SYNERGISTIC EFFECTS OF ETHYLENE DIAMINE TETRA ACETIC ACID AND IMIPENEM AGAINST *Pseudomonas aeruginosa* WERE ISOLATED FROM CHEESE IN HILLA PROVINCE.

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ABSTRACT

The study was performed to the study synergistic effect of Ethylene diamine tetra acetic acid (EDTA) and Imipenem against *Pseudomonas aeruginosa* were isolated from cheese at Hilla province. Cheese samples 70 were collected randomly from (Retail, supermarkets and dairy shops) in Hilla and were transported to the laboratory.

The result revealed (45%) *Pseudomonas aeruginous* detecte were collected from cheese. These isolates were tested for disk diffusion method for susceptibility of imipenem. Eight isolates were resistant , and (84.3%) of *Pseudomonas aeruginosa* strain were sensitive (84.3%) to EDTA alone.

Also, the result showed of Synergistic EDTA- IMP disc diffusion against (8) isolate of *Pseudomonas aeruginosa* revealed (87.5%) isolates were sensitive. However; the The result showed significant ($P < 0.05$) of susceptibility to (8) *P. aeruginosa* isolates to imipenem with and without EDTA. In conclusion, according to the present study can use oxidant agent inhibition of growth bacteria specific *P. aeruginosa* such as ethylene diamene tetra acidic acid (EDTA) and can used in preserving food specific sold cheese.

INTRODUCTION

Cheese is a stabilized raw of milk solids produced by casein coagulation and entrapment of milk fat in the coagulum (1). Its rich source of protein, vitamins, calcium, and phosphorus (2). Raw milk pasteurizion milk is used in cheese makers, this process was used for better flavor

without the addition of any starter culture (3). Cheeses losing due to microbial contamination, which could occur from various sources, including handler, packaging material and environment (4). Bacterial spoilage or growth was decreases the quality of dairy products. Dairy product had contamination with psychotropic bacteria was important the dairy industry (5). However, when dairy products, entered through post-pasteurization contamination in the factory; bacterial growth occurred when the temperature was decrease, which allows bacteria to multiply and became the dominant micro flora (6).

Pseudomonas spp. is the most common pathogenic bacteria of milk spoilage in dairy products occurred during refrigerated storage because many strains were psychrotolerant (3). In addition, produce heat-stable extracellular lipases, proteases and lecithinases produced by many of these strains, which could contribute to milk spoilage and dairy products (7). In addition, one of the most important food-change effect was food discoloration were caused by the ability of some *Pseudomonas* strains to produce colored pigments like: pyoverdine, pyocyanine, fluorescein, pyomelanin and pyorubin (8).

(EDTA) were used as an antimicrobial agent especially for bacteria. Also was an enhancer other agents, antibiotics and lysozyme, by increasing bacterial permeability outer membrane or removed or braked down in covalently bound lipid components (9). EDTA was more active in Gram negative than Gram positive bacteria when combined with antibiotics, this activity was due to the cell wall structure differences of Gram positive and negative bacteria (10).

The study was aimed to study the effects of EDTA and imipenem against *Pseudomonas aeruginosa* were isolated from cheese in Hilla province.

MATERIALS AND METHODS

Sample collection:

Seventy samples of cheese were collected randomly from different location retail, supermarkets and dairy shops in Al- Hill , Iraq and were immediately transporation by in ice (11).

Isolation of *Pseudomonas spp*.

25 g of cheese sample was added in 225ml of trisodium citrate and the mixture was homogenized for 3 minutes by stomacher (12). Six fold serial dilutions had plated onto Pseudomonas agar base (Oxoid) supplement and incubation in 24 hr. at 25 °C, three colonies from plates were inoculated on nutrient agar. Presumptive identification of *Pseudomonas spp*. was made based on ideal production, colony morphology, Gram staining and biochemical test (motility, galactose, glucose, mannitol, sucrose) (13).

Preparation of Inoculum:

A loopfull culture *Pseudomonas aeruginosa* isolates was inoculated in (5ml) sterile nutrient broth tube; then was incubated at 37^0C for 18-24 h. The turbidity was compared with 0.5 McFarland standards (14)

Imipenem sensitivity test:

All the *pseudomonas aeruginosa* isolates were subsequently tested for antibiotic sensitivity patterns by using disc diffusion method on Mueller Hinton Agar. The plates were incubated at 37°C. after (18-24) hr., an inhibitions zone were measured in millimeter (mm). then the results were interpreted (15).

EDTA Stock Solution (EDTA solution):

EDTA(0.5M) solution was achieved by dissolving 186.1 g of disodium EDTA + $2H_2O$ (Junsei Chemical, Tokyo, Japan) in one litter of distilled water and adjusting it to pH 8.0 by using NaOH. Then, the mixture was sterilized by using an autoclave (16).

Determining the synergistic Effect of EDTA with imipenem:

10 mg imipenem disc was put on the plate, a blank filter paper disc (Whatman filter paper no." 2", 6 mm in diameter) was placed at EDTA that absorbed by the disks under sterile conditions) which measured by disk diffusion method. After 18-24 hr. incubation, presence of any synergistic inhibition zone was considered as positive (17).

Statistical Analysis:

The statistical analysis was carried out to known the significant difference between groups of milk sources (super market , retail , dairy shops) by using Complete Randomize Design (CRD) method according to the (18) because it was found one variable which was effect of different of milk sources on resistance and sensitive bacteria. It was the comparison between the averages of this traits by using Duncan test (19) at the level of probability of 5% or 1% to test the significant differences between the averages of the traits and applying the SAS method (20) .

RESULTS AND DISCUSSION

Isolation and identification:

The result showed, isolation of (70) *pseudomonas aeruginosa* from cheese samples (45%) isolates analyzed were contaminated with *pseudomonas aeruginosa* (Table 1). The result

indicated the prevalence of (45%) *Pseudomonas aeruginosa* (21) reported that isolation of *Pseudomonas aeruginosa* from Damietta and kariesh cheese was 68% and 58% respectively. While high result were obtained by (22) record the contamination of cheese sample with *Pseudomonas aeruginoisa* (87.5%) in Egypt . (23) found the prevalence of *Pseudomonas spp.* from white soft cheese (22.9%) lower incidence.

Table1: Prevalence of *pseudomonas aerogenoisa* were contaminated of cheese samples in Hilla province.

Traits	Super markets Mean ±SE	Retail Mean ±SE	Dairy shope Mean ±SE
Total of samples**	25±5.77A	25±2.88A	20±2.80A
Positive isolations*	1.04±0.51B	3.400±0.50A	2.00±0.63AB
Isolation (%) :	15.6	53.1	31.2

Antibiotic susceptibility:

The determination *Pseudomonas aeruginosa* response for imipenem, disk diffusion method was used with imipenem concentration (10) µg Figure (1). (45%) of *P. aeruginosa* were isolated from cheese samples. (8) isolates of *P. aeruginosa* were resistant to imipenem while 24 were sensitive to imipenem.(24) that indicated the prevalence of *p aeruginosa* resistance to 80.4% imipenem. (25) reported approximately (10 and 50%) imipenem resistance of *P. aeruginosa* in Korea. (26) showed imipenem resistant isolates were (12) out of (162) *Pseudomonas aeruginosa* were isolated from Najaf Province. Seventy four *P. aeruginosa* strains were resistance to imipenem were identified at Zonguldak Karaelmas University (27). In addition. (28) that out of 230 isolates of *P. aeruginosa* 49.5% were resistance to imipenem in atertiary care hospital of Pakistan.

Inhibitory Effect of EDTA against *pseudomonas aeruginosa*:

The results showed that (84.3%) of *P. aeruginosa* were inhibited when EDTA used. (29) found *P. aeruginosa* sensitive to EDTA (100%). In addition (30) found *Pseudomonas aeruginosa* was rapidly lysed in EDTA. (31) reported EDTA can also kill Proteobacteria such as *Pseudomonas*

aeruginosa. The change of antibiotics permeability by EDTA caused by break down the outer membrane of the bacterial which enter and exert their effect(9).

Table2: Effect of IMP , EDTA on sensitive and resistance were contaminated of cheese samples in Hilla province.

Trails	IMP Mean±SE	EDTA mean±SE
Total of samples	35	35
S(NS)	26.66±1.76A	30.00±2.52A
R(**)	8.33±0.88A	4.00±0.80

S: sensitive R: resistance NS: no significant **: high significant at(0.01)

Double disk synergy test (DDST) of EDTA-Imipenem(EDTA-IMP) on *P. aeruginosa:*

The effect of double disk of EDTA- Imipenem on (8) isolate of *Pseudomonas aeruginosa*. These results showed inhibited (87.5%) by ETDA-Imipenem. (32) involved Double Disk Synergy (DDS) results were considered as a positive in at least one of inhibitor substrate combinations of (76.8%). Also, (26) found that the disc synergistic method showed (77.8%) isolates possessing the ability to produce the Metallo-B-Lactamase (MBLs).

(33) Imipenem and EDTA can increase the mean of inhibition zone diameter for *Pseudomonas aeruginosa*. When using imipenem disc alone the inhibition zone was 22.4mm while imipenem with EDTA the inhibition zone was 24.5mm. (25). Inhibition zone which measure from the edge of the disc was 0-3mm with imipenem alone, while zone with imipenem and EDTA discs between was 6-14mm. inhibition with EDTA alone was at least 6mm(25).

The result showed significant ($P < 0.05$) of susceptibility to (8) *P. aeruginosa* isolates to imipenem with and without EDTA are shown in the Table (3) according to (33).

Table 3. Effect of IMP , EDTA and IMP+EDTA on resistance and sensitive of *Pseudomonas aeruginosa* in cheese samples .

Trails	IMP	EDTA	IMP+EDTA
R*	6.00±1.15A	2.33±0.66B	2.00±0.57B
S**	2.33±0.33B	1.66±0.33B	6.00±0.66A
Bacterial resistance**	8.33±0.88A	4.00±0.58B	8.33±0.80A

S: sensitive R: resistance NS : No significant . * significant differences at 0.05 .

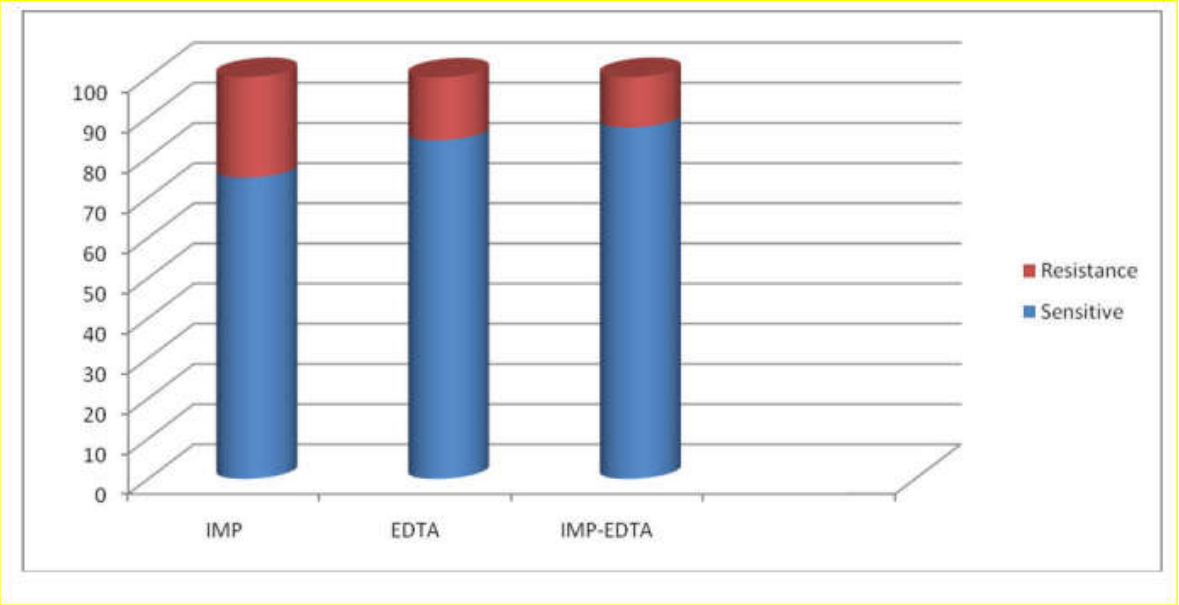

Figure (1): Percentage of resistance and sensitivity of IMP, EDTA and EDTA- IMP.

CONCLUSION

According to the present study can use oxidant agent for inhibition the growth bacteria specific *Pseudomonas aeruginosa* such as EDTA and can used preservation food specific sold cheese.

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التأثير التأزري لأثلين ثنائي امين رباعي حامض الخليك (EDTA) والامبنيم ضد *P. aeruginosa* المعزولة من الجبن في مناطق مختلفة من مدينة الحلة

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الخلاصة

اجريت الدراسة الحالية لدراسة تأثير التأزري لأثلين ثنائي امين رباعي حامض الخليك و الامبنيم ضد *Pseudomonas aeruginosa* المعزولة من الجبن في مناطق مختلفة من مدينة الحلة. ٧٠ عينة من الجبن اخذت بصورة مختلفة من الحلة (السوبر ماركت، المحلات والباعة المتجولين) ونقلت الى المختبر لغرض التحليل.

اثناء فترة البحث وجد (٤٥%) ٣٢ من مستعمرات *Pseudomonas aeruginosa* عزلت من الجبن .وهذه العزلات اختبرت بواسطة الاقراص الانتشار لغرض قياس حساسيتها الى الامبنيم ،بينت بأن (8) عزلات مقاومة الى الامبنيم ، بينما اوضحت النتائج بان(٨٤.٣%) ٢٧ من *Pseudomonas aeruginosa* حساسة ب لأثلين ثنائي امين رباعي حامض الخليك (EDTA).

ايضا وضحت النتائج بأن تأزرية اثلين ثنائي امين رباعي حامض الخليك والامبنيم (EDTA- IMP) بواسطة اقراص الانتشار ضد (٨) عزلات *Psedumonas aeruginosa* بينت ٧ (٨٧.٥ %) كانت حساسة. الاستنتاجات ، وفقا للدراسة الحالية يمكن استخدام تثبيط العامل المؤكسد للبكتيريا نمو محددة P. aeruginosa مثل اثلين ثنائي امين رباعي حامض الخليك (EDTA)ويمكن استخدامها في الحفاظ على الجبن المباع بالأغذية المحددة.

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