Assiut University web-site: www.aun.edu.eg

MOLECULAR CHARACTERIZATION OF SOME PATHOGENIC BACTERIA ISOLATED FROM MAYONNAISE

MARWA M.N. EL-GENDI and MANAL M. AMIN
Animal Health Research Institute (Assiut Provincial Lab.) Food Hygiene Department

Received: 11 February 2019; **Accepted:** 4 March 2019

ABSTRACT

The purpose of this study was to determine the molecular characterization of some pathogenic bacteria isolated from commercial and small producers mayonnaise sold in restaurants and supermarkets in Assiut governorate. In the present study, the samples were analyzed for the presence of Salmonella spp., listeria spp. and staphylococcus aureus and according to microbiological analysis, Salmonella spp., listeria monocytogenes and staph. aureus were detected in 3 of 40 (7.5%), 3 of 40 (7.5%) and 7 of 40 (17.5%) of examined samples of small producers mayonnaise, respectively. The examined samples of commercial mayonnaise were free from salmonella spp. and listeria spp. but staph. aureus could be detected in 2 samples in a percentage of 5%. The presumptive isolates were further confirmed by PCR using specific primers of *Salmonella invA* isolates, L. monocytogenes 16S rRNA gene and Staph. aureus clfA gene and serotyping of *Salmonella*. These results indicated that small producers mayonnaise samples may contain pathogenic bacteria and thereby represent a risk to the consumers in regard to foodborne diseases. Thus, it is essential to include the effective hygiene practices as an important safety measure in the production of small producers mayonnaise. Bacterial loads were detected in mayonnaise including hazardous bacteria in spite of a high acidity of the product. This high light the importance of improving production situations and hygienic status in ready to eat foods establishments.

Key words: Mayonnaise, Molecular characterization

INTRODUCTION

Recently, the issue of food contamination has triggered considerable interest in food safety, and the food industry is enforcing an increasing number of stringent regulations concerning acceptable foodborne pathogens. Mayonnaise is probably one of the most widely used sauces or condiments in the world today. It was first produced commercially in the early 1900s, and became popular in America from 1917 to 1927 (Harrison and Cunningham, 1985) and more recently in Japan where sales increased by 21 % a year from 1987 to 1990 (Brabant, 1992). Because of its low pH and high fat content, mayonnaise is relatively resistant to microbial spoilage. Mayonnaise is an oil-in-water emulsion and is traditionally prepared from a mixture of egg yolk, vinegar, oil and spices (especially mustard); it may also include salt, sugar or sweeteners, and other optional ingredients (Depree and Savage, 2001). Pasteurization causes little or no damage to the functional properties and does not affect the formation of stable mayonnaise (Palmer *et al.*, 1969).

Corresponding author: MARWA M.N. EL-GENDI
E-mail address: ahmednofel125@yahoo.com
Present address: Animal Health Research Institute (Assiut Provincial Lab.) Food Hygiene Department

Mayonnaise is a relatively microbiologically stable product due to its high fat content and addition of acidic ingredients. Organic acids and other acidic ingredients are toxic to foodborne pathogens, they also contribute to the desirable flavor and, decrease the final pH of product (Fialova *et al.*, 2008). Mayonnaise is widely consumed to such an extent that it form the foundation of one-half of all salad dressings and the basis of many other products (Xiong *et al.*, 2002). Ready-to-eat (RTE) foods by Codex Alimentarius is any food or beverage that is normally consumed in its raw state or otherwise prepared into a form in which it is normally consumed without further processing (Forsythe, 2010). Mayonnaise is ready for consumption without additional preparation and cooking by consumers. Therefore presenting a potential microbiological risks to consumers (Hwang and Tamplin, 2005).

The mayonnasie typically has a low microbial count with no or a very limited contamination with microorganisms (Michles and Koning, 2000). Commercial Mayonnaise, microbiologically, have long shelf life and are extremely safe processed foods. The safety of such products is directly associated with synergistic formulation components of which aqueous phase acetic acid and total formula pH levels are considered the most essential in inactivating foodborne pathogens such as

Salmonella and Staph. aureus (Erickson and Jenkis, 1991). The ability of pathogenic microorganisms to survive and grow at low temperatures may be important in food-borne infection, particularly when prepared foods are exposed for a long period of time in refrigeration cabinets (George and Levett, 1990). In addition, the pH of mayonnaise increases when it is added to the other foods.

The principal basis of concern is abusive handling of small producers mayonnaise by food handlers. Small producers mayonnaise could be cross-contaminated by contact with utensils, unclean table surface or raw ingredients, such as meats or vegetables. Mishandling of contaminated small producers mayonnaise enhanced the possibility of the presence of pathogenic organisms in the product (Gomez-Lucia *et al.,* 1987).

Salmonella species are found worldwide and are universally recognized as zoonotic agents. Many foods particularly of animal origin and those subjected to sewage pollution, had been identified and must be taken into consideration as a vehicle for transmitting these pathogens to human being. The primary habitat of Salmonella species is the intestinal tract of animals and humans. Egg is considered as an important vehicle for Salmonella causing human infection. Additionally, Salmonella species causes illness by means of infection, as they multiply in the small intestine, colonizing and subsequently invading the intestinal tissues, producing an enterotoxin and causing inflammatory reaction and diarrhea (ICMSF, 2006). Salmonella is the second most common of food borne illness. It is responsible for millions of cases of food borne illness a year (HGIC, 2000). The most important serotypes of Salmonella are Salmonella typhimurium and Salmonella enteritidis (Fashae *et al.,* 2010 and Hendriksen *et al.,* 2011).

Listeria monocytogenes is a foodborne pathogen that causes the severe disease listeriosis (Swaminathan and Gerner-Smidt, 2007 and Warriner and Namvar, 2009). Although the prevalence of listeriosis is low (0.52 cases per 100,000 population in the EU in 2014), the severity of the disease makes it one of the most important foodborne pathogens, both economically and with regard to public health (European Food Safety Authority, 2015). There are a variety of symptoms that may arise upon infection, including septicemia, meningitis, and gastroenteritis (Silk *et al.,* 2012). In pregnant women, it may cause spontaneous abortion, premature labor, and neonatal disease (Ferreira *et al.,* 2014). Although *L. monocytogenes* may survive mild heat treatment at <60 °C, it is relative sensitive to higher temperatures; e.g. D 71 °C<1s in foods (Lado and Yousef, 2007). Thus foods that are consumed without further heat treatment, so-called ready-to-eat foods, are the main sources for listeriosis outbreaks. The main contamination route for *L. monocytogenes* is through cross-contamination from equipment and machines to food during processing (Ferreira *et al.,* 2014). *L. monocytogenes* possesses the ability to establish itself in equipment/production environments, and single strains of *L. monocytogenes* have been found to reoccur in production environments over periods of years (Carpentier and Cerf, 2011 and Ferreira *et al.,* 2014). In addition to being able to survive a wide range of temperatures, Listeria spp. can grow in a variety of salt concentrations, high osmotic pressure, and low pH environments, but succumb to pasteurization (Milillo *et al.,* 2012).

Staphylococcus aureus food poisoning is one of the most common types of food borne diseases worldwide, which caused by an intoxication resulting from the ingestion of food containing Staphylococcal enterotoxins, which is emetic, pyogenic and mitogenic, suppresses immunoglobulin secretion and enhances toxic shock (Stewart *et al.,* 2002). On the other hand (Abeer, 1997) mentioned that Coagulase positive Staphylococcus aureus is considered the most important species of Staphylococci due to its pathogenicity and enterotoxin production which cause food intoxication. *S. aureus* have been shown to able to grow at low pH values. Nevertheless, in small producers mayonnasie, other factors besides pH should be taken into account when considering the potential risk of the product. Gomez-Lucia *et al.* (1990) demonstrated that *S. aureus* may grow at 22°C and sythesize enterotoxins in mayonnaise. Acidification is one of the methods commonly used in the food industry to control growth and survival of spoilage-causing and pathogenic microorganisms. However, it has been reported that microorganisms exposed to a moderately acidic environment may develop cells with increased resistance and longer survival time when transferred to a more acidic condition (Cheng *et al.,* 2003).

The objectives of this study were to determine the molecular characterization of some pathogenic bacteria isolated from commercial and small producer mayonnaise.

MATERIALS AND METHODS

A) Collection of samples:

A total of eighty random samples of commercial and small producers mayonnaise (40 samples of each), were collected from different supermarkets and restaurants at Assiut Governorate. The collected samples were transferred directly to the laboratory in an ice box under complete aseptic conditions. The samples were immediately examined bacteriologically for the detection of Salmonella spp., Listeria spp. and staph. aureus.

B) Isolation of Salmonella (Quinn *et al.*, 2002a):
25 ml of each well mixed mayonnaise sample were thoroughly mixed with 225 ml of sterile buffered peptone water. All samples were incubated at 35 °C for 24 ± 2 h. One hundred microliters from the pre-enriched sample was transferred to 10 ml of Rappaport Vassiliadis (RV) enrichment broth and incubated at 43 °C for 24 h. Loopfuls from enriched RV broth were separately streaked onto each of xylose lysine desoxycholate (XLD) agar and Salmonella- Shigella (SS) agar plates and incubated at 37 °C for 24 h. Two or three of typical or a typical colonies (colorless with black center on SS standard colonies with black center on XLD) were selected from each selective medium and streaked onto nutrient agar slope which incubated at 37 °C for 24 h for further biochemical and serological identification.

Serological identification of Salmonellae: This part has been done in Serology Department in Animal Health Research Institute, El-Giza, Egypt. Serological identification of Salmonellae was carried out according to Kauffman – White scheme (Kauffman, 1974 and Popoff *et al.*, 2004) for the determination of Somatic (O) and flagellar (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan).

C) Isolation and Identification of Listeria spp. according to the International Organization for Standardization (ISO11290 -1, 2017). Briefly, a 25 g mayonnaise sample was aseptically homogenized in 225 ml pre-enrichment half-Fraser broth (CM0895, Oxoid Ltd) supplemented with half-Fraser supplement (SR0166E, Oxoid Ltd) in Stomacher bags (SewardLtd, West Sussex, UK) for 30 s using a Stomacher circulator (Easy Mix, AES Laboratoire, Bruz, France), followed by incubation at 30ºC for 24 h. Then 0.1 ml half-Fraser broth was added to 10 ml Fraser broth containing Fraser supplement and incubated at 37ºC for 48 h. At the end of incubation, a loopful of Fraser broth was streaked on chromogenic Listeria agar (ALOA) supplemented with Brilliance Listeria Differential Supplement (SR0228E, Oxoid Ltd) and incubated at 35ºC for 24 to 48 h. *L. monocytogenes* appear as green–blue colonies surrounded by an opaque halo. For biochemical identification of L. monocytogenes, five suspect colonies from each plate were streaked on TSA (M290, Oxoid Ltd) supplemented by (0.6%) yeast extract (LP0021) and incubated at 37ºC for 18–24 h.

Biochemical confirmation of L. monocytogenes: Suspected colonies were verified by Gram staining, catalase, oxidase, haemolysis and CAMP tests, motility, Methyl Red-Voges Proskauer (MR-VP) reactions, nitrate reduction and the production of acids from rhamnose, xylose and mannitol for the identification as described by ISO11290 -1 (2017).

D) Isolation and Identification of Staph aureus according to Bennett and Lancette (2001): All the samples were prepared and enriched on Staphlococci broth for 20h at 35 ^{o}C and then inoculated onto Baird Parker Medium (Oxide, Basingstoke, England), and incubated aerobically at 37 ^{o}C for 24 h. The isolates were identified using established microbiological methods which included colony morphology, Gram staining and biochemical testing [catalase, coagulase and sugar fermentation (glucose, sucrose, lactose and mannitol)].

Identification and characterization of coagulase positive and negative Staphylococcus Species: the isolates were identified according to (ISO, 2003b).

Coagulase test according to (ISO, 2003b):
Five colonies typical and atypical were selected from each plate. The selected colonies inoculated into 5ml Brain Heart Infusion broth. The tubes were incubated at 37ºC for 24 hours. From which 0.1 ml was transferred to tubes containing 0.3 ml of sterile citrated rabbit plasma. Inoculated tubes were incubated at 37ºC and examined for clot formation after 4 hours.

PCR techniques:
DNA extraction. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer.

Oligonucleotide Primer. Primers used were supplied from Metabion (Germany) are listed in table (1 and 2).

PCR amplification. Primers were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products.
The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) and generuler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table 1: Oligonucleotide primers sequences.

Microbial agent	Target gene	Oligonucleotide Sequence (5\ – 3 \ )	Amplified product size (pb)	Reference
Staph aureus	*ClfA*	GCAAAATCCAGCACAACAGGAAACGA CTTGATCTCCAGCCATAATTGGTGG	638	*Mason et al., 2001*
L. monocytogenes	*16S Rrna*	ggA CCg ggg CTA ATA CCg AAT gAT AA TTC ATg TAg gCg AgT TgC AgC CTA	1200	*Kumar et al., 2015*
Salmonella	*invA*	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	*Oliveira et al., 2003*

Table 2: Cycling conditions of the different primers during cPCR

Microbial agent	Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No.of cycles Final extension	Final extension
Staph aureus	*ClfA*	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
L. monocytogenes	*16S Rrna*	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 1 min.	35	72˚C 10 min.
Salmonella	*invA*	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec	72˚C 30 sec.	35	72˚C 7 min.

RESULTS

Table 3: Incidence of the isolated bacteria from examined samples:

Examined samples	No. of the examined samples	*Salmonella spp.*		*L. monocytogenes*		*Staph aureus*	
		No.	%	No.	%	No.	%
Commercial mayonnaise	40	-	-	-	-	2	5%
Small producers mayonnaise	40	3	7.5%	3	7.5%	7	17.5%

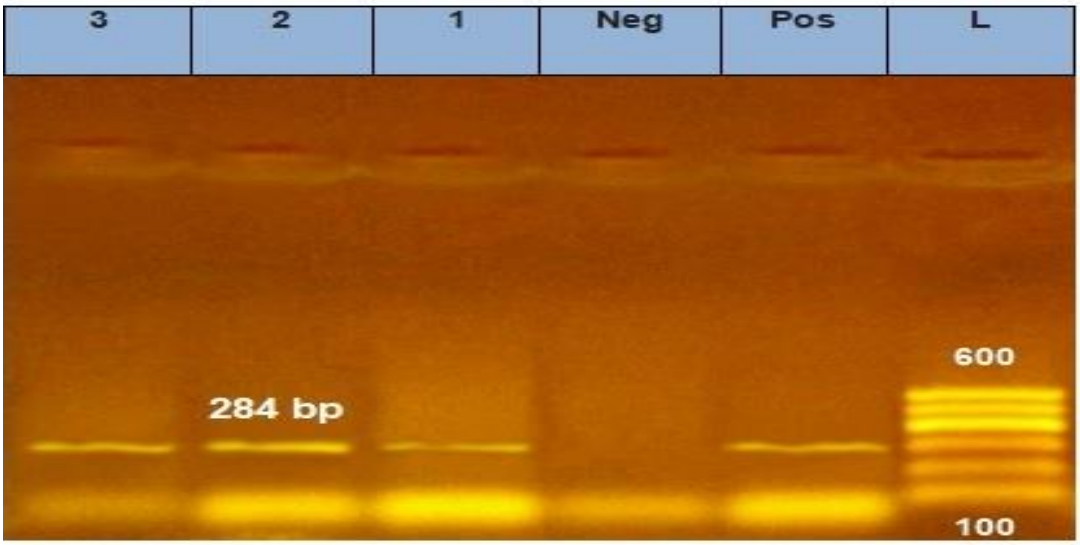

Fig. 1: PCR result of *Salmonella invA* gene among isolates. Lane L: ladder, lane pos: control positive, lane neg: control negative lane 1, 2, 3 (+ve *Salmonella invA* gene).

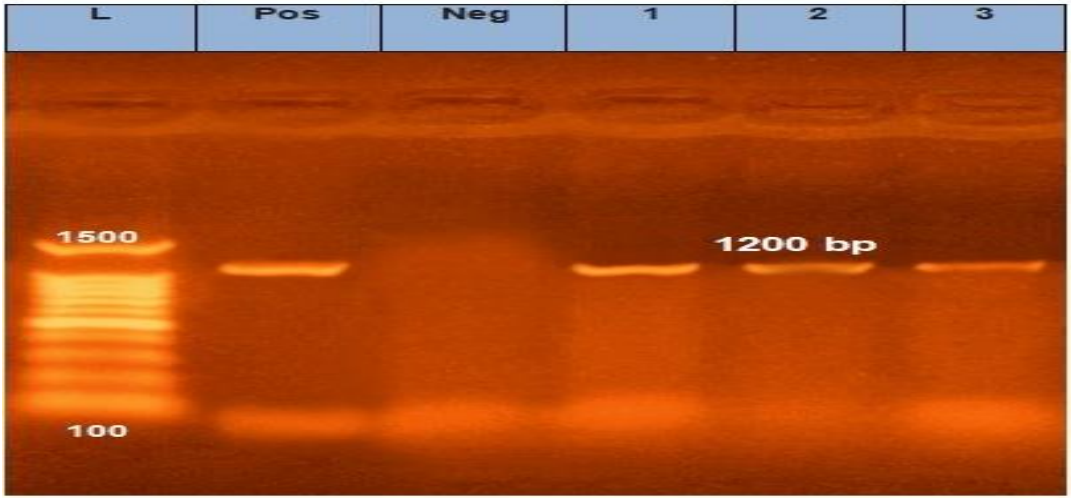

Fig. 2: PCR result of *L. monocytogenes 16S Rrna* gene among *L. monocytogenes* isolates. Lane L: ladder, lane pos: control positive, lane neg: control negative lane 1, 2, 3 (+ve *L. monocytogenes 16S Rrna* gene).

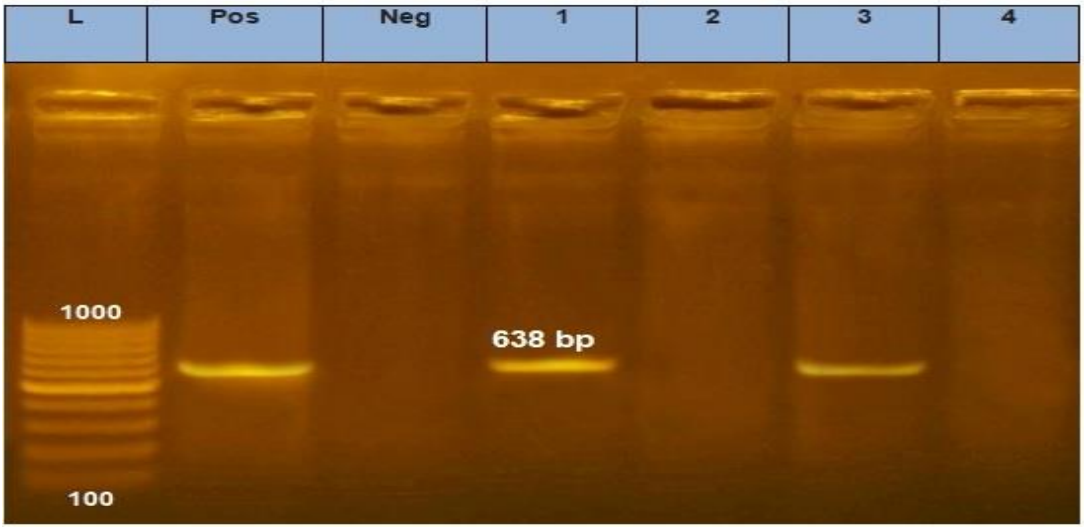

Fig. 3: PCR result of *Staph aureus clfA* gene among Coagulase positive *S. aureus* isolates. Lane L: ladder, lane pos: control positive, lane neg: control negative lane 1 and3 (+ve *Staph aureus clfA* gene).lane 2 and 4 (-ve *Staph aureus clfA* gene).

DISCUSSION

Over the past few years, food safety has become very topical subject, eliciting a great deal of public concern elsewhere. As, certain food and their products particularly become contaminated with different microorganisms, likewise, mayonnaise which are palatable, nutritious, healthful and relatively inexpensive food used as appetizer. Poor personal hygiene causes more than 90% of the sanitation problems in food service industry. Also government statistics showed that improper hand washing alone accounts for more than 25% of all food borne illnesses (Weistein, 1991).

The recorded results in Table 3 & Fig.1 showed that the percentage of salmonella spp. isolated from commercial and small producers mayonnaise were 0 and 7.5 %, respectively. Serological identification of isolated Salmonella in the examined positive small producers mayonnaise showed that the 3 isolates of salmonella spp. were Salmonella typhimurium. Contamination through food often occurs when organisms are introduced into food preparation areas and are allowed to multiply in food, e.g. due to insufficient storage temperatures, inadequate cooking or cross contamination of Ready to Eat food. Even infected food handlers may act as a cause of contamination for foodstuffs (Effimia, 2015). The genus Salmonella constitutes of two species: S. enterica and S. bongori. Salmonellosis in humans is usually described by severe fever, abdominal pain, nausea and sometimes vomiting. Symptoms are often minor and most infections are self-limiting, lasting a few days. Nevertheless, in some patients the infection may be more serious and associated with dehydration or septicemia. Mortality is typically low with less than 1% of reported cases being lethal. The usual reservoir of Salmonella is the intestinal tract of animals from where a variety of foodstuffs of both animal and plant origin may become contaminated with fecal organisms either directly or indirectly. Higher results were documented by Tayfur *et al.* (2013) who could isolate Salmonella from 26.3% from examined retail mayonnaise-base salads.

The most dangerous mayonnaise-contaminating microorganism is Salmonella. In Rio Grande do Sul (Brazil), homemade mayonnaise accounts for 17% cases of salmonellosis (Capalonga *et al.,* 2014). Changes in pH and temperature can control the growth of Salmonella enteritidis (Keerthirathne *et al.,* 2016), and the model describing the growth of this microorganism in mayonnaise at different temperatures has been proposed (Elias *et al.,* 2016).

Food poisoning cases in New south Wales restaurant by Salmonella species Potsdam strain were attributed to shell egg-based Caesar salad dressing mayonnaise, a swab of a cap from a mayonnaise bottle collected and tested positive (Unicomb *et al.,* 2003) Due to its ingredients mayonnaise regarded as source of many bacterial contaminants especially Salmonella spp. The survival of Salmonella spp. and Staph. aureus in mayonnaise is influenced by the pH of the mayonnaise and the choice of the acidulant used in preparation (Radford and Board, 1993).

Salmonella species although associated with eggs they were not isolated from any samples of commercial mayonnaise in this study**.** In the absence of heat treatment, and using much vinegar represent the main safety factor as it contributes in decreasing pH of the products especially most of mayonnaise samples were prepared from whole eggs which contain the alkaline egg white. Adding garlic (Allium sativum) also can lower bacterial content (Ross *et al.,* 2001). Lemon due to citric acid can play a good role in minimizing Salmonella species in commercial mayonnaise products as suggested by Xiong *et al.* (2002). In addition, type of vegetable oil used could also affect survival of Salmonella species in mayonnaise as shown by Lock and Board (1996). Howard *et al.,* 2012 said that the chances of outbreaks to occur are even greater, when high risk ingredients, such as raw eggs, are used to prepare a food product. Although improper storage seems to be an important risk factor for the occurrence of salmonellosis outbreaks linked to homemade mayonnaise, it should be highlighted that low cell concentrations of Salmonella can still cause infection (Gog *et al.,* 2012).

The recorded results of the genus Listeria Table 3 & Fig. 2 declared that Listeria spp. could not be detected in the examined commercial mayonnaise and *L. monocytogenes* isolated from 7.5% of the examined small producers mayonnaise. The presence of *L. monocytogenes* in small producers mayonnaise indicated that they could be potential sources of listeriosis in humans because these types of foods are commonly eaten raw. There is a need for a more strict control measures in food hygiene and processing of food. The prevalence of Listeria species in small producers mayonnaise may be linked to the presence of the Listeria genus in natural environment, soil and surface water (Nightingale *et al.,* 2004). The genus Listeria contains 10 species, *L. monocytogenes, L, ivanovii, L. seeligeri, L. innocua, L. welshimeri, L. grayi, L. marthii, L. rocourtiae, L. fleischmannii and L. weihenstephanensis* (Zhang *et al.,* 2007 and Halter *et al.,* 2012), among these species only *L. monocytogenes and L. ivanovii* are pathogenic (Liu, 2006). *L. monocytogenes* is an intracellular foodborne pathogen that causes listeriosis and severe infections in humans with high mortality rate, mainly in high risk groups including pregnant women, elderly people, babies, HIV and cancer patients.

The current results illustrated in Table 3 & Fig. 3 showed that *Staph. aureus* isolates were recovered from 5% (2/40) of commercial mayonnaise samples and 17.5% (7/40) of small producers mayonnaise samples. Similar results (17.4%) were registered (Tayfur *et al.,* 2013) by PCR by using specific primer for (*Staph aureus clfA*), confirmed the presence of coagulase positive Staph aureus DNA. Staph. aureus is the principle cause of food poisoning and clinical infections in humans and animals (Chiang *et al.,* 2006). Coagulase test was considered a primary test in identification of Staphylococcal spp. but diagnosis of these species needs many biochemical tests to guarantee a consistent results. (Quinn *et al.,* 2002b) also coagulase positive Staphylococci have the same phenotypic characters so coagulase test not considered a single species-specific biochemical test (Sasaki *et al.,* 2010). Staphylococci species isolated are a result of excessive handling during preparation of small scale producers mayonnaise and indicate the poor personal hygiene of food handlers. *Staph. aureus* is one of the major bacterial agents causing food borne diseases in human worldwide (EFSA, 2010). It is an opportunistic pathogen, which associated with food poisoning and food spoilage (Argudin *et al.,* 2010). The storage at abusive temperature and inappropriate time periods, failure in hygienic practices and cross-contamination/ recontamination appear as the major risk factors for occurrence of foodborne disease outbreaks (Todd *et al.,* 2007).

PCR play a confirmative role in detection of Salmonella spp., *L. monocytogenes* and *Staph. aureus* and focus a light on presence of pathogenic types of these isolates in small producers and commercial mayonnaise which consider a public health problem.

Our research showed that small scale producers mayonnaise was contaminated with pathogenic bacteria and confirmed by using PCR technique. This bacterial contamination of mayonnaise may come from the ingredients used in making mayonnaise like yoghurt, carrot, pepper, water, eggs, potato, and breadcrumbs. The eggs could be contaminated from the infected hens or their shells became contaminated from faecal matters from the hen, the lining of the nest or by washing water. Food handlers or food processor during cleaning and breaking of the egg shells, peeling of potatoes or garlic, carrot, or during mixing of yoghurt, vegetable oil , ketchup could be a major source of mayonnaise contamination especially in the recognized bad handling habits and low personal hygiene and sanitation conditions observed in food establishments from where samples were taken. Knifes, mixing spoons, food utensils and surfaces which were used for holding different foods e.g meat and chicken could also act as sources of cross contamination as observed during mayonnaise preparation in dusty crowded environments.

CONCLUSION

Control of the safety and spoilage of small producers mayonnaise depends on these steps: There should be usage of cooked, blanched, or pasteurized animal products; vegetables should be washed and ensured that it is clean; usage of organic acids (acetic acid) to help keep pH low, strict hygiene in preparation, mixing, and storing small producers mayonnaise production under proper refrigeration and good hygienic practices represent negligible microbiological health hazards risks to consumers. The results of the present study clearly indicated that microbial quality and safety of small producers mayonnaise was unsatisfactory. The presences of organisms not only indicate poor hygiene but also itself may be pathogenic. The pathogenic bacteria such as Salmonella, listeria and staph. aureus may pass to the mayonnaise which suggests that mayonnaise should be considered as a vehicle for the transmission of potentially pathogenic bacteria.

RECOMMENDATION

1- Raw eggs used in homemade mayonnaise must be forbidden in the food control legislation, because the shells of eggs from the farms are contaminated by faecal matter from the hens and by the lining of the nest.
2- Hygienic situation of ready - to – eat - food establishment should be improved by food handler training and health education and enforcement of food safety laws, by ministry of health and public health inspectors in the localities.
3- The food control health inspector must insure the certain signs of good hygienic standards, such as clean toilets, clean cutlery or crockery, clean walls and floors, clean uniforms and fingernails, short or covered hair, valid medical card.
4- Wash hand basins and soap must be available in the room of food processing to help food handler to clean their hands.
5- Good sanitation in Cafeterias must be enforced by the public health inspector of the locality, because insects are vectors and transmitter of microorganisms.
6- Vegetables and spices must be cleaned with clean water.
7- Eggs used in homemade mayonnaise must be pasteurized as mentioned in the literature review.

REFERENCES

Abeer, A.A. (1997): Organoleptic inspection and microbiological quality of different types of fermented milk. M.V.Sc. thesis, Fac. Vet. Med., Cairo Univ.

Argudin, M.A.; Mendoza, M.C. and Rodicio, M.R. (2010): Food Poisoning and Staphylococcus aureus Enterotoxins, Toxins 2 (7): 1751-1773.

Bennett, R.W. and Lancette, G.A. (2001): Staphylococcus aureus. In: FDA Bacteriological 1 Manual. 8th Ed. Gaither sburg, MD, AOAC International: 12.

Brabant, L.E. (1992): Please squeeze the dressings: new sales opportunities in Japan. AgExporter, 4: 12-13.

Capalonga, R.; Ramos, R.C.; Both, J.M.; Soeiro, M.L.; Longaray, S.M.; Haas, S. and Tondo, E.C. (2014): Salmonella serotypes, resistance patterns, and food vehicles of salmonellosis in southern Brazil between 2007 and 2012. J Infect Dev Ctries 8(7):811–817.

Carpentier, B. and Cerf, O. (2011): Review - persistence of Listeria monocytogenes in food industry equipment and premises. Int. J. Food Microbiol. 145, 1–8.

Cheng Hsin-Yi, Yu Roch-Chui and Chou Cheng-Chun (2003): Increased acid tolerance of Esherichia coli O157:H7 as affected by acid adaptation time and conditions of acid challenge, Food Res Int. 36:49-56.

Chiang, C.Y.; Fan, M.C.; Liao, W.W.; Kulin, C. and Tsenh, Y. (2006): Real-Time PCR Detection of Staphylococcus aureus in milk and meat using new primers designed from the heat shock protein gene htr Asequence: J. of Food Protection, 70(12):2855–2859.

Depree, J.A. and Savage, G.P. (2001): Physical and flavor stability of mayonnaise. Trends Food Sci. Tech., 12:157-163.

Effimia, E. (2015): Prevalence of Listeria monocytogenes and Salmonella spp. in Ready-to-Eat Foods in Kefalonia, Greece. J Bacteriol Parasitol**.,** 6(5): 6: 243.

EFSA (European Food Safety Authority) (2010): The community summary report on trends and sources of zoonoses, zoonotic agents and food borne outbreaks in the European Union in 2008. EFSA, 8(1): 1496**.**

Elias, S.O.; Alvarenga, V.O.; Longhi, D.A.; Sant'Ana, A.D.S. and Tondo, E.C. (2016): Modeling growth kinetic parameters of Salmonella enteritidis SE86 on homemade mayonnaise under isothermal and nonisothermal conditions. Foodborne Pathog Dis 13(8): 462–467.

Erickson, J.P. and Jenkins, P. (1991): Comparative Salmonella Spp. and Listeria monocytogenes inactivation Rates in four commercial mayonnaise products. J. Food Prot. 54(12): 913-916.

European Food Safety Authority (2015): The European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2014. EFSA J. 13, 3991.

Fashae, K.; Ogunsola, F.; Aarestrup, F.M. and Hendriksen, R.S. (2010): Antimicrobial susceptibility and serovars of Salmonella from chickens and humans in Ibadan, Nigeria. J. Inf. Dev. Count; 4: 484-94.

Ferreira, V.; Wiedmann, M.; Teixeira, P. and Stasiewicz, MJ. (2014): Listeria monocytogenes persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. J. Food Prot., 77(1):150–70. doi:10.4315/0362-028X.JFP-13-150.

Fialova, J.; Chumchalova, J.; Mikova, K. and Hrusova, I. (2008): Effect of food preservatives on the growth of spoilage lactobacilli isolated from mayonnaise-based sauces. Food Cont. 19:706-713.

Forsythe, S.J. (2010): Microbiological criteria. In: The Microbiology of Safe Food, Chapter 6, pp. 266-288, Wiley-Blackwell Publication, Oxford.

George, A.E. and Levett, P.N. (1990): Effect of temperature and pH on survival of Listeria monocytogenes in coleslaw. Int J Food Mic. 11: 345-350.

Gog, J.R.; Murcia, A.; Osterman, N.; Restif, O.; McKinley, T.J.; Sheppard, M. and Bryant, C.E. (2012): Dynamics of Salmonella infection of macrophages at the single cell level. Journal of the Royal Society, Interface (Published online).

Gomez-Lucia, E.; Goyache, J.; Orden, J.A.; Domenech, A.; Hernandez, H.F.; Ruiz-Santa Quitera, J.A. and Suarez, G. (1990): Influence of temperature of incubation on Staphyylococcus aureus growth and enterotoxin production in homemade mayonnaise. J Food Prot. 53, 386-390.

Gomez-Lucia, E.; Goyache, J.; Blanco, J.F.B.; Garayzabal, F.; Orden, J.A. and Suarez, G. (1987): Growth of Staphylococcus aureus and enterotoxin production in homemade mayonnaise prepared with different pH values. J. Food Prot. 50, 872-875.

Halter, E.L.; Neuhaus, K. and Scherer, S. (2012): Listeria weihenstephanensis sp. nov., isolated from the water plant Lemna trisulca of a German fresh water pond. Int. J. Syst. Evol. Microbiol. DOI: 10.1099/ijs.0.036830-0.

Harrison L. J. and Cunningham F. E. (1985): Factors influencing the quality of mayonnaise. J. of Food Qual., (8): 1-20.

Hendriksen, R.S.; Vieira, A.R.; Karlsmose, S.; Lo Fo Wong, D.M.; Jensen, A.B. and Wegener, H.C. (2011): Global monitoring of Salmonella serovar distribution from the world health organization global food borne infections network country data bank: results of quality assured laboratories from 2001 to 2007. Food Borne Path. Dis.;8(8):1-14.

HGIC (Home and Garden Information Center). (2000): Foodborne illness: Prevention Strategies http://www.hgic. clemson. edu.

Howard, Z.R.; O'Bryan, C.A.; Crandall, P.G. and Ricke, S.C. (2012): Salmonella in foods: evolution, strategies and challenges Salmonella Enteritidis in shell eggs: Current issues and prospects for control. Food Research International, 45(2), 755–764.

Hwang Cheng-An and Tamplin, M.L. (2005): The ınfluence of mayonnaise pH and storage on the growth of Listeria monocytogenes in seafood salad. Int. J. Food Microbiol. 102: 277-285.

ICMSF (International Commission on Microbiological Specification) (2006): Microorganism in Foods, Microbial ecology of food commodities. 2nd ed. Kluwer Academics, Plenum Publishers, UK.

ISO (2003b) ISO standard DIS 6888:2003(E) Horizontal method for the enumeration of Coagulase Positive Staphylococci (Staphylococcus aureus and other species).

ISO 11290-1 International Organization for Standarization (2017): Microbiology of the food chain – Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. – Part 1: Detection method. International Standard ISO 11290-1. Geneva, Switzerland: International Organization for Standarization.

Kauffman, G. (1974): Kauffman white scheme. J. Acta. Path. Microbiol. Sci., 61: 385.

Keerthirathne, T.P.; Ross, K.; Fallowfeld, H. and Whiley, H. (2016): A review of temperature, pH, and other factors that infuence the survival of Salmonella in mayonnaise and other raw egg products. Pathogens 5(4):63.

Kumar, A.; Grover, S. and Batish, V.K. (2015): Exploring specific primers targeted against different genes for a multiplex PCR for detection of Listeria monocytogenes. 3 Biotech (2015) 5:261–269.

Lado, B.H. and Yousef, A.E. (2007): Characteristics of Listeria monocytogenes important to food processes. In: Ryser, E.T., Marth, E.M. (Eds.), Listeria, Listeriosis and Food Safety. CRC Press, pp. 157– 213.

Liu D. (2006): Identification, subtyping and virulence determination of Listeria monocytogenes, an important foodborne pathogen. J. Med. Microbiol. 55:645-659.

Lock, J.L. and Board, R.G. (1996): The influence of acidulants and oils on autosterilization of homemade mayonnaise. Food Research International 28(6): 569-572.

Mason, W.J.; Blevins, J.S.; Beenken, K.; Wibowo, N.; Ojha, N. and Smeltzer, M.S. (2001): Multiplex PCR Protocol for the Diagnosis of Staphylococcal Infection. J. Clin. Microbiol., 39, (9): 3332–3338.

Michels, M.J. and Koning, W. (2000): Mayonnaise Dressing, Mustard, Mayonnaise Based salads, and Acid Sauces. In; The microbiological safety and quality of Food. Editors; BM Lund, TC Baird-Parker, GW Gould, Volume I, Chapter 30, pp 807-835, Aspen Publishers, Inc., Gaithersburg, Maryland.

*Milillo, S.R.; Friedly, E.C.; Saldivar, J.C.; Muthaiyan, A.; O'Bryan, C. and Crandall, P.G. (2012):*A review of the ecology, genomics, and stress response of Listeria innocua and Listeria monocytogenes. Crit Rev Food Sci Nutr., 52(8): 712–25.

Nightingale, K.K.; Schukken, Y.H.; Nightingale, C.R.; Fortes, E.D.; Ho, A.J.; Her, Z.; Grohn, Y.T.; McDonough, P.L. and Wiedmann, M. (2004): Ecology and Transmission of Listeria monocytogenes Infecting Ruminants and in the Farm Environment. Appl. Environ. Microbiol. 70:4458-4467.

Olivera, S.D.; Rodenbusch, C.R.; Ce, M.C.; Rocha, S.L.S. and Canal, C.W. (2003): Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* de-tection. Lett. Appl. Microbiol., 36: 217-221.

Palmer, H.H.; Ijichi, K.; Cimino, S.L. and Roff, H. (1969): Emulsifying characterization of hen's egg yolk proteins in oil-in-water. Food Technology 23, 148-156. C.F.Y. Mine.).

Popoff, M.Y.; Bockemuhl, J. and Gheesling, L.L. (2004): Supplement 2002 (no. 46) to the Kauffmann-White scheme. Res. Microbiol. 155: 568-70.

Quinn, P.J.; Markey, B.K.; Cater, M.E.; Donnelly, W.J.C. and Leonard, F.C. (2002a): "Veterinary Microbiology and Microbial Diseases" 1st ed. Blackwell Science, Inc., Oxford.Pp:106-107.

Quinn, P.J.; Cater, M.E.; Markey, B.K. and Cater, G.R. (2002b): Clinical veterinary microbiological Mosby- Year Book Europe Limited. Staphylococcus species. 118-127.

Radford, S.A. and Board, R.G. (1993): Review: Fate of pathogens in homemade mayonnaise and related products. Food Microbiology 10: 269-278.

Ross, Z.M.; O'Gara, E.A.; Hill, D.J.; Sleightholme, H.V. and Maslin, D.J. (2001): Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl. Environ. Microbiol. 67(1):475-80.

Sasaki, T.; Tsubakishita, S.; Tanaka, Y.; Sakusabe, A.; Ohtsuk, M.; Hirotaki, S.; Kawakami, T.; Fukata, T. and Hiramatsu, K. (2010): Multiplex-PCR method for species identification of coagulase-positive

staphylococci: J. Clin. Microbiol. 48, 765-769.

Silk, B.J.; Date, K.A.; Jackson, K.A.; Pouillot, R.; Holt, K.G. and Graves, L.M. (2012): Invasive listeriosis in the Foodborne Diseases Active Surveillance Network (FoodNet), 2004-2009: further targeted prevention needed for higher-risk groups. Clin. Infect. Dis., 54(Suppl 5): S396–404. doi:10.1093/cid/cis268

Stewart, C.M.; Cole, M.B.; Legan, J.D.; Slade, L.; Vandeven, M.H. and Schaffner, D.W. (2002): Staphylococcus aureus growth boundaries: moving towards mechanistic predictive models based on solute- specific effects. Appl. Environ. Microbiol., 68(4):1864- 1871.

Swaminathan, B. and Gerner-Smidt, P. (2007): The epidemiology of human listeriosis. Microbes Infect. 9, 1236 –1243.

Tayfur, M.; Cakır, S.; Orkun, T.; Ercan, A. and Yabancı, N. (2013): Microbial quality of retail mayonnaise-base salads**.** Afr. J. Microbiol. Res.,7 (20), 2269-2273.

Todd, E.C.; Greig, J.D.; Bartleson, C.A. and Michaels, B.S. (2007): Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 3. Factors contributing to outbreaks and description of outbreak categories. Journal of Food Protection, 70(9), 2199–2217.

Unicomb, L.; Bird, P. and Dalton, C. (2003): Outbreak of Salmonella Potsdam associated with salad dressing at a restaurant. Communicable Diseases Intelligence, 27, (4): http://www.health.gov.au/ internet/ main/publishing.nsf/content/cda-pubscdi-2003-cdi2704-htm-cdi2704l.htm

Warriner, K. and Namvar, A. (2009): What is the hysteria with Listeria? Trends Food Sci. Technol. 20, 245–254.

Weistein, J. (1991): The clean restaurant II: Employee hygiene. Restaurants inst. 15(13): 138-144.

Xiong, R.; Xie, G.; Edmondson, A.S. and Meullenet, J.F. (2002): Neural network modelling of the fate of Salmonella enterica serovar Enteritidis PT4 in home-made mayonnaise prepared with citric acid. Food Cont.13: 525-533.

Xiong, R.; Xie, G. and Edmonson, A.S. (2000): Modelling the pH of mayonnaise by the ratio of egg to vinegar. Food Control 11(1): 49-56.

Zhang, Y.; Yeh, E.; Hall, G.; Cripe, J.; Bhagwat, A.A. and Meng, J. (2007): Characterization of Listeria monocytogenes isolated from retail foods. Int. J. Food Microbiol. 113: 47-53.

التوصيف الجزيئي لبعض البكتريا الممرضة المعزولة من المايونيز

مروة محمد نبيل الجندي ، منال محمد امين

E-mail: ahmednofel125@yahoo.com Assiut University web-site: www.aun.edu.eg

اجريت هذه الدراسة علي ٨٠ عينة من المايونيز وتشتمل على ٤٠ عينة من كل من المايونيز التجاري وصغار المنتجين تم تجميعها من السوبر ماركت والمطاعم بمحافظة اسيوط. واشتملت الدراسة علي فحص هذه العينات لتواجد ميكروب السالمونيلا، الليستريا مونوسيتوجينز والمكور العنقودي الذهبي حيث تم عزل بكتيريا السالمونيلا والليستيريا مونوسيتوجينز والمكور العنقودي الذهبي من مايونيز صغار المنتجين حيث كانت النسب كالتالي 7.5، 7.5 و17.5 % على التوالي ولم يتم عزل اي من السالمونيلا اوالليستيريا من عينات المايونيز التجاري. وكانت نسبة عزل المكور العنقودي الذهبي من المايونيز التجاري 5%. كما تم التوصيف الجزيئي للميكروبات الممرضة المعزولة من انواع المختلفة للمايونيز باستخدام تفاعل البلمرة المتسلسل. وأوصت الدراسة بضرورة تطبيق الاشتراطات الصحية الجيدة أثناء عمليات اعداد وتجهيز مايونيز صغار المنتجين لتقليل تلوثها بميكروب السالمونيلا والليستيريا والمكور العنقودي الذهبي.