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DISINFECTION OF TABLE EGGS USING LEMON JUICE AS A NATURAL BIOCIDE

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ABSTRACT

Bacterial contamination of table eggs is a serious public health problem around the world due to increase the risks of food-borne illness. Disinfection of table eggs is essential to minimize the possibility egg contamination from shells. In the current study, 100 samples (table eggs) were collected from different supermarkets of Basrah city. Identification and disinfection of bacteria on shell of table eggs were done in Veterinary Medicine College, Public Health Laboratory / University of Basrah. Samples were cultured on blood agar, mannitol salt agar, macConkey agar, salmonella-shigella agar, eosin methylene blue agar, and tryptic soy agar to differentiate different types of bacteria before and after processing with lemon juice depend on its morphology and Gram's staining. The detection of organisms for genus and species were then done based on biochemical characteristics using VITEK® 2 system. The present study revealed that the major contaminant of table eggs was with Gram-negative bacteria and the minor contaminant was with Gram-positive bacteria. Gram-positive bacteria detected on shell of table eggs (*Leuconostoc species* and *Gemella bergeri*) were resistant to lemon juice. However, Gram-negative bacteria identified on shell of table eggs (*Cronobacter sakazakii*, *Raoultella ornithinolytica*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Moraxella group*, and *Serratia plymuthica*) were sensitive. In conclusion, table eggs collected from supermarkets were contaminated with pathogenic bacteria. Lemon juice was suitable to be used as an antiseptic agent to minimize the contamination of eggshells with Gram-negative bacteria.

INTRODUCTION

Eggs are one of the most important nutrition sources for sustenance and growth of human being. They are a highly nutritious product containing high levels of fat soluble, vitamins, minerals, and protein, In addition, eggs are a highly quality of saturated and monounsaturated fatty acids. Furthermore, eggs are a highly calories and consumption of eggs at the breakfast is recommended to start the day with a high energy sources, However, contamination of egg shell makes them a serious public health problem around the world due to increase the risks of food-borne disease (1).

Nutrient elements in hen's egg create an excellent environment for the development of both microfloral and pathogenic bacteria. Eggs can be infected vertically (prior- shell formation) or horizontally (post- shell formation). Through vertical transmission, pathogens (namely *Salmonella*) are introduced from the infected ovary or oviduct tissue to eggs prior- shell formation. Contamination via horizontal transmissions usually happens from cloaca to shell egg, which the excretion of faeces takes place. In addition, several factors have been responsible in contamination of egg table including cloths and hands of poultry workers and the environment vectors (dust, pets and rodents). Package, storage, transporting and marketing are other factors responsible in contamination of table egg. The common pathogenic to human beings that isolated from shell egg are *Staphylococcus, Micrococcus*, *Sarcinia, Arthrobacter*, *Bacillus*, *Pseudomonas*, *Achromobacter*, *Alcaligenes*, *Flavobacterium*, *Cytophaga*, *Escherichia*, *Aerobacter*, *Proteus*, *Serratia*, *Aeromonas* (2, 3).

The environmental condition combined with the poor hygiene is suitable area for the survival and proliferation of micro-organisms in table eggs (4).

To ensure table eggs are safe for consumers, several methods have been used to minimize the egg contamination from shells. Chlorine, ultraviolet, hydrogen peroxide, peracetic acid, quaternary ammonium compounds have been used to reduce the microbial contamination from shell egg. Chlorine and ultraviolet combined, peracetic acid and ultraviolet combined, and ultraviolet and hydrogen peroxide combined have also been used to reduce the microbial contamination from shell egg (5).

However, all these methods unable to reduce microbial contamination on egg shells completely. In addition, these disinfectants are high cost, toxic, and unfriendly to the environment. Therefore, others effective methods are required to ensure that shell egg is strong, internal egg quality is good, and eggs are sanitary as possible when they collect by consumers. Lemon juice is an alternative agent has been used a disinfectant (destroyed the bacteria on non-living objects) to clean hard surfaces (6).

In addition, the acid in lemons has been used as antiseptic (destroyed the bacteria on living skin) to eliminate a variety types of Gram- negative bacteria from war wounds, anti-inflammatory, antiparasitic (destroyed the parasites within the body) for treating malaria (plasmodium parasites), anti-oxidant, and antibacterial (destroyed the bacteria within the body) for treating cholera (*Vibrio cholera*), diphtheria (*Corynebacterium diphtheria*), typhoid (Salmonella typhi), phague (*Yersinia pestis*), Leptospirosis (*Leptospira interrogans*), and internal hemorrhage by the vitamin p (bioflavinoids) in it (7,8,9).

However, there is no study has been carried out to examine the effect of lemon juice as antibacterial to food surfaces. Thus far, this work aimed to use lemon juice as antibacterial to minimize the possibility egg contamination from shells.

MATERIALS AND METHODS

Study design

A total of 100 samples (table eggs) were collected from different supermarkets of Basrah city. Detection and disinfection of bacteria were done in Veterinary Medicine College, Public Health Laboratory/University of Basrah.

Preparation of sample

Before processing, swabs from each egg were taken from the shell using rinsed swab method (10). Briefly, sterile cotton swab was soaked in 0.1% peptone, rubbed the surface of the egg, kept in the nutrient broth medium, and incubated at 37°C for 24h. The swabs were then

streak on each of the following media: Blood agar, mannitol salt agar, macConkey agar, salmonella-shigella agar, eosin methylene blue agar, and tryptic soy agar to differentiate different types of bacteria depend on its morphology and Gram's staining.

All culture media were prepared following the manufacturer's instruction and sterilized by autoclaving at 121°C for 20 min. The detection of organisms for genus and species were then done based on biochemical characteristics using Gram-negative and Gram-positive cards by VITEK® 2 system (bioMérieux)(Table1).

After processing of egg by rub the surface of the egg with lemon juice (a pH of around 2.2), swabs from each egg were taken from the shell using rinsed swab method (10).

Briefly, sterile cotton swab was soaked in 0.1% peptone, rubbed the surface of the egg, kept in the nutrient broth medium, and incubated at 37°C for 24h. The swabs were then streak on the on the selective media mentioned above to differentiate different types of bacteria based on its morphology and Gram's staining. Detection genus and species of bacteria was then carried out by the VITEK® 2 system (bioMérieux) based on biochemical characteristics using Gram-negative and Gram-positive cards(Table 1).

Table 1: Biochemical tests on Gram-negative and Gram-positive cards

Biochemical Test on Gram-negative card	Biochemical Test on Gram-positive card
Ma-Phe-Pro-A.Rylamda.SE	D-AMYGDAUN
Adonitol	PHOSPHATfDYUNOSfTOL PHOSPHOLfPASE C
L-Pyrrolydonyl-Arylamidase	D-XYLOSE
L-Arabitol	ARGINfNE DIHYDROLASE 1
D-Cellobiqse	BETA-GALACTQSIDASE
BETA-Calactosidase	ALPHA-GLUCOSIDASE
H2S Production	Aia-Phe-Pro ARYLAMIDASE
Beta-N-AcetyL-Glucosaminidase	CYCLODEXTRIN
Glutamyl Aryiamidase pNA	L-Aspartate ARYLAMIDASE
D-Clucose	BETA GALACTOPYRANOSIDASE
Gamma-Gliitamyl -Transferase	ALPHA-MANNOSIDASE
Fermentation/ Glucose	PHOSPHATASE
Betaglucosidase	Leucine ARYLAMIDASE
D-Maltose	L-Prolirie ARYLAMIDASE
D-Mann1tol	BETA GLUCURONIDASE
D-Mannose	ALPHA-GALACTOSIDASE
Betaxylosidase	L-Pyrrolidonyl-ARYLAMIDASE
Beta-Alanine aryiamidase pNA	BETA-GLUCURONIDASE
L-Proline Aryiamidase	Alanine ARYLAMIDASE
Lipase	Tyrosine ARYLAMIDASE
Palatinose	D-SORBITOL
Tyrosine Arylamidase	UREASE
Urease	POLYMiXIN B RESISTANCE
D-Sorbitol	D-GALACTOSE
Saccharose/Sucrose	D-RIBOSE
D-Tacatose	L-LACTATE alkalinization
D-Trehalose	LACTOSE
Citrate (sodium)	N-ACETYL-D-GLUCOSAMINE
Malonate	D-MALTOSE
5- Keto-D-Gluconate	BACITRACIN RESISTANCE
L-Lactate alkalinisation	NOVOBIOCIN RESISTANCE
Alpha-Glucosidase	GROWTH IN 6.5% NaCI
Succinate alkalinisation	D-MANNITOL
Beta-N-Acetyl-Galactosaminidase	D-MANNOSE
Alpha-Galactosidase	METHYL-B-D-GLUCOPYRANOSIDE
Phosphatase	PULLULAN
Glycine Aryiamidase	D-RAFFINOSE
Ornithine Decarboxylase	0/129 RESISTANCE (comp.vibrio.)
Lysine Decarboxylase	SALICIN
Decarboxylase Base	SACCHAROSE/SUCROSE
L-HistidIne assimilation	D-TREHALOSE
Coumarate	ARGININE DIHYDROLASE 2
Betaglucoronidase	OPTOCHIN RESISTANCE
OH 29 Resistance (comp .Vibrio)	
Glu-Gly-Arg-Arylamidase	

Statistical analysis: PC LIS-compatible software used to analyse the result of the current study

RESULTS

This study revealed that the major contaminant of table eggs was with Gram-negative bacteria and the minor contaminant was with Gram-positive bacteria. 6 of the bacteria present on egg shells belong to Gram-negative and 2 belong to Gram-positive bacteria (Table 2). Gram-negative bacteria present on egg shells were *Cronobacter sakazakii*, *Klebsiella oxytoca*, *Raoultella orinthinolytica*, *Enterobacter aerogenes*, *Moraxella group*, and *Serratia plymuthica*. the Gram-positive bacteria present on eggshells were *Leuconostoc mesenteroides* and *Gemella bergeri* (Table 2). The present study also revealed that the major contaminant of table eggs was with *Raoultella ornithinolytica* and the minor contaminant was with *Gemella bergeri* (Figure 1).

Table 2: Morphologic features of the bacterial present on the surface of egg.

Bacterial present on the shell	Morphologic features
Cronobacter sakazakii group	Gram-negative rod glossy yellow
Raoultella ornithinolytica	Gram-negative bacilli smooth circular
Klebsiella oxytoca	Gram-negative rod, slightly mucoid yellow to cream
Enterobacter aerogenes	Gram-negative, Straight rods, smooth irregularly round to rough "cauliflower"
Moraxella group	Gram-negative, rods or cocci, smooth, convex, and circular whitish
Serratia plymuthica	Gram-negative, rods, a smooth, round, convex pale pink
Leuconostoc mesenteroides spss mesenteroides	Gram-positive, rods, spherical, oval, slimy, smooth, round grayish in nature
Gemella bergeri	Gram-positive cocci tiny convex pink

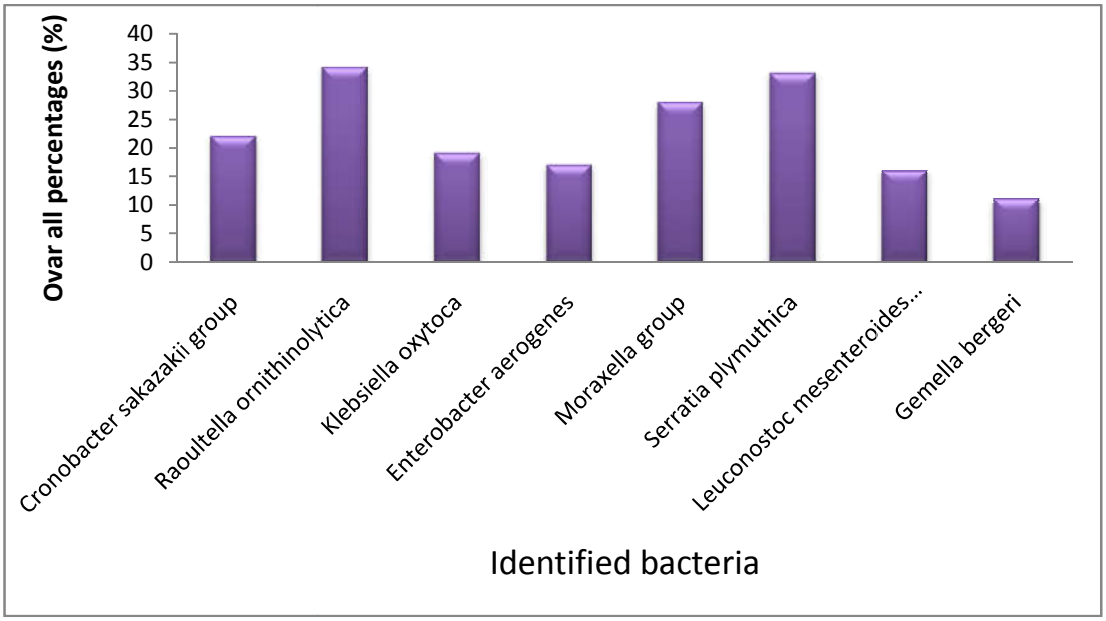

Figure 1: Percentages of bacterial detection on the surface of the table eggs

Table 3: The bacterial present on the surface of egg before and after processing with lemon juice

Bacterial present on the shell	Before processing	After processing
Cronobacter sakazakii group	+	-
Raoultella ornithinolytica	+	-
Klebsiella oxytoca	+	-
Enterobacter aerogenes	+	-
Moraxella group	+	-
Serratia plymuthica	+	-
Leuconostoc mesenteroides spss mesenteroides	+	+
Gemella bergeri	+	+

Note: (+) denotes for presence and (-) stands absence of bacteria

DISCUSSION

Bacterial contamination of table eggs is a serious public health problem all over the world due to increase the risks of food-borne illness. Disinfection of table eggs by using natural biocide is required to minimize the possibility egg contamination from shells. The present study revealed that bacterial present on the surface of egg are *Cronobacter sakazakii*, *Raoultella*

orinthinolytica, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Moraxella group*, *Serratia plymuthica*, *Leuconostoc mesenteroides* and *Gemella bergeri*. All these bacteria detected on shell of table egg play an important role in spoilage of food product, leading to food poisoning in consumers. This finding is in agreement with previous study, in which those bacteria identified on the surface of table eggs are pathogenic and spoiled food product. It has been found that eggs can be infected horizontally from cloaca to shell egg, which the excretion of faeces takes place or vertically from the infected ovary or oviduct tissue to eggs prior- shell formation(4).

Contaminated with pathogenic bacteria might also be due to the environmental condition combined with the poor hygiene (4). *Cronobacter sakazakii* (*Enterobacter sakazakii*) is a food-borne pathogen causes a life-threatening to human, predominantly in neonates. In infant and young children, Infection of *C. sakazakii* causes bacteremia , enterocolitis, meningitis, septicemia, and urinary tract inflammation (11).Infections among immunocompromised adults have also been found (12).

In adult, *C. sakazakii* infection causes appendicitis (appendix inflammation), biliary sepsis, conjunctivitis (eye inflammation), pneumonia, and urosepsis (12). *C. sakazakii* can be found in water, soil, and plant materials (12). A basic reason for hen's egg contamination in *C. sakazakii* is the flies (environment vectors) (13). *C. sakazakii* has been isolated from a wide range of food including egg, milk, meat, and seafood products (14).

Raoultella ornithinolytica is human pathogen causes a life-threatening, predominantly in adult. It causes bacteraemia, pneumonia, Cholangitis (biliary tract infection). *R. ornithinolytica* can be found in the soil, plants and water. It may also be found in the upper respiratory and intestinal tracts of healthy chickens. A basic reason for hen's egg contamination in *R. ornithinolytica* may be the fact that the egg excretes from the hen's chicken through the cloaca, where faecal material is also excreted. The adherent faeces to the egg's shell contaminate egg contents through shell penetration by microorganisms. In addition, the environmental factors (temperature and humidity) play an important role in pathogenic penetration and increase the frequency of contamination (15).

Klebsella oxytoca (Bacterium oxytocum) is a pathogenic bacteria now being isolated more frequently. It causes neonatal infections of the bloodstream, urinary tract, central nervous system, wound, lung, and soft. It is found in gut, mouth, nose of man and animals. It is also

acquired from the environment (water, soil, and insect). This bacterium can live on the surface of the housefly eggs (*Musca domestica*) and contaminate hen's egg. In addition, adherent faeces to the egg's shell contaminate egg table by this pathogen (16)

Enterobacter aerogenes (*Klebsiella aerogenes*) is a pathogenic bacterium for humans and animal. It is widespread in nature, water, sewage, soil, and on plants. It is normal inhabitants in gastrointestinal and mucosal surfaces of the animal. In animal, *E. aerogenes* causes mastitis (inflammation of udder). In humans, *E. aerogenes* causes wound infections, septic shock, bacteremia (the presence of bacteria in the blood), meningitis (inflammation of the membranes brain), septicemia (blood poisoning), lower respiratory and urinary tract infections. *E. aerogenes* infections are generally treated with antibiotics, but the effectiveness of treatments for infection with *E. aerogenes* could compromise by development of bacterial resistance (17).

Moraxella group is Gram-negative bacteria in the *Moraxellaceae* family. They are normal inhabitants the mucosal surfaces of human and other animals. The bacterium can be transmitted by flies. In human, *Moraxella* causes tracheobronchitis (inflammation of the trachea and bronch), pneumonia, otitis media (inflammation of middle ear), sinusitis (a sinus infection), and blepharoconjunctivitis (inflammation of the eyelids and conjunctiva). In animal, *Moraxella* causes infectious ovis keratoconjunctivitis (inflammation of the cornea and conjunctiva)(18).*Serratia plymuthica* is a significant pathogen in a case of chronic osteomyelitis and in case of sepsis associated with infection of a central venous catheter (19). It has been found in a variety of environments (soil, water, plants, animals and air).

Leuconostoc species are Gram-positive bacteria widely spread in water and soil and play an important role in food fermentations and several industrial (20). *Gemella bergeri* is a normal flora found in the oral cavity and digestive tract of human and other animals. It has been recently implicated in endocarditis (an infection of the endocardium) in infancy with a bicuspid aortic valve status post-intervention (21).

The current study revealed that Gram-positive bacteria detected on shell of table eggs (*Leuconostoc species* and *Gemella bergeri*) were resistant to lemon juice. This finding is in agreement with previous study, in which those Gram-positive bacteria are tolerant to acidity

(22). The mechanisms used by those Gram-positive bacteria to survive in acidic environment are F_1F_0-Type ATPase proton pumps inhibitor, macromolecular repair, cell membrane alteration, alkali production, pathways induction by transcriptional regulators, metabolism change, and cell density and signaling modification. However, Gram-negative bacteria detected on shell of table eggs (*Cronobacter sakazakii*, *Raoultella ornithinolytica*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Moraxella group*, and *Serratia plymuthica*) were sensitive to the lemon juice. This finding is in agreement with previous studies, in which those Gram-negative bacteria are susceptible to organic acid (23, 24, 25, 26).

The most of food-borne pathogen is not grown in too acidic environment, making lemon juice a natural biocide due to its rich acid content. The acid in lemon juice penetrates the cell membrane of bacteria, prompting a release of protons, which causes cell death. The structure of all macromolecules affects at high acidity. Lipid and large proteins of bacteria (enzymes) are sensitive to the pH value in their environment. Usually, lipids are hydrolysed at high acidity and an enzyme a shape alters brings about an alteration of the ionic charges on the molecule, lost the catalytic properties of the enzymes and halts the metabolism. In addition, hydrogen ion bonds holding together strands of DNA break up at high acidity. Furthermore, the production of ATP in cellular respiration are collapsed and impaired at high acidity because the proton motive power depends on the concentration of hydrogen ion across the plasma membrane. The ionization of amino-acid functional groups and disrupt hydrogen bonding can also modify by moderate alteration in hydrogen ion, promoting denaturation and destroying activity of bacteria's cell (27)

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