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Original Research

Microbial Contamination Level of Tamarind Sauce Prepared in Several Seafood Restaurants

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Abstract

Microbiological contamination of ready-to-eat food (RTE) has increased considerably due to poor personal hygiene practices and failure to maintain the cleanliness of surfaces and utensils during processing. Tamarind sauce is prepared in restaurants using a traditional method that involves soaking the pulp in water before extracting it ready for consumption. This study carried out in Jeddah city, aimed to evaluate the microbial contamination level of tamarind sauce according to the Standardization Organization for GCC (GSO). All the samples were revealed to be of unsatisfactory microbial quality, as significant levels of contamination were observed overall, as measured according to the GCC (GSO). The percentage 60% of samples containing a standard bacterial count averaged 2.98 $\log_{10}$ cfu/ml. Coliform bacteria were also detected and estimated at 80%. An unacceptable percentage was recorded for *Salmonella* sp. contamination, as it was found at a rate of 100%. Staphylococci also showed a high percentage and were estimated at 80%. Yeasts and molds were also detected 60%. The results obtained highlight the importance of hygienic auditing and managing employees' role in fulfilling commitments to health standards and legislation, in addition to raising communities' awareness of the risk of RTE products to public health.

Keywords: tamarind contamination, public health, RTE foods, food hygiene, food microbiology

Introduction

Many recent studies have confirmed that dietary habits play an important role in maintaining public health. Undoubtedly, eating habits can be implicated in the spread of chronic and sometimes fatal diseases. Indeed, the most common diseases to have occurred in the most recent century can be linked to food consumption behavior. For example, obesity, diabetes, cardiovascular diseases, microbial inflammation [1, 2], and more recently respiratory diseases such as coronavirus disease 2019 (COVID-19) [3]. A variety of different types of microorganisms are linked to pathogenic infections associated with food, such as viruses, bacteria, parasites, fungi, and yeasts. Significantly, multiple factors, in addition to eating habits, can contribute to the transmission of microbial infections through food, including levels of hygiene [4], social status [5], economic level [6], occupational health [7], level of education [8], geographical location [9], consumer behavior [10], urbanization [11], multiculturalism [12], and age and gender [13].

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According to the World Health Organization overview, approximately 600 million people – approximately 1 in 10 people worldwide – become ill from eating contaminated food [14, 15]. In addition, an estimated 420,000 people die annually caused by foodborne diseases [16]. Economically, the losses from eating contaminated food in low- and middle-income countries are estimated to be approximately 110 billion US dollars each year, calculated according to loss of productivity and medical expenses. Furthermore, 40% of children under five years old suffer from foodborne diseases, resulting in 125,000 deaths every year [17]. Notably, foodborne diseases disrupt sustainability projects as well as social and economic development by burdening healthcare systems, damaging economies, and negatively impacting national tourism and trade [18].

In recent years, the tremendous developments within the food related industries have significantly altered consumer behavior. For example, it has become increasingly common to eat special foods to support specific health needs, such as raw foods, spiced foods, and cold and warm foods or drinks. This includes meat products, seafood products, aquatic products, and plant products. Such dishes are historically associated with infectious microbial diseases, including a huge number of pathogenic microorganisms that can result in infections. The bacteria most frequently linked to food related disease are *Esherichia coli, Salmonella* sp., *Shigella* spp., *Bacillus cereus, Cronobacter sakazakii* [19]*, Listeria monocytogenes* [20], *Clostridium botulinum*, *Clostridium perfringens, Staphylococccus aureus, Yersinia enterocolitica,* and *Vibrio* spp. [21], with the most public pathogenic viruses being Hepatitis and Noroviruses [22-24]. Furthermore, several common pathogenic species of fungi and yeasts can cause food infections, such as *Mucor circinelloides* [25], *Rhizopus species* [26], and *Candida* species present as food-borne yeast [27].

Tamarind pulp is the fruit of the Tamarind leguminous tree species (*Tamarindus indica*), which is from the Fabaceae family. Tamarind trees are widespread in India and Africa, as they are typical in semi-tropical and semi-arid regions [28]. Tamarind is served with foods in a variety of forms, such as in sauces, beverages, jams, juices, and ice cream, and as a pickling agent for different foods [29]. Tamarind extract, or tamarind sauce, is one of the most popular traditional sauces in the Middle East. Tamarind pulp contains a combination of cellulosic materials, sugars, proteins, fibers, tartaric acid, and pectin, along with various minerals such as magnesium, phosphorous, potassium, and calcium [30].

Tamarind sauce is prepared by simply mixing the fruit pulp with water before blending it and leaving it to thicken for 2-3 hours at room temperature. The sauce is then extracted by percolating the suspension. Tamarind sauce is recognized as a medium in which the growth of pathogenic microorganisms can occur for diverse reasons; for example, it contains nutrients and a suitable pH level, and is served cold and stored at room temperature or in a refrigerator [31]. Thus, it is never exposed to elevated temperatures that would reduce its microbial load [32, 33]. In the present study, the microbial load of tamarind sauce was determined by collecting samples from different seafood restaurants with varying hygiene levels in Saudi Arabia- Jeddah. Ascertaining the level of contamination in this sauce was considered an important objective as it is widely consumed.

Material and Methods

Collection of Samples

Tamarind sauce samples are usually prepared in traditional ways in restaurants and are then packaged manually in small plastic bottles. A total of 60 samples were collected from six different public seafood restaurants in Jeddah City Saudi Arabia. Each 10-sauce sample was taken from a single restaurant and then immediately moved under standard conditions to the laboratory in a box containing ice before being stored at –20ºC prior to examination. The samples were divided into six sets according to the different restaurants they were collected from. The sets were numbered as Groups A, B, C, D, E, and F.

Temperature and pH Determination

The temperature of each sample was determined immediately upon collection using a portable temperature electrode (Ohaus STTEMP30) before placing it in an ice box. Meanwhile, the pH level of each sample was measured upon its arrival at the laboratory using a digital pH meter (Ohaus Starter 3100 pH Bench).

Microbiological Analysis

Standard methods were implemented to estimate the microbiological quality of the samples collected. The colony counter (Funke Gerber, Colony Star 8502-3038, Germany) was used to isolate and count the colonies of the microbial groups. The colonies obtained from duplicate samples with suitable dilution was recorded as colony forming units per gram (log_{10} cfu/ml) of sauce sample.

Determination of Total Viable Bacteria

The total bacterial count was performed using the standard methods of Aerobic Plate Count (APC) in non-fastidious nutrient agar, HiMedia M001. The estimated number of colonies were recorded as (log_{10} CFU/ml) units for each sample subjected to the pour plate (PP) method [34]. The Petri dishes were then incubated at ±37ºC for 24-48 hours.

Enumeration of Coliform

To assess the total Enterobacterales, the medium of Violet Red Bile Agar HiMedia M049S [35] was used. Then, after sample inoculation, the plates were transferred to the incubator at ±37ºC for total coliform and ±44ºC for fecal coliform, respectively, for 48 hours.

Enumeration of *Staphylococci* sp.

In order to determine the staphylococcal counts, the Baird Parker Agar Base HiMedia M043S was used [36].

Detection of *Salmonella* sp.

Detection of *Salmonella* strains was performed using Bismuth sulphite agar medium, HiMedia MU027 [37].

Molds Determination

The mold count was estimated using the Rose Bengal Chloramphenicol Agar HiMedia M640 as a selective enumeration medium for yeasts and molds [38].

Statistical Data

The data obtained was recorded using Excel software, and the graphs were displayed. SPSS software was used for the statistical data analysis for each sample and the samples overall.

Results and Discussion

The study determined the level of microbial contaminants present in tamarind sauce using 60 samples obtained from different seafood restaurants. A total of 10 samples were examined from each restaurant and labeled as either A, B, C, D, E, or F to denote the restaurant from which it originated. The temperature of the samples was found to be above refrigeration temperature, in the range of 10 to 16ºC, with a few exceptions. The pH of all the samples was in the range of 7-8, which provides alkaline growth conditions that favor bacterial growth. The results obtained showed the highest average of the microbial load was recorded in restaurant (A), with a total bacterial count of ±2.98 log_{10} cfu/ml, and the lowest average was for yeasts and molds at ±0.748 log_{10} cfu/ml. The average number of coliforms, *Salmonella* sp., and staphylococci were ±2.753, ±2.57, and ±1.869 log_{10} cfu/ml, respectively, as shown in Fig. 1.

The samples collected from restaurant (B) also showed the average bacterial total count estimated was ±2.99 log_{10} cfu/ml, giving the highest average. The yeasts and molds total count was the lowest average number at ±1.831 log_{10} cfu/ml. Total coliform was detected in all samples, but the results revealed variations between the samples, with the average number estimated as 2.9 log_{10} cfu/ ml. As presented in Fig. 2, *Salmonella* sp. and staphylococci were also isolated from samples (B) at ±2.69, ±1.782 log_{10} cfu/ml.

Fig. 3 showed the average microbial number present in the samples obtained from restaurant (C). As detailed, the bacterial total count and coliform were the highest levels of microorganisms, estimated as ±2.98 and ±2.90 log_{10} cfu/ml, respectively. After that, *Salmonella* sp. was estimated as ±2.76 log_{10} cfu/ml. The lowest average number was recorded for staphylococci as ±1.40 log_{10} cfu/ml, and then for yeasts and molds at ±2.01 log_{10} cfu/ml.

The average bacterial total count was estimated to be ±2.98 log_{10} cfu/ml in restaurant (D)'s samples. Coliform and *Salmonella* sp. were also found in all samples to

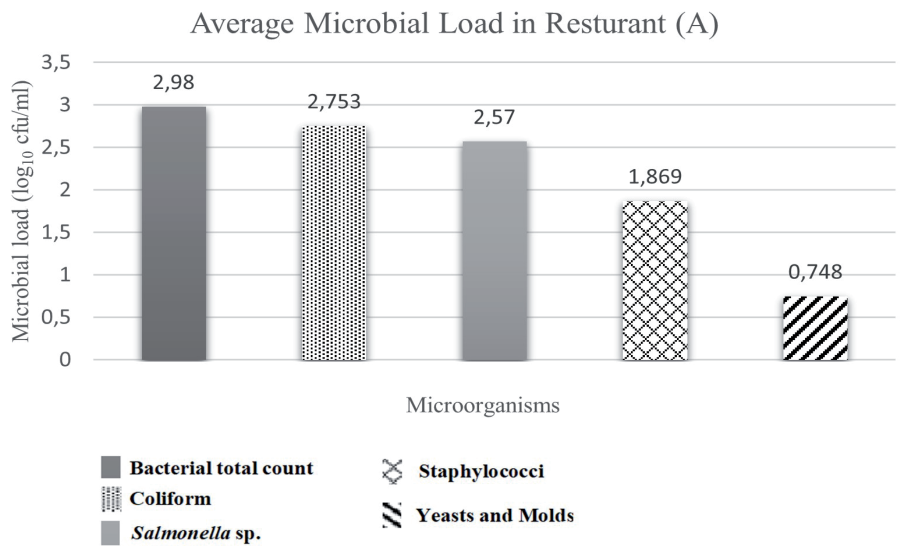

Fig. 1. The average microbial load by bacterial total count, coliform bacteria, *Salmonella* sp., staphylococci and yeast with moulds in restaurant A.

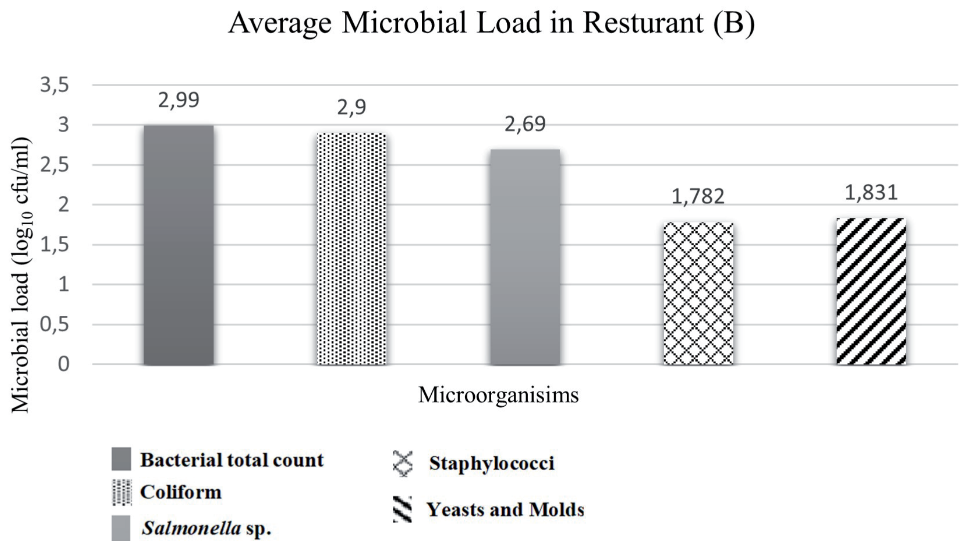

Fig. 2. The average microbial load by bacterial total count, coliform bacteria, *Salmonella* sp., staphylococci and yeast with moulds in restaurant B.

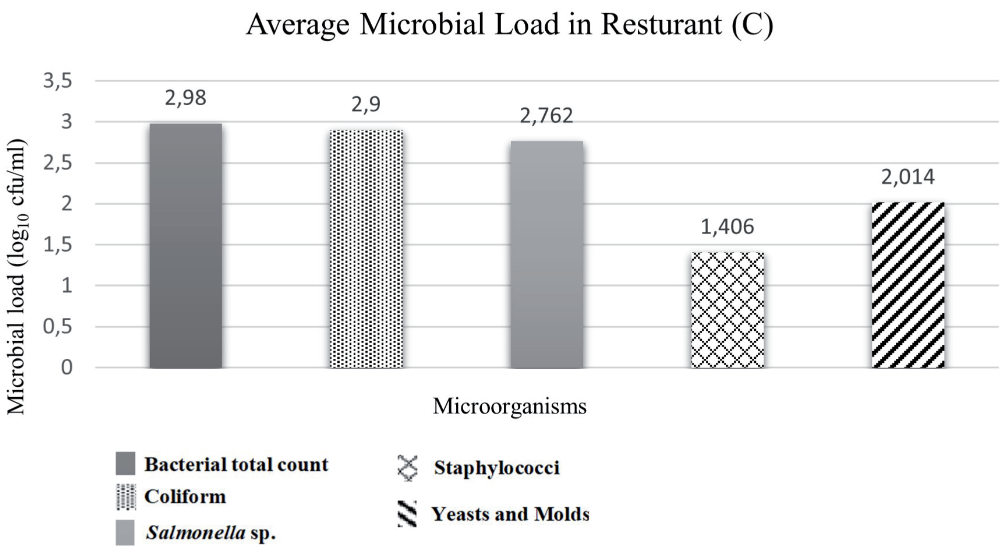

Fig. 3. The average microbial load by bacterial total count, coliform bacteria, *Salmonella* sp., staphylococci and yeast with moulds in restaurant C.

have an average count ±2.75 and ±2.67 log_{10} cfu/ml. Averages detected for staphylococci with yeasts and molds were ±1.31 and ±0.64 log_{10} cfu/ml, as shown in Fig. 4.

Together, the total bacterial total count, coliform, and *Salmonella* sp. detected in restaurant (E)'s samples by average amount were ±2.97, 2.76, and ±2.64 log_{10} cfu/ml. *Salmonella* sp. was also isolated in the samples and estimated as an average ±1.36 log_{10} cfu/ml, while yeasts and molds reportedly had the lowest average within the samples at ±0.61 log_{10} cfu/ml, as presented in Fig. 5.

As shown in Fig. 6 the samples collected from restaurant (F) did not differ significantly from the other restaurants. The bacterial total count average was recorded as ±2.97 log_{10} cfu/ml. Other microorganisms, such as coliform and *Salmonella* sp. returned averages of ±2.69 log_{10} cfu/ml and ±2.57 log_{10} cfu/ml, respectively. Staphylococci average was ±1.29 log_{10} cfu/ml, and yeast and molds averaged ±0.60.

Therefore, the results indicate that all the samples collected from the different restaurants show an abundance of bacterial contamination when compared to other yeasts and molds. As presented in Fig. 7 the highest average number for all the samples collected from all restaurants (A, B, C, D, E, and F) was recorded for the total bacterial count at ±2.98 log_{10} cfu/ml. After which the average number of coliform bacteria was ±2.79 log_{10} cfu/ml and then *Salmonella* sp. by ±2.65 log_{10} cfu/ml. The lowest average number across

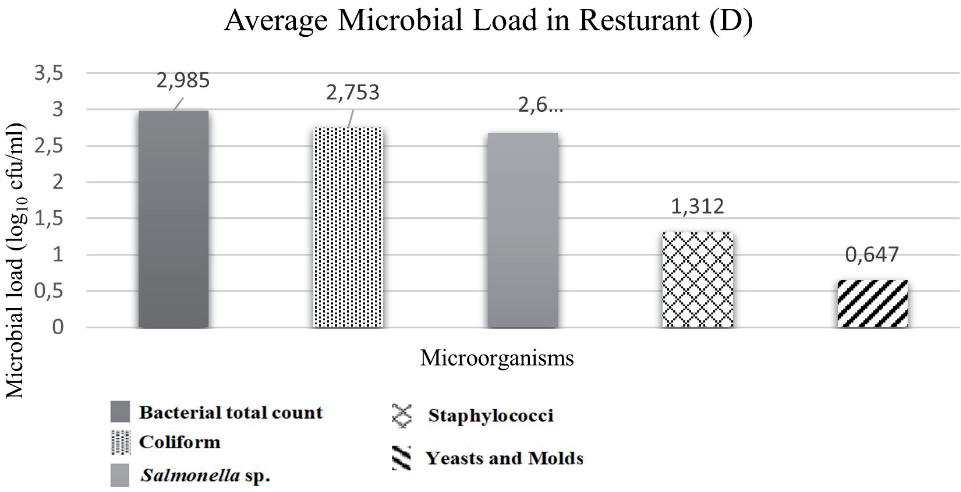

Fig. 4. The average microbial load by bacterial total count, coliform bacteria, *Salmonella* sp., staphylococci and yeast with moulds in restaurant D.

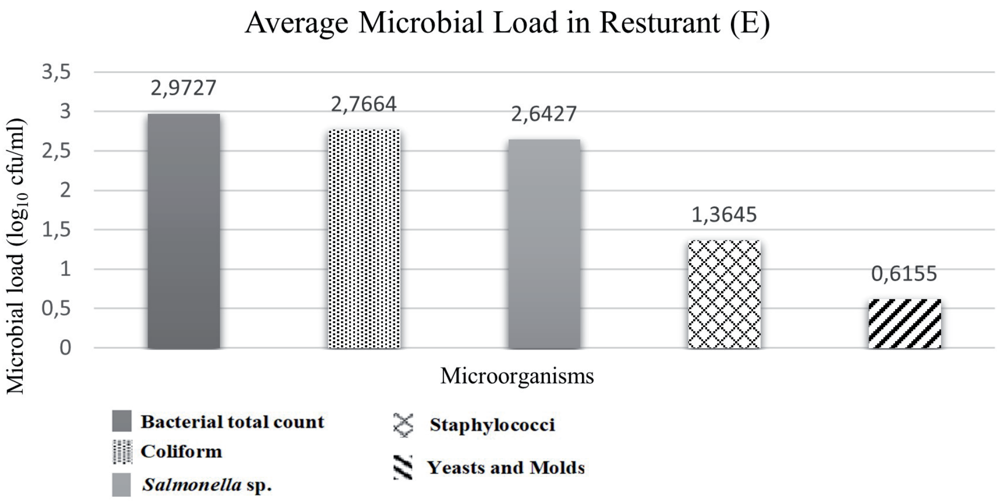

Fig. 5. The average microbial load by bacterial total count, coliform bacteria, *Salmonella* sp., staphylococci and yeast with moulds in restaurant E.

all the samples was recorded for staphylococci, yeasts, and molds, at ±1.50, ±1.07 log_{10} cfu/ml, respectively.

The study took place in Jeddah city, which is a coastal city in Saudi Arabia, throughout which seafood restaurants are broadly distributed. Tamarind sauce is consumed locally as a favorite sauce and is provided chilled with the main seafood meal, such as fish and shrimp. The microbial content of tamarind sauce manufactured with raw tamarind pulp has been acknowledged as a significant concern and public health risk, as it is prepared in a traditional way inside restaurants by workers. There are no local specific standards for tamarind sauce production. In this study, 60 samples were collected and analyzed to determine the level of pathogenic microbial contamination. The average microbial enumeration was determined for the total count of aerobic bacteria, coliform bacteria, *Salmonella* sp., staphylococci, yeasts, and molds. The results obtained from each restaurant revealed a significant rate of contamination in all 60 samples, with some differences in contamination level between samples and by type of contaminant. An average for the total microbial range for all samples was determined as 2.98 log_{10} cfu/ml, which is considered more than the desired range of GSO [39, 40], based on 60% of total samples. Although few prior studies have focused on the microbial quality of tamarind sauce, in a study of microbiological quality determination of tamarind drink in Jordan, the amount of APC was also found to be unacceptable in terms of quality and estimated at 2 to 5.8 log_{10} cfu/ml [41]. The coliform bacteria in the current study was also detected in an unacceptable range at an average 2.79 log_{10} cfu/ml for all samples, and estimated as present in 80% of total samples. The highest volume of contaminant recorded according to the GOS standard was for *Salmonella* sp. at an average 2.65 log_{10} cfu/

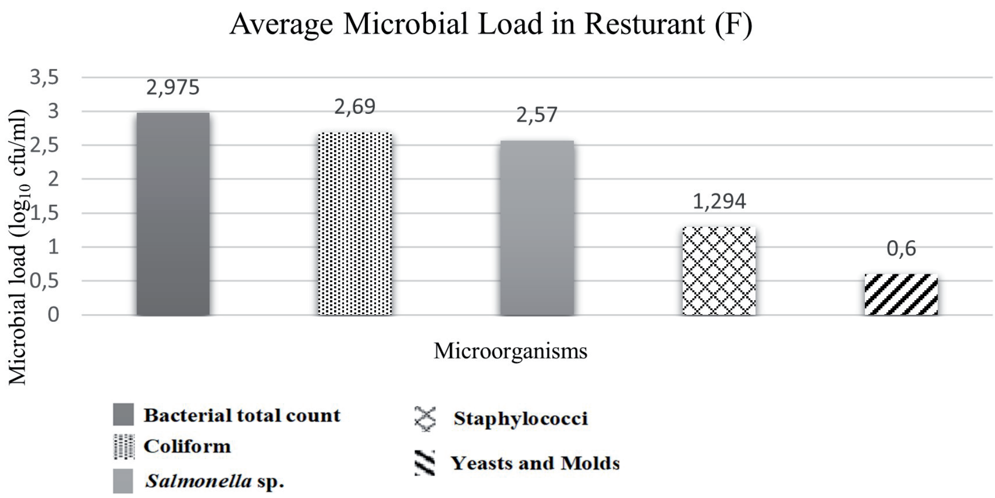

Fig. 6. The average microbial load by bacterial total count, coliform bacteria, *Salmonella* sp., staphylococci and yeast with moulds in restaurant F.

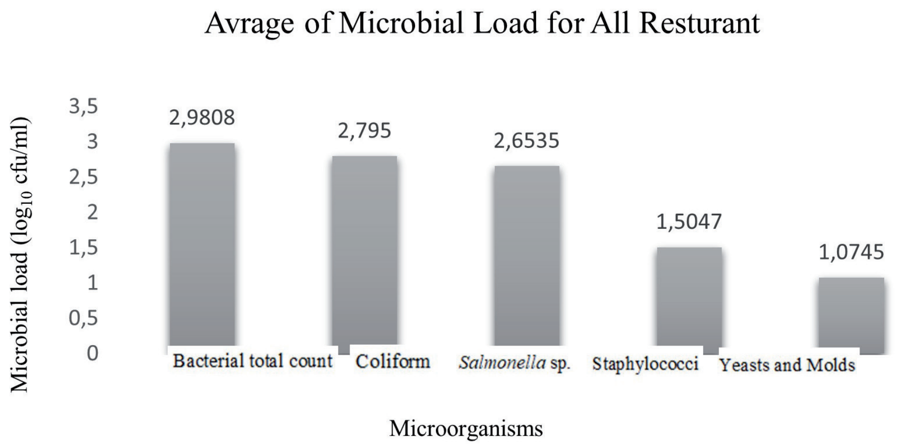

Fig. 7. The average microbial load by bacterial total count, coliform bacteria, *Salmonella* sp., staphylococci and yeast with moulds for all restaurants.

ml, with a percentage of *Salmonella* sp. detected in all samples (100%) at varying levels. These findings are attributed to the almost complete lack of personal hygiene and food handling practices [42]. Staphylococci were also shown to be high, with an average estimated at 1.50 $\log_{10}$ cfu/ml for the samples overall, being present in approximately 80% of the samples. Linked to the fact that the pH of Tamarind sauce is alkaline, a percentage of 60% of yeasts and molds was detected in the samples, estimated at 1.07 $\log_{10}$ cfu/ml.

As the sauce is served chilled, this can contribute to the spread of pathogenic microbes, especially when quality requirements are not applied [43]. The microbial quality of sauces from a takeaway restaurant in the UK found 5% of 1208 samples were of unsatisfactory microbial quality, due to containing *Escherichia coli*, *Staphylococcus aureus* at $\geq 10^2$ cfu g^{-1} and *Salmonella* sp. [44]. Unsatisfactory microbial levels of APC, *E.coli*, *S.aureus*, *Salmonella* sp., yeasts, and molds were also detected in tamarind prunes (dried plums) [45]. Meanwhile, another study determined the effect of storing tamarind juice for 180 days at room temperature (~28ºC) and showed no presence of coliform bacteria or *Salmonella* sp. [46]. It is clear that the level of microorganisms tested in this study exceeds the standard requirement for the majority of samples, despite some differences in microbial load between the restaurants, reflecting different sauce preparation protocols at each restaurant. The microbial level indicators for the tamarind sauce samples reflect a weakness in the quality of the raw ingredients used in the preparation. In addition, there is also a high possibility that there is a major defect in the application of personal hygiene conditions among workers in these restaurants [47]. However, the presence of coliforms does not only arise due to fecal contamination. Coliforms can be found in the wider environment on tools and in soil, plants, and water. Nevertheless, the study does reflect poor hygiene practices during food production leading to high levels of bacterial contamination.

Conclusions

The study revealed that the tamarind sauce sold in some seafood restaurants in Jeddah city was not safe for human consumption and could be deleterious to health, resulting in food poisoning of consumers as it contains higher than permissible standards of potential pathogenic microorganisms. The contamination may be attributable to the source of the raw materials used in its preparation, poor personal hygiene of staff, and/or improper food handling. It is recommended that proficient hygiene practices be implemented throughout the various preparation stages of the final product. Factors that can be addressed to help minimize microbial growth and contamination such as temperature and pH should also be considered. It is also necessary to focus on increasing awareness among workers to increase their knowledge of personal hygiene when handling different foods. In addition, strict application of laws and restrictions by regulatory authorities is recommended. With the gradual introduction of effective standards to regulate this field, such as (HACCP) hazard analysis and critical control points, it is hoped that food safety will improve.

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Conflict of Interest

The authors declare no conflict of interest.

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