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Microbiological Assessment of Various Ready-To-Eat Foods in Cairo, Egypt, and Studying the Possible Antibacterial Effects of Garlic and Cumin Oils as Food Additives

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READY-TO-EAT (RTE) food contamination has become a global health problem. A bacteriological survey of RTE food samples (54 barbecued chicken meat, 50 meat shawarma, 56 chicken shawarma, 44 grilled fish, 36 fesikh, 48 brackish fish, and 48 sardines) was implemented. The samples were analyzed using standard microbiological techniques. *Enterobacteriaceae* (67.26%), *Aeromonas* (5.36%), *Pseudomonas* (1.19%), *S. aureus* (9.82%), coagulase-negative staphylococci (11.90%), and *Listeria* (9.52%) were detected in the examined samples. Different *Enterobacteriaceae* species were isolated and identified. Neither *Campylobacter* nor salmonellae were detected. The count of the isolated bacteria in the different collected samples was determined. The antibacterial activities of garlic and cumin oils against different recovered isolates were investigated. The inhibition zone diameters (mm) of garlic and cumin oil were 17 and 14 against *Enterobacteriaceae*, 16 and 11 against *Aeromonas*,14 and 12 against *Pseudomonas*, 20 and 14 against *S. aureus* and 18 and 12 against *Listeria*, respectively. This study recommends the application of good hygienic practices in the preparation of RTE foods to improve their hygienic conditions and minimize their bacterial counts which will undoubtedly minimize the public health hazards. Finally, using spices like garlic and cumin as additives in RTE foods may provide better inhibition of foodborne pathogens.

Keywords: *Aeromonas*, *Enterobacteriaceae*, *Listeria*, *Pseudomonas*, Staphylococci, Cumin oil, Garlic oil.

Introduction

In Egypt, with changes in the routine lifestyle like an increase in people staying out of their homes, travel pleasure time, business, tourism, and the involvement of a huge number of women in different jobs, the consumption rate of RTE foods has risen significantly. RTE foods are foods directly consumed cooked or raw without additional handling. However, RTE foods can be involved in probable health risks due to their potential microbial contamination with foodborne pathogens at any stage of food chain preparation [1]. When RTE foods are handled in unsanitary circumstances where pathogenic bacteria exist in the equipment, workers' aprons/hands, and surrounding environment, the final RTE food end product could be contaminated [2]. The major foodborne pathogens that could infect humans through ingestion of contaminated food are, for example, *Staphylococcus aureus*, *Salmonella* spp., *Vibrio cholera*, *Streptococcus* spp., *Klebsiella pneumoniae*, *Micrococcus* spp., *Clostridium botulinum*, *Aeromonas hydrophila*, *Escherichia coli*, *Bacillus* spp., *Enterobacter cloacae*,

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Acinetobacter, Proteus mirabilis, *Pseudomonas* species, *Citrobacter freundii,* and *Serratia* species. The ingestion of food contaminated by food-borne pathogens could induce FBDs (food-borne diseases) [3]. The common manifestation of FBDs is diarrhea which is caused mainly by microbial toxins. However, manifestations of FBDs could also include abdominal pain, enteric complications, hemorrhagic colitis, fever, meningitis, bloodstream infection, kidney failure, joint infection, miscarriage, paralysis, etc [4]. Other serious consequences of FBDs have been recorded like; brain and neural disorders, liver kidney failure, and mortalities [3]. The World Health Organization (WHO) reported that globally 2.2 million individuals die annually from both FBDs and waterborne diseases, 1.9 million of whom are children [4, 5]. Annually, 30% of individuals living in developed countries are suffering from FBDs and waterborne diseases as reported by the WHO [6, 7]. While, in developing countries, people are more susceptible to illness and death due to FBDs [8]. Accurate and rapid identification of foodborne pathogens in food is important for quality assurance.

Due to the plentiful of food-borne poisoning cases caused by food-borne bacteria and their toxins, there is a need for ongoing research to control RTE food health hazards [9]. Garlic has obtained a reputation as a therapeutic and prophylactic medicinal plant. Garlic has served as a significant therapy for the Sumerians and the ancient Egyptians. In Greece throughout the prime Olympics, garlic was taken by athletes to enhance their toughness [10]. Indian and ancient Chinese medicines advised garlic to aid digestion and respiration and to treat parasitic infestation and leprosy [11]. Al Qanoon Fil Tib [12], a well-known book advised garlic as an antibiotic in the treatment of several infectious illnesses, toothache, arthritis, constipation, chronic cough, snake and insect bites, parasitic infestation, and gynecologic diseases. On the other hand, cumin (*Cuminum cyminum*) is a multipurpose plant species cultivated in China, India, many Mediterranean countries, and the Middle East. Cumin has been widely used to treat a variety of diseases, including diabetes, cancer, and hypolipidemia [13]. The aim of the current study is to detect some common foodborne pathogens in RTE food, determine the count of the isolated bacteria in the different collected samples, and to determine the antibacterial efficiency of garlic and cumin oils among the most commonly isolated pathogens.

Materials and Methods

Sampling

The samples were prepared and examined according to The ICMSF (International Commission on Microbiological Specifications for Foods) [14]. A total of 336 different RTE food samples, including barbecued chicken meat (n=54), meat shawarma (n=50), chicken shawarma (n=56), grilled fish (n=44), fesikh (n=36), brackish fish (n=48), and sardines (n=48) were collected randomly from various food restaurants in Cairo, Egypt. Samples were collected into sterile lunch boxes, placed in an insulated icebox and transported to the Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre, Cairo, Egypt where they were analyzed within one hour of collection for the presence of microorganisms.

Sample preparation

According to the FDA (Food and Drug Administration) [15], 25 grams of each sample were transmitted into a sterile flask containing 225 ml of sterile 0.1% *w/v* peptone water (LP0037, Oxoid, UK). The content of the flask was transferred into a sterile 400 ml stomacher bag (Seward Medical, UK), and homogenized at room temperature in a stomacher blender (Seward Medical, UK) for 1 min at 230 rpm. One ml of each homogenate was transported to a sterile tube containing 9 ml of 0.1% peptone water then ten-fold serial dilutions were adjusted.

Bacterial isolation

Isolation of different bacteria from each sample was carried out using the International Standards Organization (ISO) protocols. *Enterobacteriaceae* (ISO 21528-1:2017) [16], *Campylobacter* (ISO 10272-1:2017) [17], *Listeria* species (ISO 11290-1:2017) [18], *Staphylococcus* species (ISO 6888-1:2021) [19], *Pseudomonas aeruginosa* (ISO 22717:2015) [20], and *Aeromonas* (ISO, 2004) [21]. Briefly, the family *Enterobacteriaceae* was isolated using Violet Red Bile Glucose selective agar (VRBGA, CM1082, Oxoid, UK) followed by incubation for 24 h at 37℃. *Campylobacter* spp. were isolated using pre-enrichment in Preston broth (CM0067, Oxoid, UK) and then incubated under microaerobic conditions at 37℃ for 6 h, followed by incubation at 42℃ for 48 h then plating on modified Charcoal Cefoperazone Deoxycholate (mCCDA, CM0739, Oxoid, UK) agar plates with mCCDA selective supplement (SR0155, Oxoid, UK) and incubated at 42°C for 3-5 days under microaerobic conditions.

Listeria monocytogenes was isolated using pre-enrichment in half-strength Fraser broth, followed by incubation in full-strength Fraser broth (CM0895+ SR0156, Oxoid, UK) at 30℃ for 24-48 h followed by plating on PALCAM Listeria selective agar (CM0877, Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol, Oxoid, UK) followed by incubation at 37℃ for 24-48 h. *Staphylococcus aureus* was isolated by plating on Baird-Parker agar with egg yolk tellurite medium emulsion (CM0275 + SR0054, Oxoid, UK) followed by incubation for 24 h at 37℃. *Pseudomonas* spp. were isolated using Pseudomonas agar base medium (CM0559, Oxoid, UK) supplemented with Pseudomonas CFC supplement (SR0103, Oxoid, UK) and incubated for 18 h at 35℃. *Aeromonas* species were isolated using Starch Ampicillin agar (SAA, M1177, Himedia, India) with ampicillin (10 mg/l) and incubated at 28℃ for 24 h. After incubation, colonies were enumerated and the results were expressed as colony-forming units per gram of sample (cfu/g) and were statistically analyzed.

Identification of bacterial isolates

Typical colonies of each isolate were selected from the agar plates and purified by repeated subculturing on appropriate agar plates. Identification of the isolates was implemented using standard morphological, biochemical, and microbiological methods. For identification of *Campylobacter* species, the suspected colonies were tested by motility, Gram staining, catalase test, oxidase test, and growth at 25℃ [22]. *Campylobacter* species identification confirmation was achieved by using biochemical tests (API Campy, bioMérieux, France) and cephalothin and nalidixic acid sensitivity tests (Oxoid, UK). For *Pseudomonas* species, the suspected colonies were handled according to previous studies [23, 24]. *S. aureus* colonies appeared black with clear zones on Baird-Parker agar plates. Identification of staphylococci colonies was conducted according to reference studies [25]. *Staphylococcus* species confirmation was carried out using the API-Staph identification Kit (bioMérieux, France) according to the manufacturer's instructions. Colonies of purple-pink color on Violet Red Bile Glucose agar were identified as a member of the family *Enterobacteriaceae* and confirmed by the API system (bioMérieux, France) according to the manufacturer's instructions [26]. Typical colonies of *Aeromonas* species on Starch Ampicillin agar were confirmed by microscopical and biochemical identification [27]. Typical grey-green colonies with a black centre and a black halo on the PALCAM Listeria selective agar were described as *Listeria* species [28].

Antibacterial activity of Garlic and Cumin oil

The agar well diffusion method was used to screen the antibacterial effects of garlic and cumin oils (Harraz Herbs shop, Cairo) against the most commonly isolated pathogens; *Enterobacteriaceae*, *Aeromonas* spp., *Pseudomonas aeruginosa*, *S. aureus,* and *Listeria* spp. Using a sterilized borer, wells (diameter of 6 mm) were made in the Mueller-Hinton agar (CM0337, Oxoid, UK) plates. A fresh culture of the bacterial cells (100 µl 1×10^8 CFU/ml) was swabbed onto the surface of the agar plates. The wells were then filled with 100 µl of garlic oil and cumin oil. After incubation at the recommended time and temperature, the diameters of the transparent zones of inhibition around wells were measured and recorded [29].

Statistical analysis

Statistical analyses were carried out using IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 25 (2017) for Windows [30]. Results were statistically analyzed using the simple frequency table and descriptive statistics (minimum, maximum, 5% trimmed mean, and interquartile range).

Results

Bacterial isolates recovered from RTE food samples

The results reported in Table (1) and Figure (1) reveal that the prevalence rates of *Enterobacteriaceae* isolated from barbecued chicken meat, meat shawarma, chicken shawarma, grilled fish, fesikh, brackish fish, and sardines were 70.4%, 60.0%, 85.7%, 61.4%, 55.6%, 81.3%, and 50.0%, respectively. *Aeromonas* species were isolated from barbecued chicken meat and grilled fish in 11.1%, and 27.3% of the tested samples, respectively. *S. aureus* was detected in barbecued chicken meat (7.4%), grilled fish (56.8%), and brackish fish (8.3%). Coagulase-negative staphylococci were isolated from barbecued chicken meat (3.7%), meat shawarma (4.0%), chicken shawarma (28.6%), fesikh (11.1%), and sardines (33.3%). *Listeria* species were isolated from chicken shawarma (42.9%), grilled fish (9.1%), and fesikh (11.1%). *Pseudomonas aeruginosa* was isolated only from grilled fish (9.1%). No campylobacters were isolated from any of the examined samples.

Among *Enterobacteriaceae* species as presented in Table (2), *E. coli* were isolated from barbecued chicken meat (3.7%), fesikh (11.1%), brackish fish (16.7%), and sardines (14.6%). *Erwinia cacticida* was isolated from barbecued chicken meat (7.4%), meat shawarma (60.0%), chicken shawarma (85.7%), grilled fish (34.1%), and sardines (16.7%). *Enterobacter cloacae* was isolated from barbecued chicken meat (5.6%) and sardines (16.7%). *Serratia fonticola* was isolated from barbecued chicken meat (3.7%) and grilled fish (9.1%). *Enterobacter intermedius* was isolated from barbecued chicken meat (3.7%) and sardines (16.7%). *Proteus mirabilis* was isolated from barbecued chicken meat (55.6%), grilled fish (18.2%), fesikh (22.2%), and brackish fish (33.3%). *Proteus penneri* was detected in fesikh (33.3%), and brackish fish (41.7%). *Klebsiella oxytoca* was detected only in barbecued chicken meat (3.7%). *Edwardsiella tarda* was isolated only from meat shawarma (20.0%). *Citrobacter freundii* was detected only in fesikh (13.9%). *Yersinia pseudotuberculosis* was detected only in brackish fish (16.7%). *Serratia liquefaciens* was detected only in barbecued chicken meat (3.7%). *Providencia stuartii* was detected only in barbecued chicken meat (3.7%). *Proteus vulgaris* was detected only in barbecued chicken meat (3.7%). No salmonellae were detected in any of the examined samples.

Table (3) illustrates the minimum, maximum, 5% trimmed mean, and interquartile range of the count of the isolated bacteria among the collected samples. In barbecued chicken meat, *Campylobacter*, *Pseudomonas*, and *Listeria* were absent. The 5% trimmed means for *Enterobacteriaceae*, *Aeromonas*, CPS, and CNS in barbecued chicken meat were 3.024, 0.223, 0.12367, and 0.000, respectively. In meat shawarma, *Campylobacter*, *Aeromonas*, *Pseudomonas*, and CPS were absent. The 5% trimmed means for *Enterobacteriaceae* and CNS in meat shawarma were 2.399 and 0.000, respectively. In chicken shawarma, *Campylobacter*, *Aeromonas*, *Pseudomonas*, and CPS were absent. The 5% trimmed means for *Enterobacteriaceae,* CNS, and *Listeria* in chicken shawarma were 3.225, 1.023, and 0.918, respectively. In grilled fish, *Campylobacter* and *CNS* were absent. The 5% trimmed means for *Enterobacteriaceae*, CPS, *Aeromonas*, *Pseudomonas*, and *Listeria* in grilled fish were 2.465, 2.418, 0.511, 0.114, and 0.042, respectively. In fesikh, *Campylobacter*, *Aeromonas*, *Pseudomonas*, and CPS were absent. The 5% trimmed means for *Enterobacteriaceae,* CNS, and *Listeria* in fesikh were 2.061, 0.298, and 0.174, respectively. In brackish fish, *Campylobacter*, *Aeromonas*, *Pseudomonas*, CNS, and *Listeria* were absent. The 5% trimmed means for *Enterobacteriaceae* and CPS in brackish fish were 3.151 and 0.000, respectively. In sardines, *Campylobacter*, *Aeromonas*, *Pseudomonas,* CPS, and *Listeria* were absent. The 5% trimmed means for *Enterobacteriaceae* and CNS in sardines were 1.662 and 1.287, respectively.

Antibacterial activity of Garlic and Cumin oils

Data shown in Table (4) reveal that the inhibition zones' diameters (mm) of garlic and cumin oils were 17 and 14, respectively against *Enterobacteriaceae*, 16 and 11 against *Aeromonas*, 14 and 12 against *Pseudomonas*, 20 and 14 against *S. aureus,* and 18 and 12 against *Listeria*.

Discussion

Foodborne diseases due to ingestion of food contaminated with bacterial pathogens still constitute a huge global concern [31]. In the current study, it is reported that the prevalence rates of *Enterobacteriaceae, Campylobacter, Aeromonas, Pseudomonas, S. aureus,* coagulase-negative staphylococci (CNS), and *Listeria* in RTE foods were 67.26%, 0.00%, 5.36%, 1.19%, 9.82%, 11.90%, and 9.52%, respectively. In addition, RTE foods showed the existence of multiple foodborne pathogens including *S. aureus*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella* species, *Escherichia coli*, spoilage bacteria, yeasts, molds, and total aerobic where all are associated with serious public health FBDs [32, 33]. A high prevalence of *S. aureus* (12.5%) and *Listeria monocytogenes* (20%) were detected in desserts containing dairy cream. In addition, *Escherichia coli* and *Salmonella* species were detected in 8.5% and 17.5% of RTE sandwiches, respectively [34]. In another study, *Shigella, Salmonella, Listeria, Campylobacter, Clostridium*, *Acinetobacter, Nocardia, Vibrio*, *Leuconostoc, Acetobacter* species, and *Bacillus cereus* were isolated from

different RTE foods such as fried fish, salad, and bread [35]. In a study conducted to survey the contamination levels in fast-food restaurants, a high prevalence of contamination (37.5%) was reported [3]. The food handlers' hands represented a major source of food poisoning bacteria. Indeed, *S. aureus*, *Pseudomonas* species, *E. coli*, *Klebsiella* species, *Shigella* species, *β*-hemolytic streptococci, and yeasts were detected in 17.6%, 9.6%, 14.4%, 7.2%, 3.2%, 4% and 6.4% of hand samples taken from food handlers, respectively. Meanwhile, samples obtained from knives used for processing and handling of foods, showed 35% contamination by *Escherichia coli*, 5% *Shigella* species, 10% *Klebsiella* species, and 10% *Morganella* species. However, samples taken from spoons showed contamination by *Klebsiella* species (10%) and *E. coli* (40%), while plate samples showed contamination by *Shigella* species (4.55%) and *E. coli* (22.73%) [36]. Inadequate cleaning procedures and atmospheric conditions could result in such high microbial contamination [37].

Psychrophilic bacteria including *Aeromonas* are capable of surviving and growing at cold temperatures ranging from 2ºC to 10ºC that are imposed on processed food products [38]. In the current study, the results illustrate that *Aeromonas* species were isolated from barbecued chicken meat (11.1%) and grilled fish (27.3%). The results of previous studies [2, 39] coincided with ours as *Aeromonas* species were isolated from ground chicken meat. Other studies reported higher levels of contamination by *Aeromonas* species (47.17% and 57%) in chicken meat samples, respectively [40,41]. Count and types of psychrotrophic bacteria as well as storage temperature are deemed to be the primary factors responsible for the spoilage of poultry meat [42]. On the other hand, fish is a magnificent host for bacterial contamination and replication due to its favorable post-mortem pH (commonly >6.0). Contamination of fish meat can occur during slicing meat and filleting. The growth of spoilage bacteria in fish meat, especially the potentially pathogenic *Aeromonas* species is not prevented if fish are packed under modified atmosphere conditions or vacuum packed [1]. *Aeromonas* species are the causative agent for a variety of diseases in humans mainly gastroenteritis as *Aeromonas* have the ability to produce enterotoxins. In Brazil, an outbreak of gastroenteritis and acute diarrhea through ingestion of contaminated water has been reported [43].

Pseudomonas species are psychrotrophic bacteria that can reduce food shelf life even in refrigerators [1]. In the current study, *Pseudomonas* species were isolated only from grilled fish (9.1%). In previous reports, *Pseudomonas* was detected in samples of washing areas (34.62%), refrigerator handles (27.50%), preparation areas (27.27%), cutting board (50%), serving counter (23.68%), glass (11.76%), and knives (5%) [36]. *Pseudomonas* species were detected in samples of meat pie, fried rice, and salad [3]. A previous study on food contact surfaces reported contamination (1.7%) by *Pseudomonas* spp. [37]. *P. aeruginosa* is widely distributed in nature in moist environments like water baths, sinks, hot tubs, and showers and so can easily contaminate food. However, consumption of food contaminated with *P. aeruginosa* seldom causes FBDs as it is a normal flora of the gastrointestinal tract [3].

Listeria species were isolated from samples of chicken shawarma (42.9%), grilled fish (9.1%), and fesikh (11.1%) tested in this study. In a similar investigation, *L. monocytogenes* was detected in RTE foods such as hard cheeses (0.1%), fruits and vegetables (0.6%), soft and semi-soft cheeses (0.9%), meat and meat products (1.8%), salads (4.2%) and fish products and fishes (6.0%) [9]. However, no *L. monocytogenes* was detected in any of the examined samples of RTE foods at the 44 Italian airport food service facilities [6]. *Listeria* species are able to live and grow in harsh environmental conditions and can form biofilms which facilitate their cross-contamination and spreading [9]. Annually as reported by the WHO, 1 million people suffer from listeriosis [44]. Listeriosis outbreaks are caused by the ingestion of foods contaminated by high doses of *L. monocytogenes*. Listeriosis manifestations may include fever, diarrhea, myalgia, and headache, while severe Listeriosis may include high mortality rates (20–30%), especially in immunocompromised patients [44]. As reported by the EFSA BIOHAZ Report in 2010–2011, different RTE foods were contaminated with *L. monocytogenes*; 1.7% contamination was

recorded in fish, 0.43% in meat, and 0.06% in cheese samples [45]. In addition, as reported by the EFSA BIOHAZ Report in 2018, RTE salads were determined as the primary source of foodborne *L. monocytogenes* [46]. The presence of *L. monocytogenes* in RTE foods emphasizes the importance of enhancing hygiene standards and implementing regulations through the RTE food chain to ensure the safety of their consumers.

In the current study, *S. aureus* was detected in barbecued chicken meat (7.4%), grilled fish (56.8%), and brackish fish (8.3%). While coagulase-negative staphylococci were isolated from barbecued chicken meat (3.7%), meat shawarma (4.0%), chicken shawarma (28.6%), fesikh (11.1%) and sardines (33.3%). A previous study on food contact surfaces reported contamination by *Staphylococcus aureus* (4.4%) [37]. *S. aureus* was also isolated from all types of the examined food except fried rice [3]. It is well known that RTE foods are most probably prepared by direct hand resulting in a high level of contamination by foodborne pathogens such as *Staphylococcus* species [47]. Food handlers can smoothly contaminate food with *S. aureus* if they cough or sneeze during their preparation of food [3]. The presence of *S. aureus* in RTE foods represents a dangerous hazard to human life, as *S. aureus* is known as a potent toxigenic foodborne pathogen that could cause moderate to life-threatening diseases [25].

Of the *Enterobacteriaceae* members investigated in this study, *E. coli* (6.25%), *Erwinia cacticida* (31.25%), *Edwardsiella tarda* (2.98%), *Klebsiella oxytoca* (0.60%), *Enterobacter cloacae* (3.27%), *Serratia fonticola* (1.79%), *Serratia liquefaciens* (0.60%), *Enterobacter intermedius* (2.98%), *Providencia stuartii* (0.60%), *Proteus mirabilis* (18.45%), *Proteus vulgaris* (0.60%), *Proteus penneri* (9.52%), *Citrobacter freundii* (1.49%), and *Yersinia pseudotuberculosis* (2.38%) were detected in the examined samples. No salmonellae were detected in any of the examined samples. A previous study on food contact surfaces (like food containers, serving dishes, knives, cutting boards, and other utensils) reported contamination by *Klebsiella* spp. (18.7%), *Escherichia coli* (17.7%), and *Proteus* spp. (0.7%) [37]. Pathogenic bacteria like *E. coli* and *Salmonella* spp., could remain viable on food contact surfaces for a long time [37]. Contaminated food and water as well as person-to-person contact are the important sources of *E. coli* and *Salmonella* species [4]. Similar to the current study, there were no salmonellae detected in any of the examined RTE foods [6]. However, in another study, *S. typhi* was isolated from fish rolls and salad which could result in a hazardous salmonellosis foodborne outbreak [3]. A significant increase in the incidence of bacterial isolates in RTE food indicated contamination primarily from contaminated water used for washing and also from contaminated processing materials [36]. Additionally, contaminated food contact surfaces including knives and equipment are likely the primary vehicles for the elevated contamination level of RTE food end products [37].

In the current study, the 5% trimmed mean of the isolated bacteria was used instead of the mean as the mean counts were mainly around zero. The total counts of *Enterobacteriaceae* among the collected samples ranged from 0 to 6.934*, Aeromonas* (0 to 4.813), *Pseudomonas* (0 to 3.813)*,* CPS (0 to 6.7), CNS (0 to 6.71), and *Listeria* (0 to 5.04) log10 cfu/g. The variation in the counts reported in the present study may be due to the difference in hygienic levels during RTE food preparation. The high counts of *Enterobacteriaceae* are an indicator of the poor microbiological quality of the food. In a similar study, among all examined samples (sandwich, meat pie, fried chicken, chicken stew, fried Russian, fried chips, and beef stew), coliforms and *S. aureus* mean counts ranged from 1.53 to 3.58 and 1.10 to 2.68 log x cfu/g, respectively [31]. In a previous study, the counts of *Pseudomonas* spp. were 5 log cfu/g in various examined RTE sushi types [1]. If the count of *S. aureus* reaches inside the food 10^5–10^6 CFU/ml or gm at 10℃ to 46℃, it can produce heat-stable enterotoxins [25]. If the count of fecal groups such as *E. coli, Salmonella*, *Klebsiella* spp., and *Shigella* is <1000 CFU/100 cm^3 in water or food, it indicates a 54% likelihood of occurrence of food poisoning bacteria, and if the count of fecal groups >1000 CFU/100 cm^3, it indicates a 96% likelihood occurrence of food poisoning bacteria [37].

These results reveal deficient hygienic standards which could lead to foodborne

outbreaks. The contamination of RTE foods is related mainly to the overlooked sanitary measures applied throughout food handling, processing, packaging, and cold storage. To reduce FBDs, more efficient control of the microbiological quality of RTE foods should be applied like; adequate personal hygiene, food preparation and cooking, storage temperatures, and cleaning of food equipment. Applying adequate physical and chemical barriers by wearing masks, gloves, and glass shields is recommended [37]. It is recommended that food workers wash their hands adequately. Indeed, washing hands efficiently could decrease the microbial load present in the prepared meals and on surfaces and hands [6]. Periodical medical examinations of food handlers must be carried out. Additionally, food handlers should receive training before starting work in the food production system as recommended by the Food and Agriculture Organisation of the United Nations/ World Health Organisation (FAO/WHO) [48]. Adequate temperatures must be applied as well as subdividing the food into small batches so the food stays at room temperature just for 30 minutes at maximum [6]. In addition, a food safety management system based on the principles of hazard analysis and critical control points (HACCP) must be applied [6]. The requirement of accreditation certificates as well as official inspection visits by authorities are a must to improve sanitation levels in food-serving establishments [37].

The current results revealed that the inhibition zone diameters (mm) of garlic and cumin oils against *Enterobacteriaceae* isolates were 17 and 14 respectively, against *Aeromonas* isolates (16 and 11), against *Pseudomonas* isolates (14 and 12), against *S. aureus* isolates (20 and 14) and against *Listeria* isolates (18 and 12), respectively. Garlic is an organosulfur-enriched and polyphenolic nutraceutical spice its consumption is dated back to ancient history. Garlic is advised not only to increase the color, flavor, and aroma of food but it is also adopted for therapeutic objectives for its inherent neutralization of various chronic and acute diseases [49]. The probable use of garlic for the treatment and prevention of various diseases has been evidenced [50].

Various composites in garlic are thought to have anti-microbial and anti-tumor consequences as well as it has the ability to lower cardiovascular diseases and high blood glucose levels. Garlic is usually used as a therapeutic medicine in various outbreaks, such as dysentery, cholera, influenza, and typhus [51]. The curing effectiveness of garlic is primarily related to the stunning activity of its bioactive compounds like saponins [52], organic sulfides [53], polysaccharides [54], and phenolic compounds [55]. There are more than 20 recognized polyphenolic composites in garlic and it includes bioactive compounds; 17 amino acids, and 8 main amino acids [49]. The antimicrobial mechanisms of garlic encompass the dispossession of the substances required for microbial growth, the impediment of extracellular enzymes, the anti-adherence effect of bacteria on the epithelial cells, and the morphological changes of bacteria [56].

In a similar manner, the significant compounds in cumin oils are the p-cymene, monoterpenes beta-pinene, the terpenoid aldehydes cuminic aldehyde, gamma-terpinene, and the isomeric menthadien carboxaldehydes [57]. Cuminic aldehyde and key oils were examined previously using serial dilutions and agar diffusion procedures against different Gram-negative and Gram-positive bacteria, and three different isolates of *Candida albicans*. Those isolates were recovered from clinical cases, and various types of food (sausages, pork fillets, and minced meat). It was concluded that all cuminic aldehyde and cumin oils demonstrated a significant suppression impact against all examined microorganisms except *Pseudomonas* species [57].

Conclusions

This study indicates a noteworthy problem with RTE food related to the presence of infectious foodborne public health hazards (mainly *Listeria* and *S. aureus*). There is a necessity to enhance standardization and sanitation levels throughout the whole RTE food processing chain in order to guarantee safety assurance and boost the microbiological quality of RTE food. Effective application of the HACCP (hazard analysis and critical control points) is a must. Masks, gloves, hand washing, and glass shields should be applied. Effective cleaning of cooking surfaces and devices with alcohol spray is essential. Finally, using spices like garlic and cumin as additives in RTE foods may provide better inhibition of foodborne pathogens.

TABLE 1. Simple frequency table of the isolated pathogens from different ready-to-eat food samples

Bacteria	Samples															
	Barbecued chicken meat (54)		Meat shawarma (50)		Chicken shawarma (56)		Grilled fish (44)		Fesikh (36)		Brackish fish (48)		Sardines (48)		Total	
	PNo	%	PNo	%	PNo	%	PNo	%	PNo	%	PNo	%	PNo	%	PNo	%
Enterobacteriaceae	38	70.4%	30	60.0%	48	85.7%	27	61.4%	20	55.6%	39	81.3%	24	50.0%	226	67.26%
Campylobacter	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.00%
Aeromonas	6	11.1%	0	0.0%	0	0.0%	12	27.3%	0	0.0%	0	0.0%	0	0.0%	18	5.36%
Pseudomonas	0	0.0%	0	0.0%	0	0.0%	4	9.1%	0	0.0%	0	0.0%	0	0.0%	4	1.19%
S. aureus	4	7.4%	0	0.0%	0	0.0%	25	56.8%	0	0.0%	4	8.3%	0	0.0%	33	9.82%
CNS	2	3.7%	2	4.0%	16	28.6%	0	0.0%	4	11.1%	0	0.0%	16	33.3%	40	11.90%
Listeria	0	0.0%	0	0.0%	24	42.9%	4	9.1%	4	11.1%	0	0.0%	0	0.0%	32	9.52%

PNo: positive number CNS: coagulase-negative staphylococci

TABLE 2. *Enterobacteriaceae* species isolated from ready-to-eat food samples

Species	Samples															
	Barbecued chicken meat (54)		Meat shawarma (50)		Chicken shawarma (56)		Grilled fish (44)		Fesikh (36)		Brackish fish (48)		Sardines (48)		Total	
	PNo	%	PNo	%	PNo	%	PNo	%	PNo	%	PNo	%	PNo	%	PNo	%
Escherichia coli	2	3.7%	0	0.0%	0	0.0%	0	0.0%	4	11.1%	8	16.7%	7	14.6%	21	6.25%
Salmonella	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.00%
Erwinia cacticida	4	7.4%	30	60.0%	48	85.7%	15	34.1%	0	0.0%	0	0.0%	8	16.7%	105	31.25%
Edwardsiella tarda	0	0.0%	10	20.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	10	2.98%
Klebsiella oxytoca	2	3.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	0.60%
Enterobacter cloacae	3	5.6%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	8	16.7%	11	3.27%
Serratia fonticola	2	3.7%	0	0.0%	0	0.0%	4	9.1%	0	0.0%	0	0.0%	0	0.0%	6	1.79%
Serratia liquefaciens	2	3.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	0.60%
Enterobacter intermedius	2	3.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	8	16.7%	10	2.98%
Providencia stuartii	2	3.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	0.60%
Proteus mirabilis	30	55.6%	0	0.0%	0	0.0%	8	18.2%	8	22.2%	16	33.3%	0	0.0%	62	18.45%
Proteus vulgaris	2	3.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	0.60%
Proteus penneri	0	0.0%	0	0.0%	0	0.0%	0	0.0%	12	33.3%	20	41.7%	0	0.0%	32	9.52%
Citrobacter freundii	0	0.0%	0	0.0%	0	0.0%	0	0.0%	5	13.9%	0	0.0%	0	0.0%	5	1.49%
Yersinia pseudotuberculosis	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	8	16.7%	0	0.0%	8	2.38%

PNo: Positive number

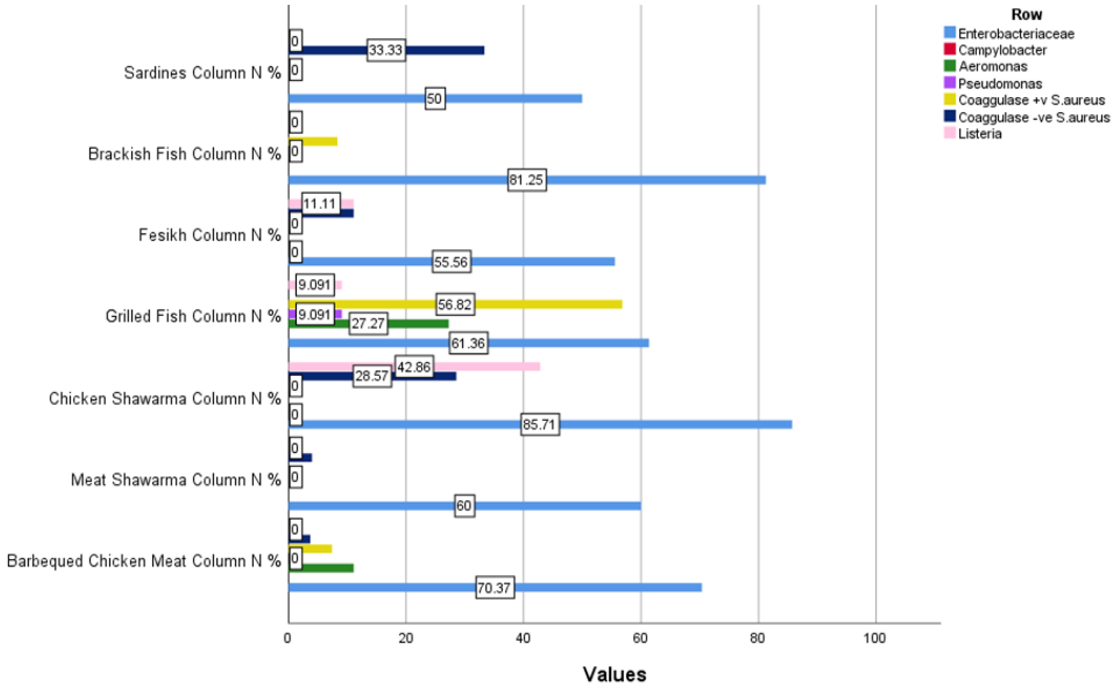

Fig.1. Percentages of pathogens isolated from different ready-to-eat food samples

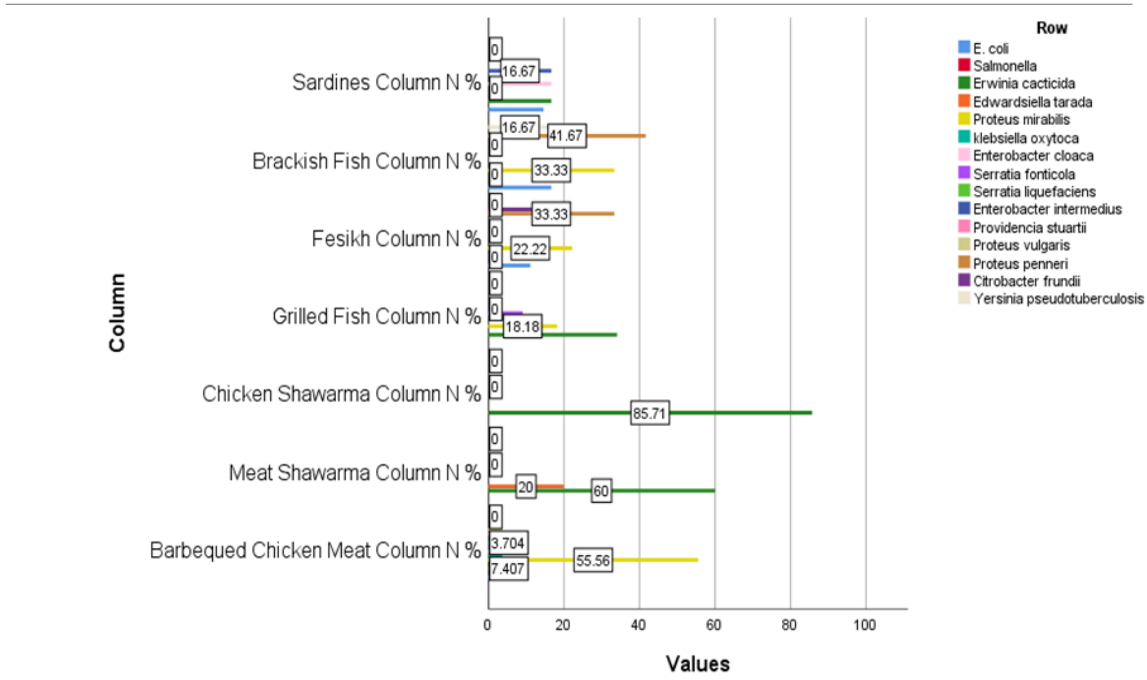

Fig. 2. Percentages of *Enterobacteriaceae* isolated from different ready-to-eat food samples

TABLE 3. Bacterial counts in the tested ready-to-eat food samples

Type of tested samples	Bacterial species	Counts (log10cfu/g)			
		Min.	Max.	5% Trimmed Mean	Interquartile Range
Barbecued chicken meat	*Enterobacteriaceae*	0.000	6.934	3.024	4.775
	Campylobacter	Absent	Absent	Absent	Absent
	Aeromonas	0.000	4.813	0.223	0.000
	Pseudomonas	Absent	Absent	Absent	Absent
	CPS	0.000	5.978	0.12367	0.000
	CNS	.000	4.792	0.000	.000
	Listeria	Absent	Absent	Absent	Absent
Meat Shawarma	*Enterobacteriaceae*	0.000	6.398	2.399	4.587
	Campylobacter	Absent	Absent	Absent	Absent
	Aeromonas	Absent	Absent	Absent	Absent
	Pseudomonas	Absent	Absent	Absent	Absent
	CPS	Absent	Absent	Absent	Absent
	CNS	0.000	5.380	0.000	0.000
	Listeria	Absent	Absent	Absent	Absent
Chicken Shawarma	*Enterobacteriaceae*	0.000	6.653	3.225	2.182
	Campylobacter	Absent	Absent	Absent	Absent
	Aeromonas	Absent	Absent	Absent	Absent
	Pseudomonas	Absent	Absent	Absent	Absent
	CPS	Absent	Absent	Absent	Absent
	CNS	0.000	6.716	1.023	2.592
	Listeria	0.000	5.041	0.918	2.360
Grilled Fish	*Enterobacteriaceae*	0.000	6.255	2.465	4.682
	Campylobacter	Absent	Absent	Absent	Absent
	Aeromonas	0.000	3.568	0.511	1.340
	Pseudomonas	0.000	3.813	0.114	0.000
	CPS	0.000	6.531	2.418	4.553
	CNS	Absent	Absent	Absent	Absent
	Listeria	0.000	2.708	0.042	0.000
Fesikh	*Enterobacteriaceae*	0.000	5.813	2.061	4.088
	Campylobacter	Absent	Absent	Absent	Absent
	Aeromonas	Absent	Absent	Absent	Absent
	Pseudomonas	Absent	Absent	Absent	Absent
	CPS	Absent	Absent	Absent	Absent
	CNS	0.000	6.301	0.298	0.000
	Listeria	0.000	2.964	0.174	0.000
Brackish Fish	*Enterobacteriaceae*	0.000	6.699	3.151	1.981
	Campylobacter	Absent	Absent	Absent	Absent
	Aeromonas	Absent	Absent	Absent	Absent
	Pseudomonas	Absent	Absent	Absent	Absent
	CPS	0.121	6.708	0.000	0.000
	CNS	Absent	Absent	Absent	Absent
	Listeria	Absent	Absent	Absent	Absent
Sardines	*Enterobacteriaceae*	0.000	6.732	1.662	3.847
	Campylobacter	Absent	Absent	Absent	Absent
	Aeromonas	Absent	Absent	Absent	Absent
	Pseudomonas	Absent	Absent	Absent	Absent
	CPS	Absent	Absent	Absent	Absent
	CNS	0.000	5.672	1.287	3.756
	Listeria	Absent	Absent	Absent	Absent

CPS: Coagulase-positive staphylococci CNS: Coagulase-negative staphylococci

TABLE 4. Antibacterial activity of Garlic and Cumin oil against the isolated pathogens

Bacteria	Inhibition zone diameter (mm)	
	Garlic Oil	Cumin Oil
Enterobacteriaceae	17	14
Aeromonas	16	11
Pseudomonas	14	12
S. aureus	20	14
Listeria	18	12

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[١] قسم الميكروبيولوجى – كليه الطب البيطرى – جامعه القاهره - القاهرة - مصر.
[٢] قسم الميكروبيولوجيا والمناعه- المعهد القومي البيطري - المركز القومي للبحوث - القاهرة - مصر.

أصبح تلوث الأطعمة الجاهزة للأكل مشكلة صحية عالمية. فى هذه الدراسة تم مسح بكتريولوجي لعينات من الأطعمة الجاهزة للأكل (٥٤ لحم دجاج مشوي، ٥٠ شاورما لحم، ٥٦ شاورما دجاج، ٤٤ سمك مشوية، ٣٦ فيسيخ، ٤٨ سمك ملوحة، و ٤٨ سردين). تم الكشف الميكروبى للعينات باستخدام التقنيات الميكروبيولوجية القياسية. تم اكتشاف وجود البكتيريا المعوية (*Enterobacteriaceae*) بنسبه 67.26% ، بكتيريا الايروموناس (*Aeromonas*) بنسبة ٥,٣٦٪ ، السودوموناس (*Pseudomonas*) بنسبة 1.19%، الاستافيلوكوكس اوريس (*S. aureus*) بنسبة ٩,٨٢٪، بكتيريا المكورات العنقودية سالبة انزيم التخسر (coagulase-negative staphylococci) بنسبة 11.90%، وميكروبات الليستريا (*Listeria*) بنسبة ٩,٥٢٪ من العينات التي تم فحصها. كما تم عزل وتصنيف أنواع مختلفة من البكتيريا المعوية. اسفرت نتائج العزل عن عدم وجود ميكروبات الكامبيلوباكتر(*Campylobacter*) وايضا عدم وجود ميكروبات السالمونيلا (salmonellae) فى العينات المختبرة. تم تحديد العد البكتيري فى العينات التي تم جمعها. كما تم التحقق من النشاط المضاد للبكتيريا لكل من زيت الثوم وزيت الكمون كمضاد للمعزولات. كانت أقطار منطقة التثبيط البكتيرى (مم) لكل من زيت الثوم وزيت الكمون ١٧ و ١٤ ضد البكتيريا المعوية، و ١٦ و ١١ ضد بكتيريا الايروموناس ، وكانت ١٤ و ١٢ ضد السودوموناس ، و ٢٠ و ١٤ ضد الاستافيلوكوكس اوريس ، وكانت ١٨ و١٢ ضد الليستريا على التوالي. توصي هذه الدراسة بتطبيق الممارسات الصحية الجيدة في إعداد الأطعمة الجاهزة للأكل لتحسين جودتها الصحية ولتقليل العدد البكتيري و التي بالتالى ستقلل بلا شك من مخاطر الصحة العامة على الانسان. أخيرًا، قد يوفر استخدام التوابل مثل الثوم والكمون كمواد مضافة في الأطعمة الجاهزة للأكل تثبيطًا أفضل لمسببات الأمراض البكتيرية و المنقولة من خلال الغذاء.

الكلمات الدالة: البكتيريا المعوية، الليستريا، السودوموناس، الاستافيلوكوكى، زيت الكمون، زيت الثوم.