Comparative prevalence of pathogenic and spoilage microbes in chicken sausages from Egypt and Greece

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ABSTRACT

This study investigated the spread of food-borne pathogens: *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Salmonella* in chicken sausage samples collected from retail markets in Greece and Egypt during 2006 and from Egypt through 2010. Other microbiological parameters; total viable count (TVC), lactic acid bacteria (LAB), pseudomonads (PS), staphylococci (STAPH), *Brochothrix thermosphacta* (BT), *Enterobacteriaceae* (EN), *Escherichia coli* (EC), yeasts and moulds (Y&M) were also counted. Egyptian chicken sausage samples were found to harbor *L. monocytogenes*, *Staph. aureus* and *E. coli* O157:H7; with frequencies equivalent to 24%, 60% and 26% of the total samples during 2006 and 37.87%, 64.44% and 41.11% of the total samples during 2010, respectively, while Greek samples were entirely free of theses pathogens. Enrichment techniques indicated the absence of *Salmonella* from both Greek and Egyptian samples. The obtained results may mobilize food producers and handlers in developing countries to take the due measures reducing food-borne pathogen risks and spoilage flora alongside the poultry chain.

Keywords: Chicken Sausage; Food-Borne Pathogens; Contamination; *Listeria*

1. INTRODUCTION

Food safety is of utmost importance all over the world. During food processing, a major risk of contamination may occur directly prior to filling or packaging. So, controlling the microbial load of such products is very crucial in ensuring a safe quality end-product. The European Commission regulation on the hygiene of foodstuffs [1] provides a risk-based approach to ensure food hygiene through the implementation of HACCP (hazard analysis and critical control point) procedures. The described measures represent the prerequisite conditions required in food manufacturing facilities [2,3]. Risk management programs including HACCP system is not yet applied in most food processing units and many kinds of traditional foods are still manufactured under somewhat modestly controlled environments especially in the developing countries including Egypt.

Listeria monocytogenes is a food-borne pathogen that can annually cause 2500 cases of meningitis encephalitis, sepsis, fetal death, abortions and about 500 deaths in the United States [4]. Between 1994 and 2002, an enormous amount of chicken and turkey products were recalled because of possible *L. monocytogenes* contamination in United State [5]. Relatively high prevalence of *L. monocytogenes* in contaminated poultry products has been also reported in Belgium [6], Norway [7] and Northern Ireland [8]. Additionally, a number of poultry products and commercially manufactured foods associated with sporadic cases of listeriosis have been reported [9-12]. *Escherichia coli* O157:H7 is associated with retail meat products [13,14] and there was a relatively high prevalence of *E. coli* contaminated poultry products in Slovakia [15]. A more general overview of factors contributing to meat-borne disease outbreaks in England and Wales [16] indicated that inappropriate storage was implicated in 32% cases, inadequate heat treatment in 26% cases and cross-contamination (most commonly, raw-to-cooked) in 25% cases. Cross-contamination during cutting, slicing and packaging of meat products leads to an increase of total viable microorganisms and reduced shelf life [17].

Spoilage of processed meat products prior to the selling date can limit its distribution options inflicting con-

siderable economic hardships on the manufacturers [18, 19]. At the retail level, meat products are liable to cross-contamination through further handling including slicing into individual parts (e.g. ham, sausages, and pâtés) and packaging. Epidemiological data from Europe, North America, Australia, and New Zealand [20] indicate that substantial proportions of food-borne diseases and spoilage microorganisms can also occur during food preparation practices used in the domestic environment. Several types of psychrotrophic bacteria including *Brochothrix thermosphacta*, lactic acid bacteria (LAB) and *Pseudomonas* spp. may grow to levels causing meat spoilage [21].

The aim of the current study was to follow the magnitude of contaminating microflora in the final poultry products collected from retail market of a developing country (Egypt) as compared to a developed one (Greece) to evaluate their liability to contamination by pathogenic and spoilage bacteria in relation to the hygienic status in each case in order to assure or improve the hygienic measures in each case and reach appropriate scientific recommendations. This may mobilize the concerned parties to take the appropriate actions achieving better hygienic measures.

2. MATERIALS AND METHODS

2.1. Sampling Procedure

Fifty samples of chicken sausages produced by a Greek food manufacturing company were purchased from the retail markets in Athens, Greece one month after their production and one month before their expiry date during 2006. A similar set of samples produced by an Egyptian food manufacturing company was purchased from the retail markets at Cairo Egypt with the same specifications during the years 2006. Other 90 chicken sausage samples produced by three different Egyptian food manufacturing companies were purchased from the retail markets at Cairo, Egypt one month after their production and one month before their expiry date during the year 2010. All samples were transported to the laboratory immediately after collection and stored at 0˚C until analysis.

2.2. Bacterial Strains

Reference strains of *L. monocytogenes* Scott A, *L. innocua* FMCC141 and *Staph. aureus* ATCC6538 were used in this study. All stock cultures were maintained at −80˚C. Each strain was aseptically sub-cultured in Tryptic Soy broth (TSB) and checked for purity onto Tryptic Soy agar plates (TSA), incubated for 24 h at 37˚C.

2.3. Direct Microbiological Counting

An aliquot (25 g) of each sample was transferred asep-

tically to a stomacher bag, combined with 225 ml of sterile Ringer's solution (Lab 100 Z) and homogenized for 60 s in a stomacher at room temperature (Lab. Blender 400; Seward Medical, London, UK). The samples were decimally diluted in Ringer's solution and 0.1 ml from duplicate samples of appropriate dilutions were spread onto the surface of solid media. Determinations were carried out as follows: total viable counts (TVC) on Plate Count agar (PCA, Merck, 1.05463) incubated at 30˚C for 72 h; yeasts and moulds on Rose Bengal Chloramphenicol Agar (Lab M 36, supplemented with chloramphenicol, X009) incubated at 25˚C for 5 days; pseudomonads on Pseudomonas agar Base (Lab M, supplemented with Cetrimide-Fucidin-Cephaloridene, X109) incubated at 25˚C for 48 h; *B. thermosphacta* on Streptomycin Thallous Acetate Actidione agar (Oxoid M0881, supplemented with streptomycin sulphate, thallous acetate and cycloheximide SR0151) incubated at 30˚C for 72 h; staphylococci on Baird Parker agar (Biolife, 401116 supplemented with egg yolk) incubated at 37˚C for 48 h. *E. coli* on Harlequin Tryptone Bile X-Glucuronide agar (TBX) (LAB HAL003) incubated at 44˚C for 24 h. Particularly, *E. coli* O157:H7 on TBX agar incubated at 37˚C for 4 h then incubated at 44˚C for 37 h. *Listeria* spp. was enumerated on Polymyxin-Acriflavin-Lithium Chloride-Ceftazidime-Aesculin-Mannitol agar (PALCAM, Biolife 401604) after incubation for 48 h at 35˚C. *Salmonella* was counted on Xylose Lysine Deoxycholate agar (XLD) (Merck, 1.05287) after incubation for 24 h at 37˚C. For Lactic acid bacteria (LAB) and Enterobacteriaceae enumerations, 1 ml sample was inoculated into 10 ml of molten de Man Rogosa Sharpe agar (MRS, Biolife 401728) and Violet Red Bile Dextrose Agar (Biolife 402188). After setting, a 10 ml overlay of the same molten medium was added. The incubation was carried out at 25˚C for 72 h and at 37˚C for 24 h, respectively. All plates were examined for typical colony types and morphological characteristics associated to each culture medium. *S. aureus* colonies were further tested for positive coagulase reaction (Bactident Coagulase Biolife).

2.4. Post Enrichment Pathogenic Detection

A two-stage enrichment procedure was used for the detection and isolation of *Listeria* spp. according to ISO 11290 [22]. An amount of each sample (25 g) was added to Half Fraser broth (225 ml) and incubated at 30˚C for 24 h. Then an aliquot (0.1 ml) of the primary enrichment media was transferred to a tube containing 10 ml of a secondary enrichment medium (Frazer broth) and incubated for 48 h at 35˚C - 37˚C. Aliquots from both media were inoculated into PALCAM dishes and incubated at 35˚C for 48 h. Presumptive colonies were subcultured on tryptone soya agar (Lab. M) and incubated for 24 h at 35˚C. Simplified genus identification was conducted

using Gram stain, catalase and oxidase reactions, motility at 25˚C, *β*-haemolysis reaction, and biochemical identification by fermentation of D-xylose, L-rhamnose, *α*-methyl-D-mannoside and D-mannitol [23]. To identify several fastidious strains, API-*Listeria* Kit was used. For *E. coli* O157:H7, the samples were analyzed using the method described in ISO 16654 [24]. Aliquots of the samples (25 g) were added to 225 ml volumes of modified tryptone soy broth with novobiocin (Merck) and incubated at 41.5˚C for 18 - 24 h. Following incubation, 0.1 ml of each enrichment sample was transferred onto Tryptone Bile Glucuronide (TBX) agar and incubated at 37˚C for 4 h, then subsequently at 44˚C for 20 h.

The detection of *Salmonella* spp. was achieved by ISO 6579 [25] by suspending 25 g of the product into 225 ml buffered peptone water (BPW) (Merck), and incubating at 37˚C for 20 h. Then, 0.1 ml of each BPW medium was transferred into a culture tube containing 10 ml of Rappaport Vassiliadis (RV) enrichment broth and incubated again at 42˚C for 24 h. From both steps, a portion of the sample was streaked on XLD agar (Merck, 1.05287) and incubated at 37˚C for 24 h.

2.5. Statistical Analyses

Data from microbiological analyses were entered into Excel and transformed to $\log_{10}$ values. All presented values are the averages of three replicates plus the standard deviation.

3. RESULTS AND DISCUSSIONS

3.1. The Overall Distribution of Microbial Group

The data in **Figure 1** delineate the overall distribution

of different microbial groups in 100 chicken sausages samples collected from the retail markets in each of Egypt and Greece one month after the date of their production during the year 2006. The number of samples positive to any microbial was related to the total tested samples giving the relative overall distribution. The total viable count (TVC) and lactic acid bacteria (LAB) were counted and detected in 100% of Egyptian samples while in only 68% and 62% of Greek samples, respectively. All other tested microbial groups were detected by direct counting or enrichment techniques in Egyptian samples where the most spread microbial groups were *B. thermosphacta* (BT), *Enterobacteriaceae* (EN), staphylococci (STAPH), yeasts & moulds (Y&M) and pseudomonads (PS) being distributed in 100, 96, 92, 82 and 82% of the tested samples, respectively. Greece samples were only positive for few microbial groups, *i.e.* BT, Y&M and PS accounting for 20%, 12% and 6% relative distribution in the tested samples, respectively. Other groups were totally absent by enrichment techniques in Greek samples. Enrichment technique did not reveal any *Salmonella* in either Egyptian or Greek samples. In Brazil, *Salmonella* was not isolated from chicken sausage [26]. Therefore, the plant environment, packaging and chill storage may be unfavorable to its persistence and growth. This finding is similar to the results of Yılmaz *et al*. [27] on Turkey chicken sausage showing no recovery of *E. coli*, *Staph. aureus* and *Salmonella* spp. The presence of *L. monocytogenes* and *Salmonella* spp. were not detected in any of the ready to eat meat in Greece [28].

3.2. Relative Distribution of Spoilage Microbes

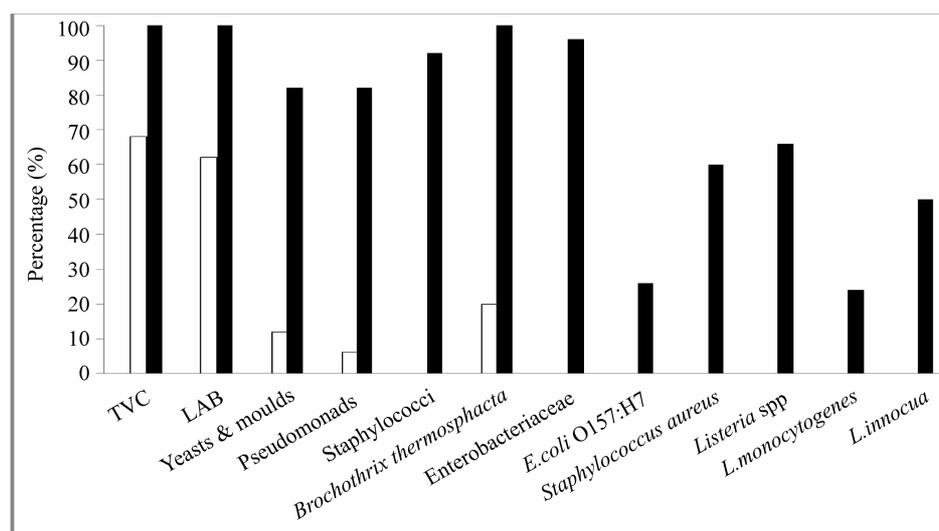**Figure 2** shows the relative distribution of TVC, LAB,

Figure 1. Relative quantitative distribution of spoilage microbes in chicken sausage collected from Egypt (black columns) and Greece (white columns) during the year (2006).
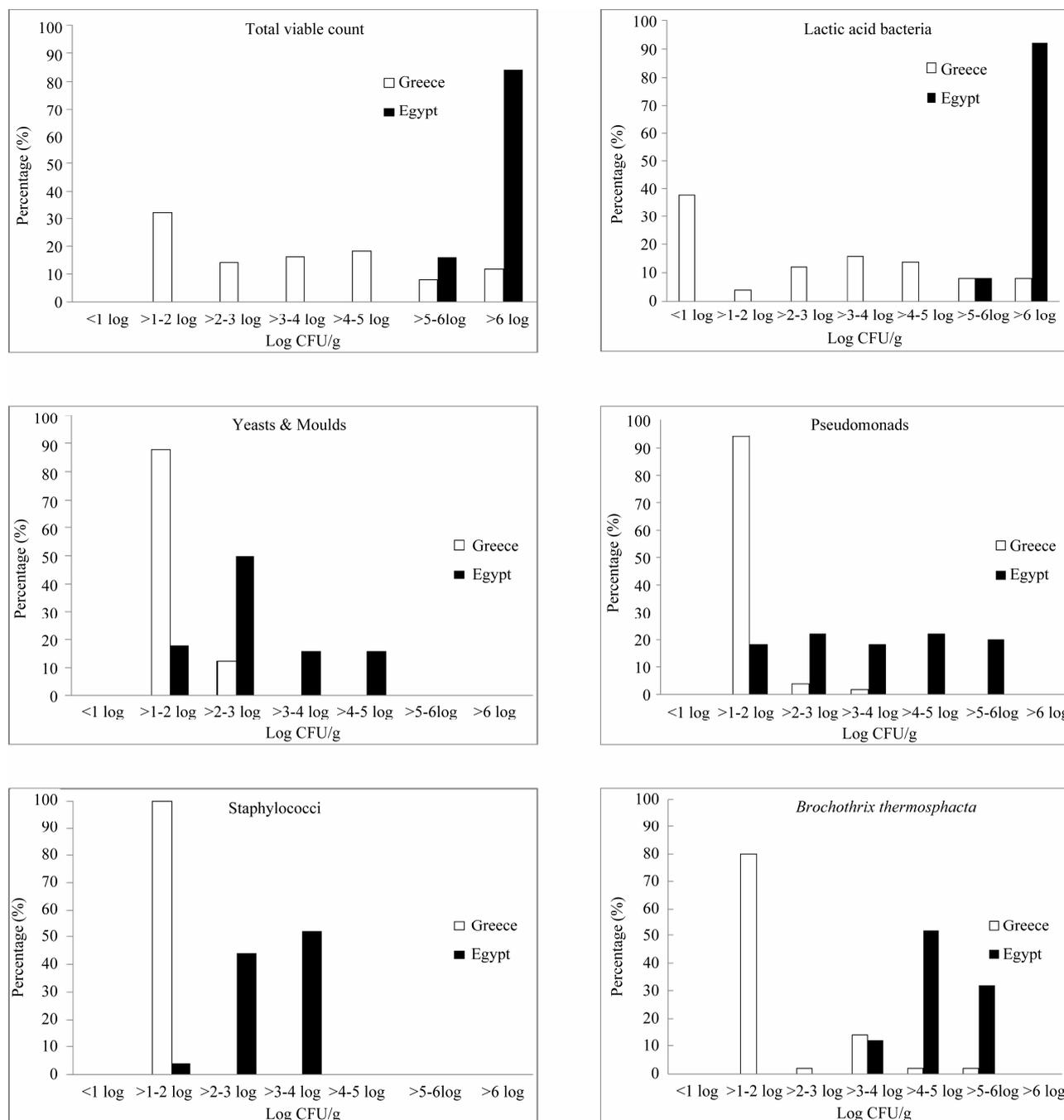
Figure 2. Relative quantitative distribution of indicator and pathogenic bacteria in chicken sausage from Egypt and Greece during the year 2006.

Y&M, PS BT and STAPH in chicken sausage samples collected from Greece and Egypt. Sausage samples were categorized into 7 levels according to the microbial load; <1, >1 - 2, >2 - 3, >3 - 4, >4 - 5, >5 - 6, >6 log CFU/g. The relative distribution of any microbe was calculated by dividing the number of the samples within certain level by the number of the total samples and multiplying by 100. Egyptian samples were most distributed in the high TVC load category (>6 log CFU/g) recording 84%

of the total. The other 16% were distributed in the second highest level of bacterial load (>5 - 6 log CFU/g). On the other hand the TVC in Greek samples followed nearly a normal distribution pattern, except that the highest distribution (32%) was recorded in the lowest bacterial load level (<2 log CFU/g). The same trend of quantitative distribution in both Egyptian and Greek samples was also true for LAB except that the highest bacterial load level (>6 log CFU/g) was spread in about 92% of the

total Egyptian samples while the lowest bacterial load level in Greek samples was spread in about 38% of the total Greek samples. Since the level >6 log CFU/g is the maximal permissible level for the aerobic plate count in meat products [29] about 92% of Egyptian samples can be classified as unacceptable for human consumption. Previous studies on chicken products, such as chicken sausage from Egypt have reported that the total bacteria count ranged from 7 to 8 log CFU/g [30,31]. In Spain, chicken sausage had total bacterial counts of mesophiles and psychrotrophs from 7.14 to 7.28 and 7.72 to 7.87 log CFU/g, respectively and about 80% of the chicken sausage were regarded unacceptable [32]. In contrast, in USA, [33] found the aerobic plate count of chicken sausage was in the level 4.65 log CFU/g. Thus, the product's microbial load depends largely on the geographical location, the associated environmental and hygienic status. However, factors such as handling, processing, packaging, storage and display may influence the microbiological load of food products at the sale points [34].

Yeasts and moulds in Egyptian samples followed the normal distribution pattern, indicating the highest distribution at the medium level (>2 - 3 log CFU/g). Greek samples distribution deviated towards the lowest detection limit (<2 log CFU/g) which recording 88% distribution of the total. This trend was also applied for the quantitative distribution of pseudomonads group in the samples of the two countries. The distribution of staphylococci group in the Egyptian samples was centered in the medium level (>2 - 3 log and 3 - 4 log CFU/g) achieving 44% and 52% distribution of the total samples, respectively, while all Greek samples were under the detection limit. The distribution of BT was exclusively condensed between the medium and high limit (>3 - 6 log CFU/g) in Egyptian samples while it was located in the low detection level (80%) in all Greek samples. The pH values of chicken sausage were 6.25 - 6.38 and 6.33 - 6.87 in the samples from Egypt and Greece, respectively, *i.e.* the pH of Egyptian samples was relatively lower, compared to Greek samples.

3.3. Relative Distribution of Pathogenic Microbes

Figure 3 shows the relative distribution of EN and bacterial pathogens (*E. coli* O157:H7, *Staph. aureus*, *Listeria* spp., *L. monocytogenes* and *L. innocua*) in chicken sausage from Egypt and Greece. The relative quantitative distribution of EN in Egyptian samples followed nearly the normal distribution pattern while all Greece samples were under the detection level. The presence of EN in processed food may give a strong indication of inadequate treatment or environmental post-process contamination, and may also indicate the extent of faecal contamination. The distribution of the pathogenic bacteria (*E.*

coli O157:H7, *Staph. Aureus* and *Listeria* spp., *L. monocytogenes* and *L. innocua*) seems concentrated in the lowest two levels (>1 - 2 to >2 - 3 log CFU/g) in Egyptian samples while all Greek samples were totally under the detection limit (>1 - 2 log CFU/g). Nørrung *et al.* [35] recommended zero tolerance for *L. monocytogenes* in 25 g of ready to eat meat products when the shelf life is longer than a week, but when shorter, the tolerance level could be <100 CFU/g, so the level 2 - 3 logs CFU/g at sale points may represent a potential risk to health. Counts of this level may also indicate a significant failure of hygienic standards in the preparation and storage of sausage. Additional, problems due to the growth of pathogenic bacteria in sausages such as *L. monocytogenes* may cause serious food safety problems for consumers. All Greece samples were under the lowest pathogenic detection limit in contrast to 30% - 75% of Egyptian samples, which were in that level. That may imply that 25% - 70% of the Egyptian samples harbored pathogens at a medium bacterial load of >2 - 3 log CFU/g. The most common pathogens associated with meat products are *L. monocytogenes, E. coli* O157:H7, *Staph*. *aureus* and *Salmonella* [36,37]. The production process of cooked meats includes a heating step that is probably sufficient to eliminate any *L. monocytogenes* and other present pathogens, so its presence in the tested samples is most likely due to post-process contamination [38]. Additionally, the ubiquitous presence of *Listeria* spp. in the environment and poor handling can lead to their spread in food-processing environments and the food chain. Taking into account that storage conditions may allow for rapid growth of pathogen [39], its presence in processed meat products could pose serious health problems. Noack and Jockel [40] reported that between 1990 and 1992, official testing of 1235 samples of meat and meat products yielded *L. monocytogenes* in 3.7% of cooked sausage. *Listeria* spp. including *L. monocytogenes* was isolated from chicken meat products [12,41]. *L.monocytogenes* was detected in 8.8% cooked meat products in Northern Spain [39], 18% of retail chicken in Northern Ireland [8], 38% in Belgium [6] and 37% in Japan [42]. Moreover, *L. monocytogenes* was a contaminant in slaughterhouses and poultry processing plants [43]. Previous studies on chicken sausage in Spain [33] and chicken frankfurter sausage in Egypt [30] have reported incidences of *S. aureus* of 100% and 40%, respectively. The levels of this pathogen in chicken frankfurter sausage from Egypt are similar to those re-ported by [32] in chicken sausage, with average of 3.15 - 3.23 log CFU/g. The presence of *Staph. aureus* in meat products may arise from food handlers through packaging and storage of the product at high temperature [44-46]. The presence and the number of *E. coli* in food are important as indicators of cross-contamination [47]. It has been suggested that several factors may contribute to the
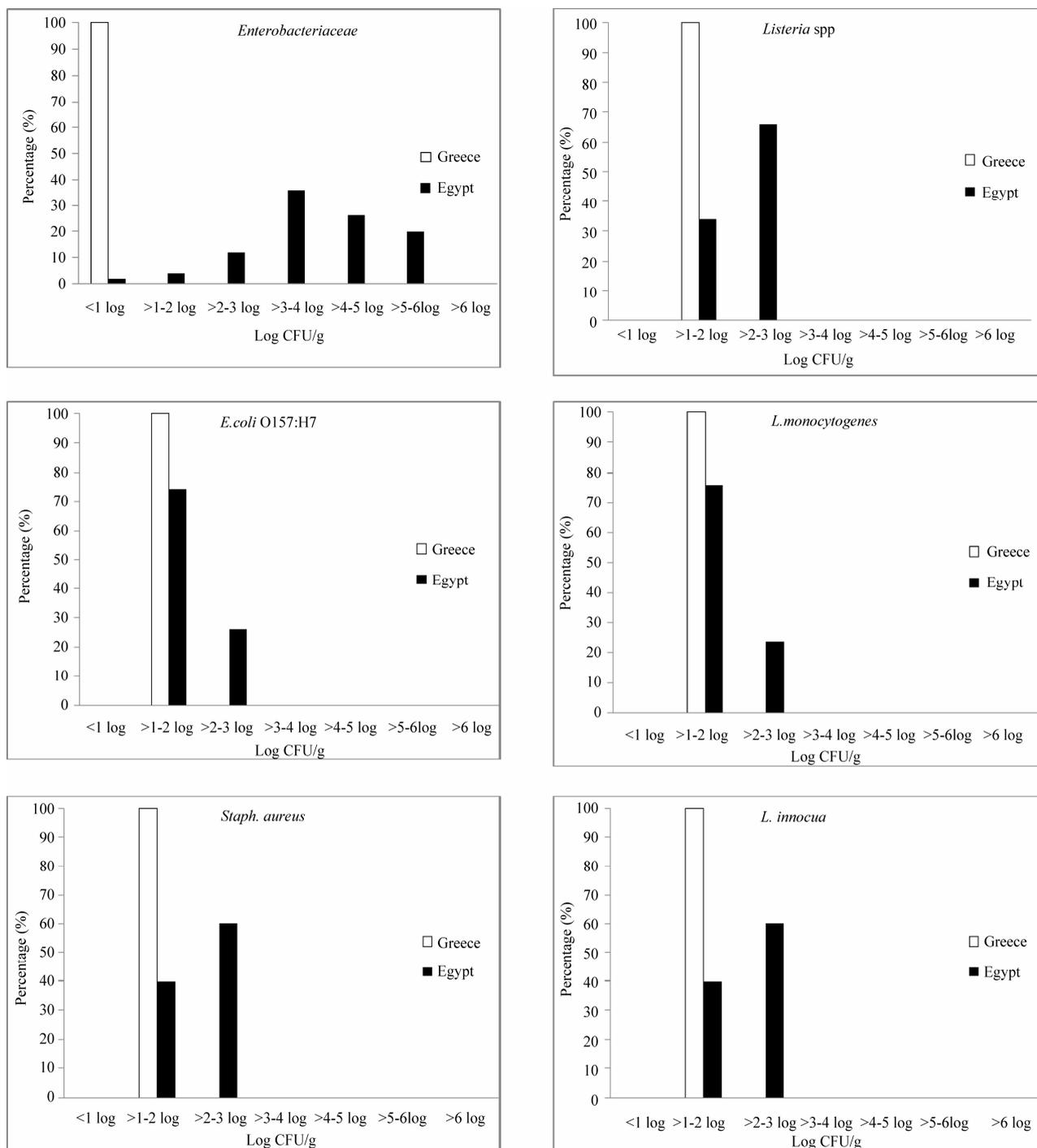
Figure 3. Relative total microbial distribution in chicken sausages from Egypt and Greece during the year 2006.

presence of these pathogens, including poor handling, poor hygiene practices, cross-contamination from food handlers and storage conditions [46]. Chicken sausage samples from Spain were reported to contain *E. coli* groups range from 3.48 to 4 log CFU/g [32]. Taulo *et al.* [48] found positive correlation (*p* < 0.05) between the presence of *E. coli* and *Staph. aureus* in food samples. Several authors found that *E. coli* O157:H7 was associ-

ated with derived retail meat products [13,14]. Of the 1750 ground beef samples analyzed, 20 (1.1%) of these were positive for *E. coli* O157 [49].

3.4. Absolute Microbial Counts of Spoilage and Pathogenic Microbes

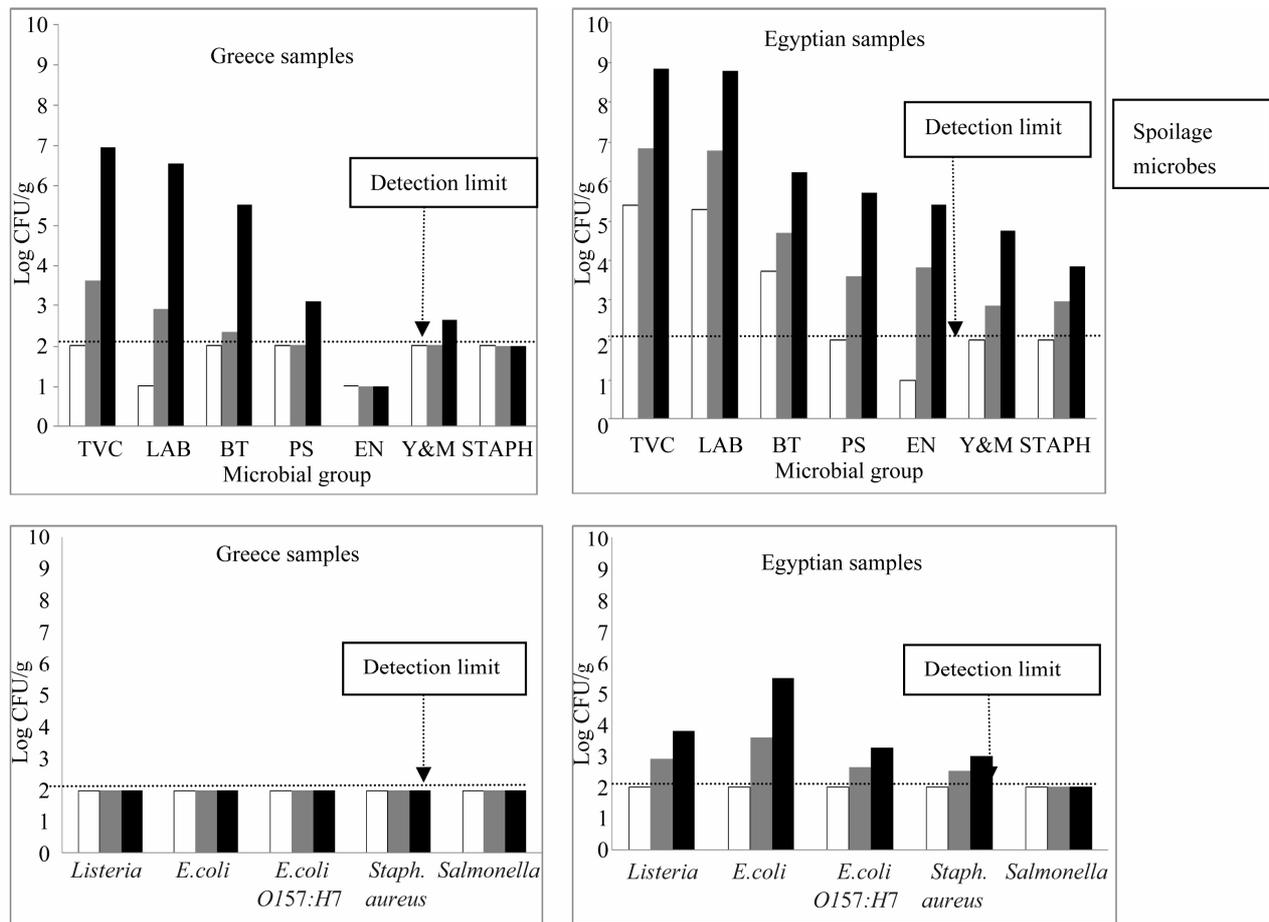**Figure 4** shows that the microbial counts in chicken

Figure 4. Microbial counts (TVC, total viable count; LAB, lactic acid bacteria; BT, *Brochothrix thermosphacta*; PS, pseudomonads; EN, *Enterobacteriaceae*; Y&M, yeasts and moulds; STAPH, staphylococci) in chicken sausage from Greece and Egypt (black columns = the maximum, gray columns = the average and white columns = the minimum) during the year 2006.

sausage from Egypt and Greece as characterized by maximum and minimum as well as the average of the whole samples. It can generally be observed that the maximum levels of the different spoilage microbes were higher in Egyptian samples than in Greek samples. The maximum values of all Egyptian samples were over the detection limit, compared to only 5 groups in Greek samples. All minimum levels in Greek samples were under the detection limit, while there were three microbial groups in Egyptian samples whose minimum were over the detection limit. The average values of the microbial groups in Greek samples were either below the detection limit (4 groups) or slightly over the detection limit (3 groups) but in all Egyptian samples the averages of all microbial groups were evidently over the detection limit. All these results indicate a more hygienic and safety correlated conditions during production and distribution of Greek sausages. The distribution of pathogenic microbial groups shows that, maximum, minimum and average values were all under the detection limit for Greek samples while most of Egyptian samples (4 out of 5) had the

maximum and average levels over the detection limit, particularly *Listeria* spp., *E. coli* O157:H7 and *Staph. aureus*. So these three groups may represent pathogenic hazards in Egyptian samples. *Salmonella* level was under the detection limit and hence does not pose hygienic problem. The presence of low number of microorganisms in chicken sausages from Greece, may be a synergistic result of quality of raw materials, ingredients, cooking, vacuum packaging and chill storage as well as good hygienic quality and practices. In addition to microbiological quality of the products, factors such as handling, processing, packaging, storage and delivery may also influence the microbiological load of food products at the point of sale [34].

3.5. Pathogenicity of *Listeria* Isolates Collected from Egyptian Samples

Listeria isolates were subjected to haemolytic test on blood agar to disclose their pathogenicity. In control samples (**Figure 5**), *L. monocytogenes* ScottA (B) in-

duced a clear zone on blood agar indicating haemolysis while *L. innocua* (C) does not. *Staph. aureus* (A) normally induces a clear zone on the outer border of the growth (colony). 34 isolates producing clear zones similar to *L. monocytogenes* Scott A and 36 isolates producing zones corresponding to *L. innocua*. From the 70 examined *Listeria* isolates could be separated into 34 pathogenic *L. monocytogenes* and 36 *L. innocua*. This is quite an alarming result revealing the hazardous hygienic status of Egyptian sausage samples while Greek sausage samples were safe. None of the samples produced zones comparable to that of the *Staph. aureus*. The 34 pathogenic *L. monocytogenes* isolated from 12 Egyptian sausage samples, *i.e.* they were distributed in about 24% of the total samples. Sporadic cases of listeriosis have been reported in a number of poultry products [9-12].

Following the magnitude of contaminating microflora in the final poultry products collected from retail market of a developing country (Egypt) as compared to a developed one (Greece), could evaluate the bacteriological quality and estimate the hygienic status in every case in order to improve the hygienic measures and motivate concerned parties to take the appropriate actions achieving this objective.

3.6. Comparative Prevalence of Pathogenic Bacteria in Chicken Sausages from Egypt during 2006 and 2010

L. monocytogenes, *E. coli* O157:H7 and *Staph. aureus* were detected in chicken sausages produced by three food manufacturing companies (B, C and D) in Egypt during 2010 and compared by food manufacturing company (A) during 2006 (**Table 1**). *L. monocytogenes*, *E. coli* O157:H7 and *Staph. aureus* were detected in about 24%, 26% and 60% of the samples of the producing company A from Egypt during the year 2006 compared to respective values of 37.78%, 41.11% and 64.44% of the samples of the producing companies B, C and D from Egypt dur-

ing the year 2010. This indicates clearly that the microbial pathogens are highly prevalent in Egypt and there was no improvement in the general hygienic status between 2006 and 2010, due probably to the insufficiency of the means and measures required for microbial control. Generally, the same trend found in 2006 applied also to the samples collected in 2010, irrespective of the producing company. This result reveals that the hygienic status was still very poor and no serious actions were taken to improve it.

4. CONCLUSION

The samples of chicken sausage from Egypt collected
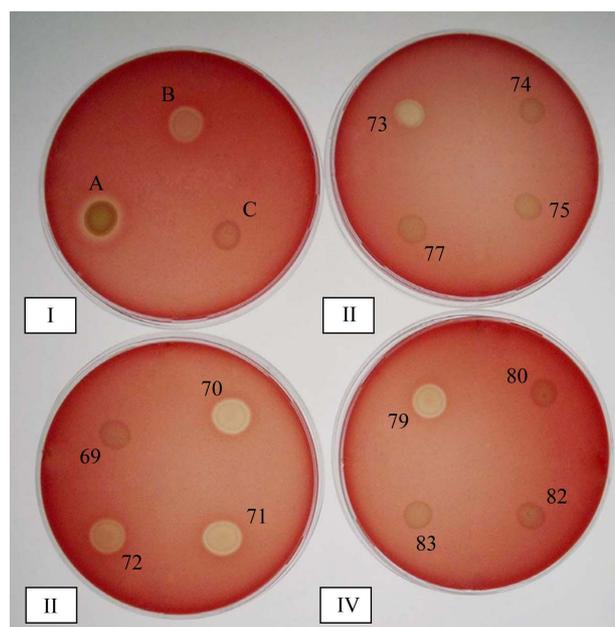

Figure 5. Heamolytic activity of some *L. monocytogenes* isolates collected from Egyptian sausage samples during 2006 and 2010. The control plate (I) contain *Staph. aureus* (A), *L. monocytogenes* (B) and *L. innocua* (C) and the other plates (II-IV) contain random samples.

Table 1. The prevalence of *Salmonella*, *L. monocytogenes*, *E. coli* O157:H7 and *Staph. aureus* in chicken sausages from Egypt during 2006 and 2010.

Company and number of samples	*Salmonella*	*L. monocytogenes*	*E. coli* O157:H7	*Staph. aureus*
		No. of positive samples during 2006		
A (50)	ND	12 (24.00%)	13 (26.00%)	30 (60.00%)
		No. of positive samples during 2010		
B(30)	ND	12 (40.00%)	10 (33.33%)	15 (50.00%)
C (30)	ND	13 (43.33%)	15 (50.00%)	21 (70.00%)
D (30)	ND	9 (30.00%)	12 (40.00%)	22 (73.33%)
Total (90)	ND	34 (37.78%)	37 (41.11%)	58 (64.44%)

ND, not detected.

in 2006 were found to harbor *Listeria* spp., *Staph. aureus* and *E. coli* O157:H7, while these pathogens were not detected in Greek samples. The pathogenic *L. monocytogenes* was exclusively detected in 24% and 37.78% of the total Egyptian samples during 2006 and 2010, respectively. While *Salmonella* was absent in both Greek and Egyptian samples as revealed by enrichment techniques. The prevalence of pathogenic bacteria in Egyptian samples was confirmed after 4 years from the original study. The obtained results will mobilize the concerned parties to take the necessary measures reducing food-borne pathogen risks and spoilage flora alongside the poultry chain.

5. ACKNOWLEDGEMENTS

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