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Microbiological Evaluation of sauces in Fast food Restaurant Chains and Independent Fast Food Restaurants: Study case the city of Alexandria

By

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التقييم الميكروبيولوجى للصوصات فى سلاسل مطاعم الوجبات السريعة العالمية و مطاعم الوجبات السريعة المستقلة دراسة حالة: مدينة الإسكندرية

الملخص

المقدمة: إن تناول الغذاء خارج المنزل أصبح يزداد بصورة أساسية وعلى نطاق واسع. المصدر الآمن والمعتمد الخالى من الملوثات الممرضة ضرورى للصحة العامة للإنسان ولحياته اليومية وللمستوى الإقتصادى وصورة المجتمع والدولة. الصوص الملوث المصنوع يدوياً دائماً ما يتهم بسبب الإصابة بالأمراض خاصةً فى الدول النامية بالنظر للصحة العامة للمستهلك.

هدف الدراسة: هذه الدراسة أعدت لتقييم التلوث الميكروبى للصوصات المعدة يدوياً فى سلاسل مطاعم الوجبات السريعة ومطاعم الوجبات السريعة المستقلة. **المنهجية:** تم جمع ٣٢ عينة من مطاعم الوجبات السريعة بنوعيها المتناولين فى الدراسة تم عمل عد كمى للميكروبات وذلك بعد تخفيف العينة ثم التعرف والتمييز بين أنواع الميكروبات المختلفة باستخدام الوسط المناسب لها. كل البيانات المتعلقة بالعد الميكروبى تم تجميعها و تحليلها كميًا. **النتائج:** معظم عينات الصوصات تحتوى على البكتيريا

الهوائية الميزوفيلية. **الخلاصة:** استهلاك الصوصات له أهمية كبيرة فى مطاعم الوجبات السريعة فى مدينة الإسكندرية مما يلقى الضوء على ضرورة اهتمام القائمين على إعداد الوجبات بتوخى الإجراءات الصحية و تدريب العاملين على تطبيق ممارسات صحة وسلامة الأغذية.

الكلمات الدالة: مطاعم الإسكندرية، الفحص الميكروبى، صوصات الساندويتشات، الأمراض المتسببة بالغذاء، سلامة الغذاء.

Abstract:

Introduction: Food away from home (FAFH) has become increasingly integral to large scale of people. The reliable supply of safe food that is free from harmful contaminants is important for the people's general health and daily life, economic development and social stability and the government and countries' image. Contaminated handmade sauces are often claimed to occur food-borne diseases, especially in developing countries, Therefore, considering the public health issue. **Aim of the study**: This study was conducted to assess the microbial contamination of handmade sauces in two restaurant categories. **Methodology:** A total of 32 samples of sauces were collected from Fast food Restaurant and Independent Fast Food Restaurant in Alexandria. The quantitative microbial tests were done by dilution plate technique. Identification of

particular bacterial group or species was performed using selective media. All the data related to microbial count are collected and analyzed. **Results:** Most of sauces samples contained viable *Enterobacteriaceae* cells and Total bacterial count (T.B.C) and free from *E. coli* and *Salmonella* spp.. **Conclusion:** The consumption of sauces is of great concern in Alexandria Fast food restaurants. Making the food preparing persons aware of sanitary practices is too crucial that could be achieved through training them food safety and hygienic practices.

Key words: Alexandria restaurants, Microbiological examination, Sandwiches sauces, foodborne diseases, Food safety.

Introduction

Foodborne illnesses are among the most widespread problems in today's world. The spectrum of diseases and pathogens recognized to be foodborne has broadened considerably. Several studies have demonstrated the presence of important and potential foodborne bacteria, including *Salmonella spp*., *Campylobacter jejuni*, *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus*, *Yersiniaenterocolitica*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and others in ready-to-eat (RTE) cooked foods (Notermans & Borgdorff, 1997).

Due to modern life-style and modern eating habits, consumers are more interested in Ready-To-Eat (RTE) meals. Therefore, production of several types of foods has shifted from home-made to commercial practice, e.g. for sauces and food based sauces (Commission Regulation, 2005).

Sewage and contaminated water have been claimed to occur frequent microbial food contamination with *Salmonella spp*., Shigella spp., Campylobacter spp., and *Escherichia coli (Odonkor & Addo, 2018)*.

Unhygienic practice during peeling, slicing, handling, trimming, packaging, etc. can result in bacterial contamination of foodstuffs (Barreto de Deus et al., 2017).

E. coli is a member of the genus *Escherichia* with the family *Enterobacteriaceae*; it is a facultative anaerobe non-sporing. Members are widely distributed in the environment and contaminated food and water are the major sources on which the bacteriaare spread, selected strains can cause a wide variety of infections in hospitals and community settings (Donnerberg et al., 2005; Tsikritzi et al., 2015).

Tomato based sauce, white sauce and gravy are three commonly used sauces aside from the bottled sauces applied at the table (e.g. Ketchup). Tomato sauces are typically served on pasta or with meat or fish, with the tomatoes being an important source of carotenoids. White sauces are produced using fat, a thickener and milk. They are widely used in the UK within fish recipes and, to a lesser extent, within pasta. Gravy sauce would be particularly energy or protein dense, while white sauces are generally more energy and protein dense.

Herbs and spices are generally considered safe and proved to be effective against certain ailments. They added to sauces, they are also extensively used, particularly, in many Asian, African and other countries. In recent years, in view of their beneficial effects, use of spices/herbs has been gradually increasing in developed countries also Spices and herbal plant species have been recognized to possess a broad spectrum of active constituents that exhibit

antimicrobial (AM) activity (Indu et al., 2006). These active compounds are produced as secondary metabolites associated with the volatile essential oil (EO) fraction of these plants (Kuorwel et al., 2011).

There a study showed that garlic 3% has the highest inhibitory effect against *E. coli* O157:H7 at the 3rd day of storage with reduction rate of 100% (Abd EL-Malek, 2005)

There is an increasing interest in applying natural antimicrobial compounds in the food industry. Consumers are increasingly avoiding the consumption of foods treated with chemicals. Natural alternatives are needed to achieve a high level of safety with respect to foodborne pathogenic microorganisms (Rauha et al., 2000). The natural sanitizers, such as organic acids, have been investigated because of their bactericidal activity (Uyttendaele et al., 2004).

Citric acid has the highest inhibitory effect due to its ability to diffuse through the cell membrane, penetrating the weak non dissociated acid, and lactic acid decreases the ionic concentration within the bacterial cell membrane of the exterior cell wall of the bacterial organism. This leads to an accumulation of the acid within the cell cytoplasm, acidification of the cytoplasm, disruption of the proton motive force, and inhibition of substrate transport. Efficient decontamination results have been obtained through immersion or drenching in solutions of organic acids such as oleic acid, acetic acid, citric acid and lactic acid (Bradley et al., 2011).

There is a study indicates that the acetic acid antimicrobial agent can reduce the pathogenic bacteria and increase the shelf life of food products (Wali & Abed, 2019).

Aim of the study

- To assess the sauces microbial counts in Chain and Independent Fast Food Restaurants in Alexandria
- To recommend how to prevent food borne illness as most of people have an attachment to high intake of energy contained foods and low intake of nutrients in their daily diet such as sauces especially young children and youth.
- To make a comparison between Fast food restaurants chains and Independent Fast Food Restaurants in sauces microbial contamination.

Material and Method

Sample collection: A total of 32 sauces samples. From the period of December 2019 to February 2020 were collected each in separate package as a consumer would buy them. They obtained from 32 different Fast food restaurants. The samples were collected in cold packs under aseptic condition and taken to the Pioneer laboratory (Private lab. in Alexandria) for microbiological examination within one hour of collection. The quantitative microbial tests were done by dilution plate technique.

Sample preparation

In order to obtain a homogenized sample the sauce sample mixed well and then 1 gm of each sample was homogenized with 9 ml of sterilized distilled water there for, 1 ml homogenized sample was serially diluted for 2 times in case of enumeration of *Enterobacteriaceae*, *E. coli*,

Salmonella spp. and diluted for four times in case of Total plate count.

Enumeration of Enterobacteriaceae

From each dilution tube, 0.1 ml liquid was spread on the Volatile Red Bile Glucose Agar (Hi Media. India) was used and the round purple colonies surrounded by a purple halo were considered to be *Enterobacteriaceae* colonies (FDA, Food and Drug Administration (FDA) (2002); Hossain & Dey, 2019).

Presence of more than 10^3 and 10^4 Colony Forming Unit(CFU)/g of *Enterobacteriaceae* in each sauce sample, respectively has been considered as unsatisfactory markers as recommended by CFS (2014).

Enumeration of E. coli

The dilution procedure was the same as previously described in the section of "Enumeration of *Enterobacteriaceae*". Two fold dilutions was performed.1 ml liquid from each dilution was mixed with 9 ml of sterilized distilled water 0.1 ml liquid was spread on MacConkey Agar (Hi Media. India), and incubated at 44 °C for 48 hr. *E. coli* colonies were detected depending on their morphological characteristics and confirmed through further tests including biochemical tests for *E. coli*, the presence of more than 10^2 CFU/g sauce has been considered as unsatisfactory marker according to CFS (2014).

Detection of *Salmonella spp.*

Detection of *Salmonella spp*. was performed in accordance with Buchanan and Gibbons (1974) where Bismuth Sulfite Agar (Hi Media. India) was used. The

dilution step was the same as previously described in the section of Enumeration of *Enterobacteriaceae*. The dilution was performed up to 10^2. Salmonella colonies were detected depending on their morphological characteristics (dark colonies with green hallow ring) and confirmed by further tests including biochemical tests. For *Salmonella spp*. Presence of more than zero CFU/25g sauce has been unsatisfactory according to (Centre for Food Safety (CFS), 2014).

Aerobic mesophilic plate count agar

Aerobic plate count (APC) was performed by pour plate method using plate count agar (PCA), 1ml of each dilution was seeded in duplicate which was incubated at 37 C for 48h 35±100C for 48±2h (Anandsynal et al., 2018; Jaja et al., 2018). Literatures indicates that the acceptable limit of Total Viable Count are $<10^4$ CFU/g (Anandsynal et al., 2018).

Results

All restaurant sauces samples were free from *E. coli* and *Salmonella spp*, except one sample in one of Independent Restaurant (Tomia) was suspected to be polluted with *Salmonella spp*., then Biochemical Tests have been done to make sure and the results were negative for all tests.

Biochemical Tests (Mikoleit, 2014):

- T.S.I (Growth on Triple sugar iron)
- Urease Test
- Indole Test.

Table 1: Microbial counts of the handmade sauce samples collected from Independent fast food restaurants

Test	**T.B.C (CFU/g)**	***Enterobacteriaceae* (CFU/g)**	***E. coli* (CFU/g)**	***Salmonella spp* (CFU/g)**
Minimum	1×10^3	2×10	-ve	-ve
Average	1.16×10^5	3.6×10^2	-ve	-ve
Maximum	2.3×10^5	7×10^2	-ve	-ve

According to table 1

In independent fast food restaurants, the minimum number of Total bacterial count was 1×10^3 CFU/g in Mayonnaise Am Ibrahim, while the highest count was in Taverna Mayonnaise 2.3×10^5 CFU/g which is unaccepted. The minimum and the highest both counts of *Enterobacteriaceae* 2×10 CFU/g and 7×10^2 CFU/g tomia Khawater Demashkia which is accepted, the average number of *Enterobacteriaceae* count was 3.6×10^2 CFU/g which is accepted.

Table 2: Microbial counts of the handmade sauce samples collected from Chain fast food restaurants

Test	T.B.C (CFU/g)	*Enterobacteriaceae* (CFU/g)	*E. coli* (CFU/g)	*Salmonella spp* (CFU/g)
Minimum	2.4×10^3	4×10	-ve	-ve
Average	6.91×10^5	6×10	-ve	-ve
Maximum	1.38×10^6	8×10	-ve	-ve

According to table 2:

in Chain restaurants, the minimum number of total bacterial count was 2.4×10^3 CFU/g in Hardees cheese sauce, while the highest count was in Pizza Hut Mayonnaise 1.38×10^6 CFU/g which is unaccepted, the minimum number of *Enterobacteriaceae* count 4×10 CFU/g Kentucky Fried Chicken Mayonnaise while the highest count was 8×10 CFU/g Mayonnaise Pizza Hut, the average number of *Enterobacteriaceae* count was 6×10 CFU/g which is accepted.

The results indicated that the average number of total bacterial count is unaccepted in Independent fast food restaurants as the number is 1.16×10^5 CFU/g. The average number of T.B.C in Chain fast food restaurant was 6.91×10^5 CFU/g which is unaccepted. The total number of total bacterial count was high and hazardous in both independent and Chain fast food restaurant. *Enterobacteriaceae* count was higher in independent fast food restaurants than Chain fast food restaurant, that mean that sauces in independent restaurant polluted by the

handler more than the Chain restaurant and the T.B.C was higher in Chain fast food restaurant more than independent fast food restaurants that means bacteria polluted sauces in the surrounding environment more than the handling by workers.

MacDonald's sauces handmade ore preserved were free from *Enterobacteriaceae*. Domino's Pizza Sauces were free from *Enterobacteriaceae*, but they are contaminated by Total bacterial count as Barbeque sauce has 1×10^4 CFU/g and Ranch sauce 5×10^3 CFU/g which is unaccepted. Hardees cheese sauce was free from *Enterobacteriaceae* contamination.

Antibacterial effect of acetic acid against *E. coli*

Due to Mixed Mayonnaise and Ketchup sauce (with pickled cucumber) Pizza Hut was free from T.B.C, *Enterobacteriaceae*, *E. coli*, and *Salmonella spp*. Antimicrobial sensitivity test has been done by using three different concentrations of acetic acid (1%, 2.5% and 5%) inoculated in Muller Hinton agar plate (MHA), after swabbing of isolated ***E. coli*** colonies and the petri dish incubated at 37°C for 24 hr. The zone of inhibition were measured in diameter according to Lingham (2013) The diameter of the inhibition zone was (-ve, 20 mm and 24mm) respectively (Figures 3&4). This indicate that acetic acid of high concentration 5% has the antibacterial action against *E. coli* and this result is agree with Wali & Abed (2019).

Figure 1: MacConkey agar enrichment media for *E. Coli*

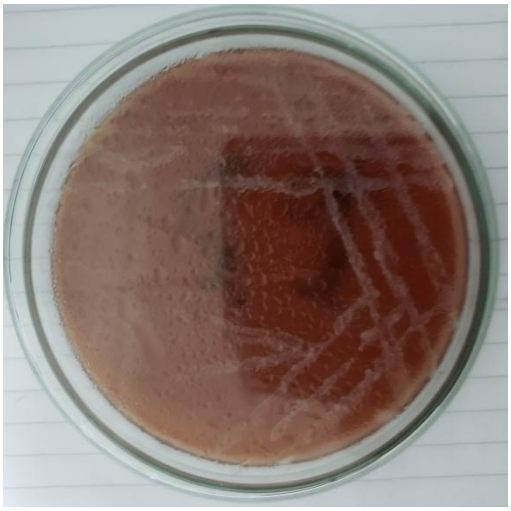


Figure 2: *E. Coli* isolated colonics

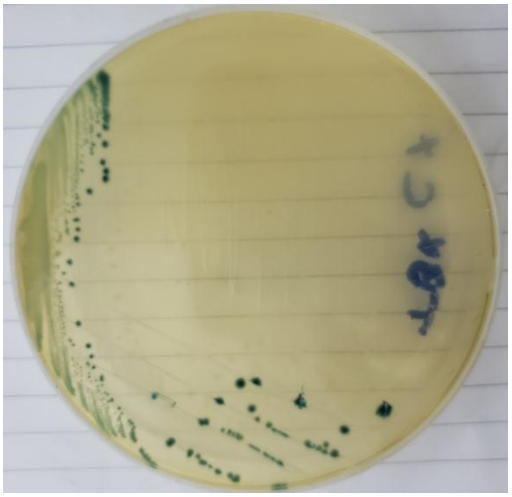


Figure 3: Before incubation

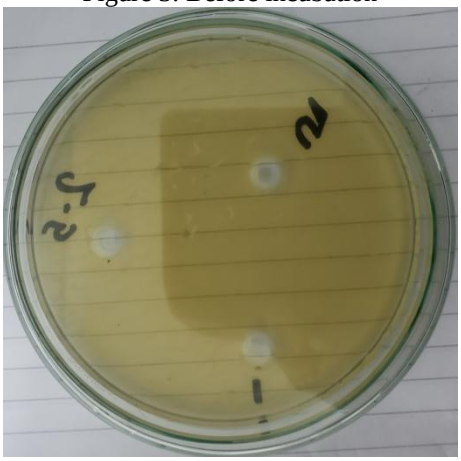
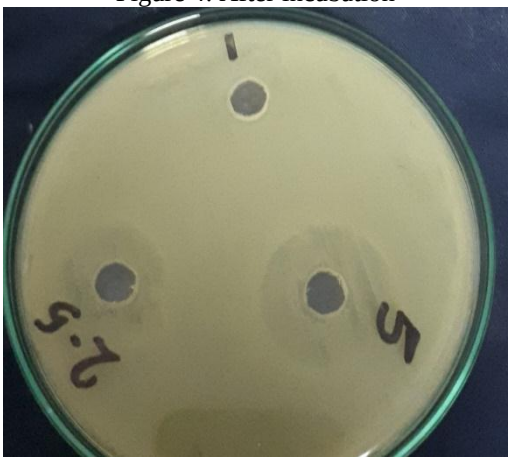


Figure 4: After incubation



1% Acetic acid Concentration.
2.5% Acetic acid Concentration.
3.5% Acetic acid Concentration.

Discussion:

The results in another study indicated that all the sauce samples contained viable Enterobacteriaceae cells; whereas 80% and 83.33% of the total samples were found to be contaminated with *Salmonella* spp. and *Escherichia coli*, respectively. Total viable bacterial cells found in the samples ranged from 1.2×103 to 4.2×109 Colony Forming Unit (CFU)/g. In addition, total Enterobacteriaceae and *E. coli* counts ranged from 30 to 2.0×107 and from 0 to 7.0×105 CFU/g, respectively. Although Plam sauce samples contained a higher amount of Enterobacteriaceae and *E. coli* compared toTomato sauce samples TS, no significant difference (p>0.05) was found. (Hossain & Dey, 2019).

There is a study resulted in Standard plate count of different types of sauce and ketchup varied from 100 - 300 CFU/g But no Coliform was detected in the selected sauces and ketchups(Anandsynal et al., 2018). There is a study was performed for study antibacterial effects of acetic acid against different types of bacteria causes food spoilage bacteria The isolates studied showed sensitivity to the range (16 mm to 18 mm) at concentrations (1%), the concentrations (1.5%) to the range (20 mm to 22 mm). However, the concentrations (2%) to the range (22 mm to 27 mm) and (27 mm to 35 mm) at concentrations (2.5%) Wali & Abed (2019) and this is agree with the side results of our study.

Conclusion

- The results of this study revealed that the consumption of sauces foods is of great concern in Alexandria restaurants. The microbial quality of sauces used for the preparation of sandwiches must be improved to reduce of health risk in consumers.

- All sauces samples were free from *E. coli* bacteria and *Salmonella spp*. This is due to high concentration of spices, herbs and acetic acid which used in making the handmade sauces in Chain and Independent Fast Food Restaurants in Alexandria.

- The total aerobic mesophilic plate count in both Chain fast food restaurants and Independent fast food restaurants was unaccepted as the average number was 6.91×10^5 CFU/g and 1.16×10^5 CFU/g respectively.

- The total *Enterobacteriaceae* was accepted in both Chain fast food restaurants and Independent fast food restaurants as the average number was 6×10 CFU/g and 3.6×10^2 CFU/g respectively.

- Independent Fast Food Restaurants has higher count of *Enterobacteriaceae* contamination it is due to unhygienic personnel practices during sauce preparation, while Chain Fast Food Restaurants has

higher count of total bacterial count due to post-contamination, between preparation and consumption. Independent Fast Food Restaurants has higher rate of sauces consumption rather than the Chain Fast Food Restaurants.

Side result:

- Antibacterial action of Acetic acid used in mixed sauce of Ketchup and Mayonnaise with (pickled cucumber) Pizza hut makes the sauce free from all examined bacteria in this study.

Recommendations

- Addition of Acetic Acid 5% concentration has strong antibacterial action and effective against T.B.C, *Enterobacteriaceae*, *E. coli* and *Salmonella spp*.
- Ensure good hygienic practices especially in Independent Fast Food Restaurants.
- The area of storage and surroundings the sauces must be clean and free from any contamination especially in Chain Fast Food Restaurants and insure sanitary preparation of sauces.
- The workers in both Chain and Independent Fast Food Restaurants have to take courses about hygienic practices in sauces preparation.

Limitation

According to the results Suggested researches for further study: antimicrobial action of acetic acid against *Salmonella spp*.

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