ANTIBACTERIAL ACTIVITY OF ACETIC ACID AGAINST DIFFERENT TYPES OF BACTERIA CAUSES FOOD SPOILAGE

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Abstract

The study was performed for study antibacterial effects of acetic acid against different types of bacteria causes food spoilage bacteria. 120 of samples were collected, including 40 raw milk, 40 cheeses and 40 yoghurt were collected randomly from Babylon province. 5 (4.1%) isolates were positive to *Streptococcus* spp., 10 (8.3%) of *Staphylococcus aureus,* 15 (12.5%) *E. coli,* 14 (11.6%) *Pseudomonas* aeruginosa and 5 (4.1%) *Proteus* spp. respectively. Determination of the antibiotic of isolates showed that *Streptococcus* spp., *Staphylococcus aureus, E.coli Pseudomonas aeruginosa and Proteus* spp. were sensitive to Gentimycin and Ciprofloxicin. *E. coli* and *Pseudomonas aeruginosa* showed resistance to Oxacillin and Erythromycin. The isolates of *Streptococcus* spp*., Staphylococcus aureus* and *Pseudomonas aeruginosa* was resistance to Amikacin. While isolating of *Proteus* spp. was resistance to Amoxicillin.

The present study showed that effects acetic acid against isolates were resistant to different types of antibiotics at different concentrations (0.5%,1%, 1.5%,2% and 2.5%) respectively. The mean of three replicates of the diameter of inhibition zones (in millimeters) around each well with acetic acid solution at concentrations (0.5%) to the range from (13mm to 18mm). The isolates studied showed sensitivity to the range (16mm to 18mm) at concentrations (1%), the concentrations (1.5%) to the range (20mm to 22mm). However, the concentrations (2%) to the range (22mm to 27mm) and (27mm to 35mm) at concentrations (2.5%).

Key words *:* Acetic acid, Antibiotic and Food spoilage.

Introduction

Microbial pathogens in food may cause spoilage and contribute to the foodborne disease incidence, and the emergence of multidrug resistant and disinfectant resistant bacteria—such as : S*taphylococcus aureus (S. aureus), Escherichia coli (E. coli), and Pseudomonas aeruginosa (P. aeruginosa*) has increased rapidly, causing the increase of morbidity and mortality (Miladi*, et al.,* 2016).

Morbidity and mortality are mainly caused by infectious diseases all over the world. The World Health Organization reported that 55 million people died worldwide in 2011, with one-third of the deaths owing to infectious diseases (Nabavi, *et al.,* 2015). Antibiotic resistant microorganisms can increase mortality- rates because they can survive and recover through their ability to acquire and transmit resistance after exposure to antibiotic drugs, which are one of the therapies to infectious diseases (Marchese, *et al.,* 2016). Antibiotic resistant bacteria threaten the antibiotic effectiveness and limit the therapeutic options, even for common infections (Paphitou, 2013).

Organic acids are increasingly used in food safety as preservatives (Lingham, *et al.,* 2012) and generally recognized as safe substances (GRAS) by the FDA and are approved as food additives by European Commission, FAO/WHO and FDA (Surekha, *et al.,* 2000). The antibacterial effect of organic acids on different types of pathogenic bacteria tartaric, citric, lactic, malic, propionic, and acetic acids (Lingham, *et al.*, 2012). Acetic acid has been investigated as an antimicrobial agent for use in meat, including poultry, beef and pork to extend its shelf-life and decontamination of bacteria, such as *Salmonella* or *Escherichia coli* (Sakhare, *et al.,* 1999). The objects of the study, antibacterial effects of acetic acid against different types of bacteria cause food spoilage.

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Materials and Methods

Sample collection

A total of 120 samples was collected, including 40 raw milk, 40 cheeses, and 40 yoghurt was collected randomly from Babylon province. All samples kept in the icebox at 4°C and were transported to the laboratory Vet. Public Health for analyzes.

Isolation and identification of bacteria

Ten milliliters of samples were taken with 90 ml of normal saline and homogenized to make an initial dilution (10^{-1}). Serial dilutions up to 10^{-6} were made for each sample, then (1 ml) from each corresponding dilution (10^{-5} and 10^{-6}) were inoculated into various selective media such as nutrient agar, mannitol salt, Eiosin methylene blue (EMB) blood and MacConkeys agar then incubated at 37°C for 24 hours (Khalil and Anwar, 2016). Isolates were identified to the species level based on the standards biochemical and microbiological methods (Macfaddin, 2000).

Preparation of stock solutions

The stock solution of acetic acid was prepared (0.5%, 1%, 1.5% and 2%) of the acid dissolved in enough sterile distilled water to make the final volume of (10 ml) (Pundir and Jan 2011).

Antibiotic susceptibility

The antimicrobial drugs used were Amoxicillin (10μg), Gentamicin (10μg), Erythromycin (25mg) Ciprofloxacin (5μg), Amikacin (30μg) and Oxacillin (30 μg). The antimicrobial susceptibility testing was done by the agar disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2016). In brief, a 0.5 Mac-Farland standardized suspension of the bacteria was prepared in 0.85% sterile normal saline solution. A sterile cotton swab was dipped into the standardized suspension of bacteria and then uniformly streaked over the entire surface of the Mueller- Hinton agar. Then, the paper discs impregnated with a fixed concentration of antibiotics were placed on the agar surface and incubated in an inverted position at 37°C for 24 hours. after incubation for 24 hours, clear zones of inhibition were produced by the bacterial growth and diffusion of the antibiotics and these were measured in millimeter using a caliper and interpreted as susceptible, intermediate and resistant (CLSI, 2016).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration was determined using different concentrations (0.5%, 1%, 1.5%, 2% and 2.5%) of the acetic acid. Sterile cork borer of 8.0mm diameter was used to bore well in the presolidified Mueller Hinton agar (MHA) plates and 100μl volume of each dilution was added aseptically into the wells made in MHA plates in triplicate that had food- associated bacteria. 100ìl distilled water introduced into the well in place of acetic acid was used as a control. All the test plates were incubated at 37°C and were observed for the growth after 24 hrs. (Pundir and Jan 2011). The zones of inhibition were measured in diameter according to the (Lingham et al., 2012).

Results and Discussion

Isolation and identification

The present study showed in (table 1) 120 samples were collected, including 40 raw milk, 40 cheeses and 40 yoghurt were collected randomly from Babylon province, 5 (4.1%) isolates were positive to *Streptococcus* spp., 10(8.3%) *of Staphylococcus aureus*, 15(12.5%) *E. coli,* 14(11.6%) *Pseudomonas* aeruginosa and 5(4.1%) Proteus spp. (Talukder and Ahmed, 2016) found the presence of *E. coli* in 36 (66.67%) of raw milk samples. (Yuen,*et al.,* 2012) *E. coli* in 47% of raw milk samples in Malaysia. (Pant, *et al*., 2013) found *E. coli* in 100% raw milk samples in India.(Leriche, *et al.,*2004) isolated *Pseudomonas* spp. from 54.5% of raw milk cheeses and (Morales *et al.,* 2005) isolated *Pseudomonas* spp. from 50% of one day old raw milk cheeses. (Singh, *et al.,* 2011) found the present 100 (17.8%) of *Proteus* spp. in raw milk samples. (Murad, *et al.,* 2016) who reported the *Streptococcus* spp. 12 (6.36%) from raw milk. Several studies have indicated the assortment of pathogenic bacteria, especially *Staphylococcus* spp. may rise in raw milk and cheese samples may responsible for nausea, vomiting, abdominal cramps and diarrhea like disease (Marjan, *et al*., 2014).

Table 1: Prevelance contamination of milk, cheese, and yoghurt by pathogenic bacteria.

Isolate	Raw milk milk	Cheeses	Yoghurt	Percentage (%)
Streptococcus spp.	4	1	0	5 (4.1%)
Staph. aureus	7	2	1	10 (8.3%)
E. coli	8	2	5	15 (12.5%)
Pseudomonas aeruginosa	9	2	3	14 (11.6%)
Proteus spp.	1	3	1	5 (4.1%)
Total (120)	29	10	10	49 (40.83%)

Antibiotic susceptibility

Our study showed that in fig. 1 isolates of *E. coli* were sensitive to Amoxicillin (79%), Gentamycine (84%), Ciprofloxacin (70%) and Amikacin (90%) while resistance

to Erythromycin (60%) and Oxacillin (70%). This result agrees with (Sadek, *et al.,* 2014) found that *E. coli* isolates sensitive to Ciprofloxacin (100%), Gentamicin (100%) and resistance to Oxacillin (100%) and Erythromycin (100%). The *Staphylococcus aureus* isolates were sensitive to Amoxicillin (80%), Gentimycin (89%), Ciprofloxacin (83%), Oxacillin (70%) and resistance to Erythromycin (90%) and Amikacin (70%). This result similar to (Mohanty, *et al.,* 2013) shows that isolates of *Staphylococcus aureus* sensitive to Gentimycin and Ciprofloxacin. (Murad, *et al.,* 2016) reported that *S. aureus* resistance to Erythromycin. The *Streptococcus* spp. isolates were sensitive to Amoxicillin (90%), Ciprofloxacin (85%), Gentamycin (50) and Erythromycin (80%). Also *Streptococcus* spp. resistance to Oxacillin (60%) and Amikacin (75%) respectively. This result agrees with (Mohanty, *et al.,* 2013) who found the *Streptococcus* spp. sensitive to Gentamycin, Ciprofloxacin and resistance to Amikacin isolates from clinical and subclinical cases of bovine mastitis. (Tomazi, *et al.,* 2018) found S. spp. isolates were sensitive to Erythromycin. (Pieterse, *et al.,* 2010) found that Stre. spp. Resistance to Oxacillin. *Pseudomonas aeruginosa* isolates were sensitive to Amoxicillin (91%), Gentamycin (85%), and Ciprofloxacin (75%). Also resistance to Oxacillin (90%), Erythromycin (90%) and Amikacin (75%). Our results agree with (Kamel, *et al.,* 2014) found that *Pseudomonas aeruginosa* were sensitive to Ciprofloxacin and resistance to Amikacin. from Pregnant Genital Tract and Delivery Room in Erbil Hospital. (Swetha, *et al.,*2017) have reported 100% resistance to Oxacillin by the isolate of *P. aeruginosa*. (Arslan and Özdemir, 2011) found that *Pseudomonas* spp. had the highest sensitivity to Gentamycin. In addition, *Proteus* spp. strains were sensitive to Gentamicin (68%), Oxacillin(83%) Ciprofloxacin (65%), Amikacin (80%) and Erythromycin (60%), while resistance to Amoxicillin (70%). This result agrees with (Abdullah and Al-shwaikh, 2009) reported that *proteus* spp. Isolates were sensitive to Amikacin, and Ciprofloxacin. However, isolates sensitive to Erythromycin and Gentimycin (*El Zubeir and El Owni*, 2009). (Perim, *et al.,* 2015) proteus spp. isolates resistance to Amoxicillin.

Antibacterial effect of acetic acid against food bacteria

The antimicrobial activity of acetic acid was investigated against bacterial that resistance to different types of antibiotics *(Streptococcus* spp*., Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and Proteus* spp.) using the well diffusion method in at different concentration (0.5%, 1%, 1.5%, 2% and 2.5%). The mean
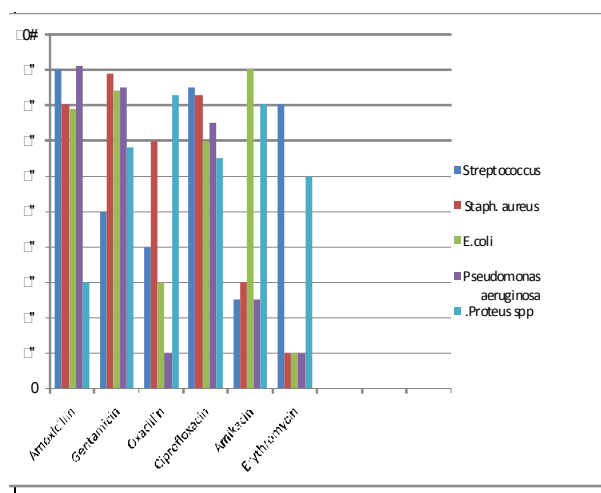

Fig. 1: The percentage of bacteria sensitive to different types of antibiotics.

of three replicates of the diameter of inhibition zones (in millimeters) around each well with an acetic acid solution is represented in table 2. It was found that at all concentrations. Acetic acid was able to inhibit bacterial growth at concentrations (0.5%) to the range from (13mm to 18mm). The isolates studied showed sensitivity to the range (16mm to 18mm) at concentrations (1%), the concentrations (1.5%) to the range (20mm to 22mm). However, the concentrations (2%) to the range (22mm to 27mm) and (27mm to 35mm) at concentrations (2.5%). These results were agrees with (Abdullah and Al-shwaikh, 2009) who reported that the minimum inhibition zone of acetic acid at concentrations (1%) range between (10mm to 15mm), the concentration at (2%) the inhibition zone from 14mm to 20mm) respectively against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus* spp. Also (Pundir, *et al*., 2011). The inhibition growth of acetic acid ranged between 0.5 and 1.0% (v/v) against (*Staphylococcus aureus* isolates I, III, *E. coli* II) was found to be the most sensitive. According to (Carpenter, *et al*., 2011) acetic acid displays residual activity to prevent the growth of pathogens. Acetic acid at the level of 0.1% - 0.5% in commercial significantly reduced numbers of *S. aureus* by 1.2-2.3 $\log^{10}$ CFU/ml at 10^0C (Olaimat, *et al.,* 2017). (Raftari, *et al*., 2009) reported that effects of organic acid against *Staphylococcus aureus* more pronounced than *E. coli* at concentrations 1%, 1.5% and 2%. Also found bacterial growth of *Clostridium* spp. decreased more than *E. coli* in same treatment. The gram positive bacteria high sensitive to different types of antibacterial than gram negative bacteria because possess an outer membrane. The bacterial growth (*Streptococcus agalactiae*) affected by acetic acid at high concentration. Also, this bacteria sensitive to weak acids, certain lactobacilli and bacteria are can to increasing growth in

low PH (Ewadh, *et al.*, 2013).

Table 2: Minimum inhibitory concentration (MIC) of acetic acid against food bacteria.

Isolations	Mean of inhibition zone millimeter determination(mm)				
	0.5	1%	1.5%	2%	2.5%
Streptococcus spp.	+	+	+	+	+
Staph. aureus	+	+	+	+	+
E. coli	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+
Proteus spp.	+	+	+	+	+

(+) : No growth.

Conclusion

This study indicates that the acetic acid antimicrobial agent can reduce the pathogenic bacteria and increase the shelf life of food products.

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