cells (Craig, 2008). Moreover, cinnamon can be useful in the treatment of type 2 diabetes; as well as lowering triglyceride levels and serum cholesterol (Khan *et al.*, 2003). Also, it is useful as a food preservative to inhibit the growth of common foodborne bacteria as *Staph. aureus* and MRSA (Chang *et al.*, 2001; Yuste and Fung, 2003; Prabuseenivasan *et al.*, 2006 and Ağaoğlu *et al.*, 2007).

This work was undertaken to throw light on the effect of adding different concentrations of nisin and cinnamon on viability of enterotoxigenic MRSA in sterile milk and ice cream.

## MATERIALS and METHODS

### I- Effect of different concentrations of nisin on the growth and survival of enterotoxigenic MRSA in laboratory prepared sterile milk:

**- The organism:**

Enterotoxigenic MRSA strain used was previously isolated and well identified from the examined milk samples.

**- Experimental procedure:**

Raw milk was laboratory sterilized by autoclaving. The sterile milk inoculated with MRSA in concentration $1x10^7$cfu/ml and nisin was added in concentration of 0.00 (nisin-free), 100 and 200 IU/ml. Each sample was divided into two portions, the first portion was kept at room temperature (20±2°C) and the other was stored at refrigerator temperature (4±2°C). The effect of nisin on the growth and survival of MRSA was determined by using ORSAB after 3 h and then every 12 h and compared with nisin-free samples.

**- Measurement of pH value:**

The pH value of each sample was determined according to standard methods of A.P.H.A. (1992) with a pH meter (Orion Research model 3200 A/digital analyzer) previously standardized with buffer solutions of pH 3.0 and pH 7.0 buffer solutions.

**II- Effect of different concentrations of ground cinnamon on the growth and survival of enterotoxigenic MRSA in laboratory made ice cream:**

**- Organism:**

Enterotoxigenic MRSA strain used was previously isolated and well identified from the examined ice cream samples.

**- Experimental procedure:**

Ice cream samples were prepared in the laboratory according to the manufacture (Egyptian Dairy & Food Company) and then the mixture inoculated with MRSA culture to contain $2x10^7$ cfu/ml. After that, the ground cinnamon was added during manufacture of these samples in concentrations of 0.00 (cinnamon-free), 0.3 and 0.6%. Each sample was divided into two portions, the first portion was kept at freezing temperature (-4±2°C) and the other was stored at deep freezing temperature (-18±2°C).

Samples from the two portions were taken before and after addition of ground cinnamon to determine the initial count of the organism by using ORSAB, the count after hardening of ice cream samples, after the first, second and third day. Then, the samples were tested every week up to 4 weeks of storage for MRSA count.

## RESULTS

The obtained results were recorded in Figures 1-4.

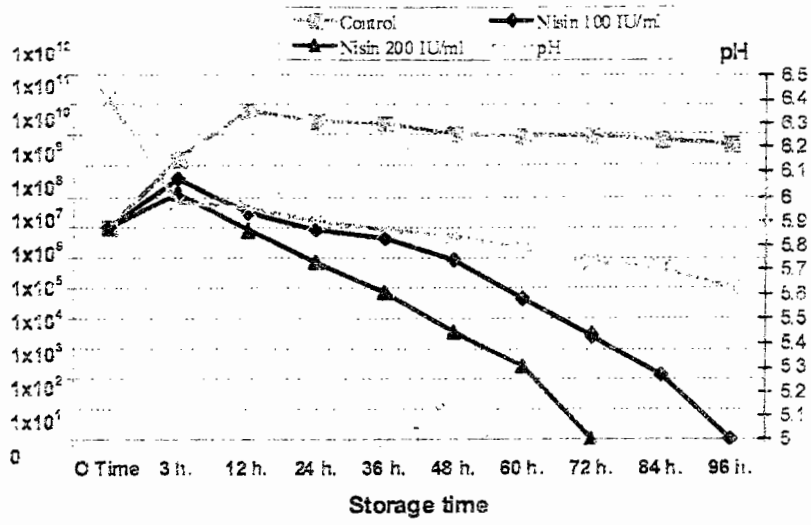


**Fig. 1:** Effect of different concentration of added nisin on the growth and survival of MRSA in sterile milk stored at room temperature (20±2°C).

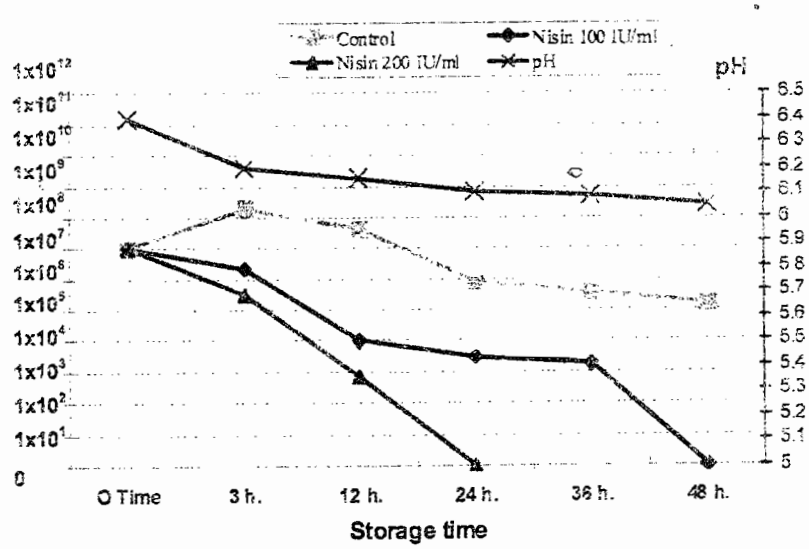


**Fig. 2:** Effect of different concentration of added nisin on the growth and survival of MRSA in sterile milk stored at refrigerator temperature (4±2°C).

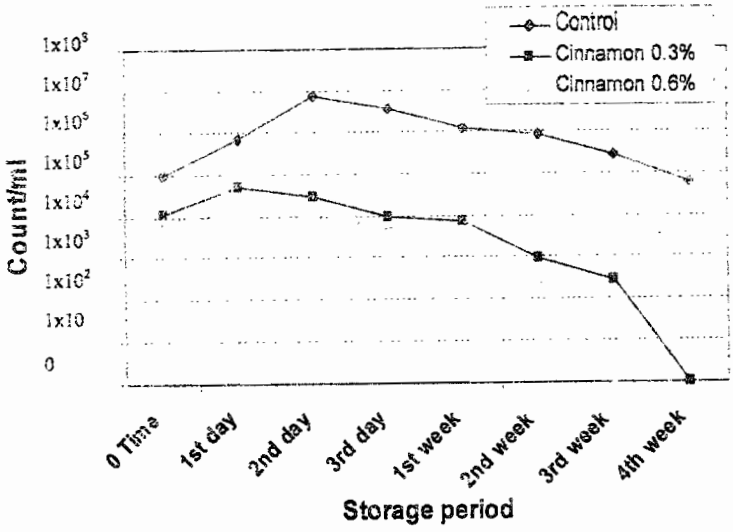


**Fig. 3:** Effect of different concentration of added ground cinnamon on the growth and survival of MRSA in laboratory made ice cream stored at freezing temperature (-4±2°C).

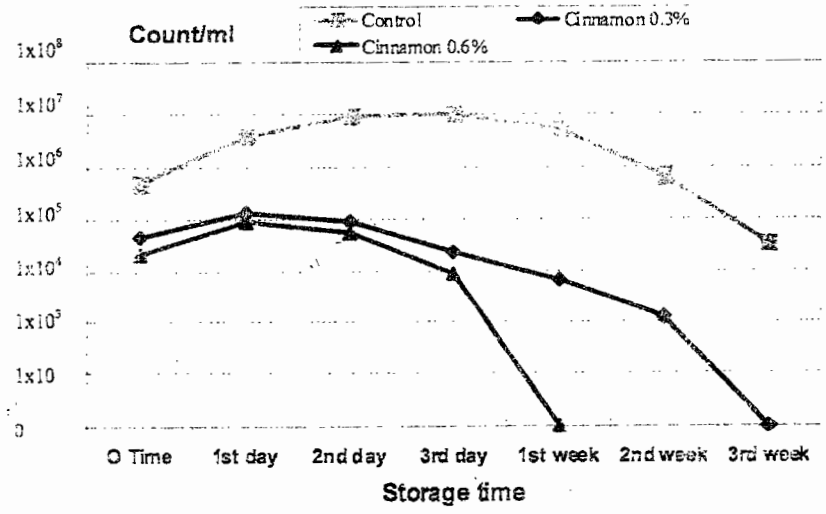


**Fig. 4:** Effect of different concentration of added ground cinnamon on the growth and survival of MRSA in laboratory made ice cream stored at deep freezing temperature (-18±2°C).

# DISCUSSION

## I- Effect of different concentrations of nisin on the growth and survival of enterotoxigenic MRSA in laboratery prepared sterile milk:

The data presented in Fig. 1 showed that the count of MRSA in sterilized milk stored at room temperature containing nisin 100 IU/ml was increased to $4.0x10^8$ cfu/ml at the 3rd h of the storage, then it reached $3.0x10^7$ at the 12th h of the storage. Then at the end of first 24 h of the storage the count gradually decreased and reached $8.5x10^5$ cfu/ml. The count of MRSA decreased until the pathogen failed to be detected on the plate and could not be isolated at the end of 96 h. While, sterile milk containing nisin 200IU/ml, the count of MRSA increased $1.4x10^8$ cfu/ml after 3 h of storage. The count gradually decreased until the pathogen failed to be detected on the plate at the end of 72 h of the storage and failed to be isolated from the milk. On the other side, in control sample, MRSA increased from the initial count of $1x10^7$ to $4.5x10^9$ cfu/ml in 96 h. Gradual decrease of pH value of the sterile milk occurred from 6.40 to 5.62 in 0 time to the end of 96 h, respectively.

Result in Fig. 2 depicted the effect of nisin on the survival of MRSA in sterile milk stored at refrigerator temperature (4±2°C). The initial count of MRSA was $1.0x10^7$ cfu/ml decreased to $2.0x10^6$ and $3.0x10^5$ cfu/ml after 3rd h after storage100 and 200 IU/ml nisin containing samples, respectively. The organism failed to be detected on the plate and could not be isolated from the samples containg 100 and 200 IU/ml nisin after 48 and 24 h storage at (4±2°C), respectively. While, the pathogen survived in the sample free from nisin up to 48 h of the storage at refrigerator temperature. Slight decrease of pH of the sterile milk from 6.40 to 6.06 at the end of 48 h of the storage.

From the aforementioned results, it was evident that the increased concentration of nisin and the storage of the sterile milk at refrigerator temperature had an inhibitory effect on the survival of *Staph. aureus*. These results agree with the theory which pointed out that *Staph. aureus* is a robust bacterium and can survive for long period at low temperatures below those which permit growth. Yet, refrigeration at <4°C may be considered the only viable method for control of growth and toxins production (I.C.M.S.F., 1996 and Ryser, 2001).

Also, the obtained results agree with thoserecorded by Rilla *et al.* (2004), who found the inhibitory effect of *Lactococcus lactis subsp. lactis* IPLA 729 on MRSA inoculated into milk in different inoculums ($1.8x10^4$

and $7.2x10^6$ cfu/ml). While, MRSA when growing alone, it reached levels of $6.4x10^8$ and $1.9x10^9$ cfu/ml, respectively. In both cases, a slight growth of MRSA occurred within the first 3 h of incubation. However, complete disappearance of the pathogen occurred at the end of the incubation period.

Many investigators recorded the inhibitory effect of nisin on *Staph. aureus* as Aman and Ahmed (1997), they added nisin in concentration of 50, 100, 150, and 200 IU/ml of nisin in cheese milk had a highly bactericidal effect against *Staph. aureus*. Also, Narasimhan *et al.* (1988) and Choi *et al.* (2000) reported the inhibitory effect of nisin on *Staph. aureus*.

Numerous studies have been made on the mode of action of nisin on susceptible vegetative cells. The target of the inhibition effect of nisin is the cytoplasmic membrane where it depolarizes energized bacterial membranes (reduces transmembrane potential) and form voltage-dependent multistate pores. The result of a pore formation is the loss of accumulated amino acids and the inhibition of amino acid transport (Shehata, 1981). Delves (1990) indicated that nisin causes disruption, either resulting in leakage of essential cellular material such as adenosine triphosphate from the cell or in more severe cases lysis of the cell.

**II- Effect of different concentrations of ground cinnamon on the growth and survival of enterotoxigenic MRSA in laboratory made ice cream:**

In ice cream samples stored at freezing temperature (-4±2°C) and containing 0.3% ground cinnamon (Fig. 3), the counts of MRSA diminished gradually from $1.1x10^4$ to $2.8x10^2$ cfu/ml by the end of 3rd week, and failed to recover from the samples by the end of the 4th week. The ice cream samples containing 0.6% ground cinnamon, the counts of MRSA decreased gradually from $8.0x10^3$ to $3.0x10^2$ cfu/ml by the end of the 2nd week, and failed to be detected in the samples by the end of 3rd week. Concerning cinnamon free ice cream, the number of MRSA decreased gradually during the storage period to reach its minimum level $6.0x10^4$ cfu/ml by the end of the 4th week.

As presented in Fig. 4, the numbers of MRSA ($4.7x10^3$ and $2.1x10^3$ cfu/ml) reduced in ice cream samples with 0.3 and 0.6% added ground cinnamon during the weeks of storage at the deep freezing temperature (-18±2°C). No viable MRSA could be detected in the samples by the end of 3rd and 1st week, respectively.

These findings were in agreement with the theory that cinnamon had antibacterial activities against many of bacteria including methicillin resistant *Staph. aureus* (Chang *et al.,* 2001). Also, many other investigators reported the inhibitory effect of cinnamon against *Staph. aureus* as Yuste and Fung (2003), Prabuseenivasan *et al.* (2006) and Ağaoğlu *et al.* (2007).

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