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COMPARATIVE STUDY OF EDTA AND TIO_2 AGAINST METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS* ISOLATED IN SOFT CHEESE

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Abstract

A total 50 random samples of locally soft cheese were collected from different location of Al Hilla province. The samples were collected (10 samples\weekly) were examined for the presence of *Staphylococcus aureus* organisms. The incidence of *Staph. aureus* were 27 (54%) in the examined cheese samples. Our study showed that *Staphylococcus aureus* isolates were variably resistance to antibiotic disk method. *Staphylococcus aureus* was highly resistant to Cefoxitin, Tetracycline, Oxacillin, Vancomycin (80%), Erythromycin, Gentamycin and Ampicillin (75%), while sensitive to Chloramphenicol and Sulphamethoxazole-trimethoprim respectively. Our study showed that the effects of titanium dioxide nano particles (Tio2) and EDTA in different concentration against Methicillin resistant *Staphylococcus aureus* isolates in different concentration (0.4mg/ml, 0.8mg/ml, 1mg/ml, 1.5mg/ml, 2mg/ml and 3mg/ml) respectively. The current study showed that titanium dioxide nano particles (Tio2) were able to inhibit the bacterial growth in different concentrations and inhibition zones had increased from 9.00±0.57 mm at concentration 0.4mg/ml to maximum 25.00±0.57 at concentration 3mg/ml respectively. While the inhibition zone of EDTA against MRSA of minimum 11.00±0.57 mm at concentration 0.4mg/ml to maximum 25.00±1.15 mm at concentration 3mg/ml respectively. The ethylene diamen tetra acetic acid (EDTA) and titanium dioxide nano particles (Tio2) had high antimicrobial effects against Methicillin resistance *Staphylococcus aureus,* also and EDTA can increase shelf life of dairy products.

Key word: Soft cheese, antibacterial activity, Tio2, EDTA

Introduction

Cheese has stabilized a curd of milk solids performed by casein coagulation and a mix-up of milk fat in the coagulum (Fernandes, 2008). Microbial contamination of cheese result from different sources, including handler, packaging material and environment (Pal *et al.,* 2014b). The occurrence of these pathogenic bacteria in dairy products can lead to many health issues to consume as they are highly sensitive to many of bacterial because of highly complex chemical composition and nutritional value (Thaker, *et al.,* 2013). *Staphylococcus aureus* presence in cheese constitutes a potential health problem since many strains of *Staphylococcus aureus* produce enterotoxins lead to food poisoning (Amal, *et al.,* 2014). The Methicillin-Resistans *S. aureus* strains have spread worldwide and are resistance to an antibiotic that associated with considerable mortality and injury (Liu, *et al*., 2009).

The bacterial resistance to antibiotics and it's contagious are serious health difficulties problems (Schit, 2006). There has been increasing importance in the use of the inhibitors of antibiotic resistance for association therapy (Abdulrahman and Nssaif, 2016).

Titanium dioxide (TiO2) nanoparticles have been of importance in different applications as a nanomedicine and antimicrobial agents (Jameel, 2014). The antimicrobial activity of TiO2 occurs by the damaging the membrane when NPs electrostatically attach to the membranes and bacterial cell wall, leading to the alter of membrane depolarization, membrane potential and loss of principle (Díaz-Visurraga, *et al.,* 2011).

EDTA as chelating agent have shown different degrees of antibacterial effectiveness, either by

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themselves or in association with other antimicrobial agents (Shen, *et al.,* 1999). EDTA had been demonstrated to have antimicrobial property against Gram-positive and Gram-negative organisms (Murdock and Matthews, 2002). It was found that 70% of strains turned from resistance into sensitive (Mohamed, *et al.,* 2006). The objective of the study, was an antimicrobial effects of EDTA and Tio2 against Methicillin resistant *Staphylococcus aureus* isolated from cheese.

Materials and Methods

Sample collection

Fifty soft cheese samples were collected randomly from Al Hilla city and transported in ice box to the laboratory of Dep. Veterinary Public Health for isolation of *Staphylococcus aureus*.

One gram of samples was taken and diluted in 10 ml of distilled water then mixed and transferred on Mannitol salt agar and further incubated at 37^{O}C for 24 h, the colonies of *Staph. aureus* appear tiny pinhead golden yellow, the colonies were identified using biochemical tests (motility, Gram staining, catalase, (Bezalwa, *et al*., 2014).

Antibiotic Susceptibility Test

Antibiotic susceptibility test was carried out for *S. aureus* isolates using on Mueller-Hinton Agar medium by disk diffusion method in accordance with (CLSI, 2016). The antimicrobial agents tested included Cefoxitin (30µg), Tetracycline (30µg), Gentamycin (10µg), Oxacillin (1µg), Chloramphenicol (30µg), Vancomycin (30µg), Ampicillin (10µg), Sulphamethoxazole-trimethoprim (25µg) and Erythromycin (15µg) by a sterile cotton swab and the antibiotics discs were laid on the surface (CLSI, 2016).

Preparation of TiO2 NPs suspension

Commercially synthesized TiO2 nanoparticalis from Sigma-Aldrich, St. Louis, MO. The subsequent dilutions were made in autoclaved double-distilled water. Different concentrations of TiO2 NPs (0.4 mg/ml, 0.8 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml and 3 mg/ml) were taken and added to 10 ml of sterile water and shaken vigorously (Jesline, *et al.,* 2015).

The antimicrobial efficiency of TiO2 nanoparticles

On Muller Hinton agar Media 0.1ml of bacterial culture was spread and the discs were placed and kept in the center of wells at room temperature. Sterile cork borer of 8.0mm diameter was used to bore well in the pre-solidified Mueller Hinton agar (MHA) plates and 100µl volume of each dilution was added aseptically into the wells on MHA plates in triplicate that had wells were made in each plate, then filled with various concentrations of TiO2 nanoparticles (0.4 mg/ml, 0.8 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml and 3 mg/ml) and incubated the discs at a temperature of 37°C for (18 hours). The zone of inhibition measured by mm after 24 hours of incubation (Jesline, *et al*., 2015).

Effects of EDTA against bacteria

Ethylene diamine tetra acetic acid (EDTA) were perpetrated in different concentration (0.4 mg/ml, 0.8 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml and 3 mg/ml) against *Staphylococcus aureus* strains. On a Mueller Hinton agar plate about 0.1ml of *Staphylococcus aureus* culture and use bore well were made on media and filled with EDTA. After incubation at 37^{0}C for 24 hours the strains were observed for the formation of the zone of inhibition against the EDTA (Bezalwa, *et al*., 2014).

Statistical analysis

Complete randomized design was followed to the treatment and using the least significant difference test (LSD) the data was analysied on the SPSS (SPSS, 2008).

Results and Discussion

Fifty soft cheese samples were collected randomly from different location of Babylon city. All samples collected (10samples/weekly) were kept in the icebox and transported to laboratory vet. Public health/veterinary medicine/ University of Al Qasim Green.

The present study showed that 27 (54%) samples analyzed were contaminated with *Staphylococcus aureus*. Which in agreements with finding (Garbaj, *et al.,* 2007) who reported that 36 and 62% *Staphylococcus aureus* isolates in soft cheese. While higher findings was reported by (Agban and Ahmed, 2012) found 92.8% of *Staphylococcus aureus* in kariesh cheese samples and lowest value who reported than these by (Eid and Eltalawy, 2014) detected that 13.3and 26.6 % *Staphylococcus aureus* in white soft cheese.

Antibiotic susceptibility

Our study showed that *Staphylococcus aureus* isolates were variably resistant. *Staphylococcus aureus* was highly resistant to Cefoxitin (90%), Tetracycline (70%), Oxacillin (85%), Vancomycin (80%), Erythromycin (90%) Gentamycin (80%) and Ampicillin (75%). While

Table 1: Prevalence of *Staphylococcus aureus* in cheese samples in Hilla province

Samples	No. positive isolates	Percentage (%)
Cheeses	27	54%
Total	50	100%

sensitive to Chloramphenicol (80%) and Sulphamethoxazole-trimethoprim (75%) respectively. The findings of the present study similar to the to (Thaker, *et al.,* 2013) report that *Staphylococcus aureus* was resistance to Oxacillin, Ampicillin. Also (Abdulrahman and Nssaif, 2016) detected that *Staphylococcus aureus* was highly resistant to Gentamycin, Tetracyclin, Ampicillin and Erythromycin. (Özdemir and Keyvan, 2016) reported isolates of *Staphylococcus aureus* sensitive to Chloramphenicol and Sulphamethoxazole-trimethoprim and highly resistant to Vancomycin.

Effects of TiO2 NPs and EDTA against MRSA

The result showed out of isolate 19(70.3%) were resistant to the antibiotic. This result agrees with (Al-Hasan, 2011) who found that (81%) the rate of Methicillin-resistant *S. aureus* (MRSA). Also, (Abdulrahman and Nssaif, 2016) showed that (83%) Methicillin resistant *Staphylococcus aureus*.

The effects of titanium dioxide nanoparticles (Tio2) and EDTA against the isolates of Methicillin-resistant *Staphylococcus aureus* in different concentrations (0.4mg/ml, 0.8mg/ml, 1mg/ml, 1.5mg/ml, 2mg/ml and 3mg/ml) in presented. In the table 2 showed that the mean of inhibition zones (in millimeters) to three replicates around each well using sterile cork borer of 8.0mm diameter. It was found that titanium dioxide nanoparticles (Tio2) were

Table 2: Antimicrobial efficiency of TiO2 nanoparticle and EDTA against Methicillin resistant *Staphylococcus aureus*.

Concentration of EDTA and TiO2	Zone of inhibition (mean of three replicates)	
	EDTA	Tio2
0.4 mg/ml	11.00±0.57 Ea	9.00±0.57 Da
0.8 mg/ml	15.00±1.15Ca	10.00±1.15Db
1 mg/ml	20.00±1.15 Ba	11.00±1.15 Db
1.5 mg/ml	23.00±1.15 Ba	14.00±1.15Cb
2 mg/ml	23.00±1.73Ba	20.00±0.57 Bb
3 mg/ml	25.00±0.57 Aa	25.00±1.15Aa

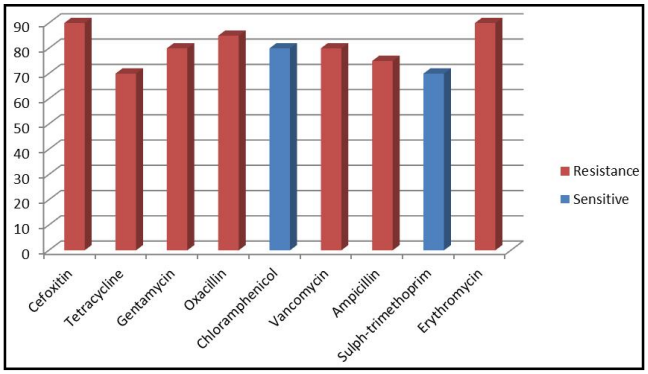

Fig. 1: Antibiotic susceptibility pattern of *S. aureus* isolates.

able to inhibit the bacterial growth in different concentrations, the inhibition zone of Tio2 against bacteria (9.00±0.57,10.00±1.15,11.00±1.15, 14.00±1.15, 20.00±0.57 and 25.00±1.15) respectively. While the inhibition zone of EDTA against MRSA of (11.00±0.57, 15.00±1.15, 20.00±1.15,23.00±1.15, 23.00±1.73 and 25.00±0.57) respectively. (Roy, *et al.,* 2010) reported that 1.9 times decrease the optical of growth bacteria as compared to control at concentration 0.5% of Tio2, while decrease 4.5 times of bacterial growth at concentration 1% of Tio2. However, nanosized Tio2 at different concentrations have the most effective antibacterial against Methicillin *Staphylococcus aureus*. (Babaei, *et al.,* 2016) observed that the death rate of bacteria depends not only on the toxic effect of photocatalytic TiO2 but also on the cell wall thickness and cell structure, also, the decomposition of bacterial components also plays role in bacterial death beside the toxic effect of photocatalytic TiO2 nanoparticles. Many studies reported that the germicidal mechanisms of TiO2 nanoparticles involving the release of positively charged ions to the reaction medium linked to (negative charges) cytoplasmic membrane(-SH) (Jayaseelan, *et al.*, 2013).

However, this reaction lead to change the cell wall and increased permeability and damages deform the structure of cellular components such as DNA, cellular enzymes and ribosomes then finally death of microbial cells .(Senarathna, *et al.,* 2017) indicated that Tio2 are non carcinogenic and non-toxic and used in self-cleaning ceramics and extending in food packaging and textile industry.

EDTA inhibited the growth tested bacteria at the concentration of 2.5 to 5.0%, Depending on the indicator strain the size of inhibition zones caused by EDTA reached 18 – 20 mm in the presence of 1.0% of this substance, 20 – 25 mm at a concentration of 2.5% and 20 – 27 mm at concentration 5.0% (Borowicz, *et al*., 2016). (Ko, *et al.,* 2008) clear zones were observed at 1.5 and 2.0 mg/ml of EDTA against *Listeria monocytogenes*. EDTA can increase cell permeability because of the effect on the outer membrane of the bacterial cells (Tajik, *et al.,* 2014). (Finnegan and Percival 2015) EDTA is a known antimicrobial compounds and also commonly used in the case of poisoning of heavy metals such as mercury and lead. This compound is also used for the treatment of biofilm occurred on medical devices, in dentistry and in veterinary medicine (Stoodley, *et al.,* 2013).

Conclusion

Ethylene diamine tetraacetic acid (EDTA) had high antimicrobial effect against Methicillin-resistant

Staphylococcus aureus, also EDTA can increase shelf life of dairy products.

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