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ORIGINAL ARTICLE

Prevalence of Enteric Pathogens in the Prepackaged Salads in Makkah City, Saudi Arabia

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ABSTRACT

Background: *Ready-to-eat foods are exposed to contamination in several ways either during harvesting, gathering, or processing. Food contamination with pathogens is a serious concern.* ***Objective:*** *The study amis to detect pathogenic bacteria in ready meals especially the prepared salads in restaurants in Makkah city. Methodology: A total of 110 samples of two types of salads, (55 sliced green salad and 55 crushed salad) were randomly collected from 55 different restaurants. Several types of culture media were used for the isolation of the pathogenic bacteria from salads samples and the identification of pathogenic bacterial strains was done using traditional and automated methods (VITEK2).* ***Results:*** *The current study results showed that Enterococcus faecalis recorded the highest percentage (89%), while Acinetobacter hemolyticus recorded the lowest percentage (1.8 %) among the isolated pathogenic bacterial strains from crushed and sliced green salad samples.* ***Conclusion:*** *This study revealed that most of the salad samples were contaminated with pathogenic bacteria and the highest percentage was recorded among the sliced green salad samples. The high pollution rates indicate a failure to implement health requirements during the preparation of meals in restaurants.*

INTRODUCTION

Ready-to-eat foods are raw foods prepared after processing and supplied to the public that can be used without further processing. These foods are exposed to contamination in several ways, either during harvesting, gathering, or processing. These contaminated foods have a more significant threat to human beings leading to an increase in the food-borne illness[1]. Several outbreaks are reported due to the consumption of contaminated ready-to-eat foods. A study was conducted on Hajj pilgrims exposed to enteric pathogens such as Enteroaggregative *Escherichia coli*, Enteropathogenic *E. coli*, and Enterohemorrhagic *E. coli* [2,3]. Different risk factors are involved in contaminating the ready-to-eat food, enhancing the microbial load in the food. These risk factors include temperature, water, equipment, and workers. The varying temperature during transferring or storage of food promotes the growth of specific pathogens. Poor water quality and contaminated water also facilitate microbial growth.

The hygienic status of equipment and workers also plays an essential role in promoting microbial reproduction. While processing the vegetables, contamination may occur during rinsing, wiping, clippings, peelings, slicing, sterilizing, and packaging[4]. Food handling may consider a source of contamination because the workers carrying infections can contaminate the food through their hands and finger nails. Almost 80 % of human illness are linked to poor hygiene both personal and domestic[5,6]. In developing countries, food contamination due to foodborne pathogens has been a serious concern due to gastrointestinal problems in human beings [7]. These enteropathogenic organisms include Acinetobacter species, Citrobacter species (*Citrobacter koseri* and *Citrobacter freundii*), *E. coli* O157: H7, and Enterobacter species (Enterobacter *sakazakii* and *Enterobacter cloacae*), Klebsiella species (*K. oxytoca and K. pnemoniae*), Salmonella species (*S. typhimurium, S. enteritidis*), Shigella, Vibrio, Campylobacter, and Yersinia[8-10].

An investigation of ready-to-eat food in public places of Yogyakarta in Indonesia showed several enteropathogens. Apart from the entero pathogens mentioned above, some other pathogens found in this study are *Yersinia enterocolitica*, Pantoea species, *Aeromonas hydrophila*, Serratia species, *Proteus mirabilis*, and *Ewangella* americana[11] . Contamination due to poor hygiene and poor temperature control while preparing food is one of the root causes of transferring enteric pathogens to food.

In 2017, the Sudanese population had an outbreak of gastrointestinal infection in Jeddah city. After investigations, the enteric pathogens found were *Bacillus cereus* and *Clostridium perfringens* due to poor temperature control and hygienic problems [12]. Given the widespread foodborne pathogens in ready meals, the current study aimed to detect pathogenic bacteria in ready meals, especially salads in the restaurants of Makkah city, Saudi Arabia.

METHODOLOGY

Sample collection:

In February 2022, 110 samples of two types of salad (55 sliced green salad and 55 crushed salad) were randomly collected from 55 different restaurants in Makkah city. All samples were collected in sterile plastic bags and transferred in iceboxes to the Microbiological Research Laboratory at the Faculty of Medicine, Umm Al-Qura University, for microbiological examination.

Culture media:

The samples were cultured on selective culture media (HiMedia), Bile esculin agar, MacConkey agar, Mannitol salt agar, Sorbitol MacConkey agar, and Salmonella Shigella agar. As well as Muller Hinton Broth to help identify enteric pathogenic bacteria in the salads ready to eat.

Cultivation and identification:

Under aseptic conditions, a cotton swab was dipped in each sample of the salads used in this study and mixed with 1ml Mueller Hinton Broth in an Eppendorf tube to obtain a homogenized suspension. 100 µl of this suspension was transferred to each type of culture media and streaked with a sterile plastic loop; a vancomycin disc of 30 µg was applied on the bile esculin agar to determine whether the food sample was polluted with *Enterococcus faecalis* and resistant to vancomycin. All inoculated plates were lifted to dry for 10 minutes, then incubated for 24 hours at 37°C. After the incubation period, the plates were visualized for the presence of pathogenic bacteria according to the standard microbiological identification techniques. VITEK2 Compact system (Bio-Merieux) was used to identify pathogenic bacterial species.

RESULTS

A total of 110 salad samples were collected from 55 restaurants in Makkah city, including 55 samples of crushed salad and 55 samples of sliced green salad. Results of bacterial examination of these samples showed that the number and percentage of contaminated samples (positive samples) of crushed salad were 50 (91%) out of the total 55 samples. The contaminated samples of sliced green salad were 52 (94.5%) out of the whole 55 samples as shown in table 1.

Table 1. Geographical distribution of collected salads samples from restaurants in Makkah city

Distribution of samples	Collected samples according to geographical dimensions of Makkah city								Positive samples N. (%)
	North N=13		South N= 24		West N=5		East N=13		
	Positive samples N. (%)	Negative samples N. (%)	Positive samples N. (%)	Negative samples N. (%)	Positive samples N. (%)	Negative samples N. (%)	Positive samples N. (%)	Negative samples N. (%)	
Type of salads	13 (100%)	0 (0.00%)	20 (83%)	4 (17%)	5 (100%)	0 (0.00%)	12 (92%)	1 (8%)	50 (91%)
Sliced green salad	13 (100%)	0 (0.00%)	22 (92%)	2 (8%)	5 (100%)	0 (0.00%)	12 (92%)	1 (8%)	52 (94.5%)

N= Number

Results of the bacterial examination of the isolated pathogenic bacterial species on the selective culture media by traditional and automated identification methods (VITEK 2 system) confirmed the isolation of eight bacterial strains, *E. coli*, *K. pneumoniae*, *K. oxtoca*, *E. cloacae*, *A. haemolyticus* and *A. baumannii* were isolated on MacConkey agar as shown in table.2. As well as *Enterococcus faecalis* which was isolated on bile esculin agar. *Staphylococcus aureus* was isolated on mannitol salt agar.

The results showed that crushed salads were contaminated with six pathogenic bacteria species that included *E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. faecalis*, *S. aureus*, and *E. cloacae*, and the sliced green salads were contaminated with eight pathogenic bacteria, which included *E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. faecalis*, *S. aureus*, *E. cloacae*, *A. hemolyticus*, and *A. baumannii*. The results recorded the highest percentage of the isolated bacteria from crushed salads these were *E. faecalis* (89%), *E. coli* (36.4%), and *S. aureus* (10%),

while the highest percentage of the isolated bacteria from a sliced green salad were *E. faecalis* (92.7%), *E. coli* (63.6%), and *S. aureus* (45.5%).

The lowest percentage record of isolated bacteria from crushed salad was *E. cloacae* (3.6%), and the lowest percentage of bacterial isolates from sliced green salad was (1.8%) of both *A. haemolyticus* and *A. baumannii*, as shown in (table.2).

Table 2: Distribution of pathogenic bacterial isolates from salad samples

Bacterial isolates	Bacterial isolates from crushed salad (N=50)		Bacterial isolates from sliced green salad (N=52)	
	N.	%	N.	%
Escherichia coli	20	36.4 %	35	63.6 %
Klebsiella pneumoniae	4	7.2 %	3	5.5 %
Klebsiella oxytoca	6	10.9 %	4	7.2 %
Enterococcus faecalis	49	89%	51	92.7 %
Staphylococcus aureus	5	10 %	25	45.5 %
Enterobacter cloacae	2	3.6 %	9	11 %
Acinetobacter hemolyticus	0	0.00%	1	1.8 %
Acinetobacter baumannii	0	0.00 %	1	1.8 %

DISCUSSION

Improper practice during food preparation can lead to food contamination with pathogens. The widespread presence of germs demonstrates the high level of contamination in food, especially in fresh salads. The current study was designed to detect pathogenic bacteria in ready salads, especially salads, in the restaurants of Makkah city.

Our results showed that *S. aureus*, *E. coli*, and *E. faecalis* recorded as the highest percentage of isolates from salads samples, whereas *E. coli* was isolated in 36.4% of sample, *E. faecalis* 89%, and *S. aureus* in 10% of the organisms isolated from the crushed salad. These three bacterial strains recorded the highest percentage of isolated bacterial strains from sliced green salads at rarates3.6%, 92.7%, and 45.5% , respectively. While, *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, *A. hemolyticus*, and *A. baumannii* recorded the lowest contamination in both crushed and sliced green salad. Previously, researchers demonstrated that when vegetables were irrigated with sewage water and the soil was treated with manure, this leads to contamination with germs [13].

Contamination of food occurs when vegetables are washed in polluted water, utensils are improperly washed or salad is prepared in a contaminated setting also, because of the contamination of hands or the cooks and the lack of application of health precautions during the preparation of food [14,15].

A study on five salad vegetables, lettuce, cucumber, parsley, carrots, and green onion, collected from local markets in Riyadh, Saudi Arabia, revealed high levels of fecal coliform bacteria [13]. A study on five salad vegetables, lettuce, cucumber, parsley, carrots, and green onion, collected from local markets in Riyadh, Saudi Arabia, revealed high levels of fecal coliform bacteria. The same study revealed high levels of fecal coliform from 200 salad samples taken from Riyadh restaurants. *E. coli* was found in 87.5% of Mutable salads, 67.5% of Fattoush and Hummus, and 50% of Tabbouleh. These results were consistent with the results of our study.

According to a study conducted in Pachuca, Mexico, 99% of salad samples from all restaurants were contaminated with fecal coliform[16]. The prevalence of fecal coliforms is higher than that reported for ready-to-eat (RTE) salads in other developing countries, such as Costa Rica and Brazil, where the prevalence of fecal coliforms in salad samples was recorded at 89% and 73% respectively[17].

Coliforms in general and in particular *E. coli* are indicators of fecal contamination. Lettuce has been identified as a significant source of Shiga toxin-producing *E. coli* (STEC) among diverse plants. One probable explanation for the high incidence of *E. coli* isolates in leek, radish, and lettuce is that these vegetables could be contaminated by animal manure through agricultural soil, which is one of STEC's main sources [18.] Due to vegetables being frequently irrigated with contaminated water and being in close contact with soil and animal feces, *E. coli* is spread by dirty water, soil, human excrement, and animal dung, and vegetables can readily become infected in the field.

Cross-contamination can also happen during the harvesting and processing phase. Shiga toxigenic *E. coli* (STEC) infections can cause serious clinical consequences like hemolytic uremic syndrome and

hemorrhagic colitis. Many studies reported that *S. aureus* was found in most salad vegetables, this is due to its importance in causing a wide range of diseases, particularly food-borne poisoning[19]. *S. aureus* infection has been associated with carriage in the nasal passages of food handlers and infected personnel; however, *S. aureus* was isolated from 50% of personnel's nasal cavities, 60% of their kitchen jackets, and 50% of their gloves, respectively[20].

In Australia, almost 1300 cases of food poisoning caused by *S. aureus* are documented each year [21]. A study conducted in Quetta, Pakistan, showed a 54% prevalence of *S. aureus* in salads, with 59% of isolates being coagulase-positive[22]. Bacterial contamination of fresh foods high in carbohydrates provides a perfect environment for germs to multiply[23]. In comparison to the aforementioned, *Enterococcus faecalis* was isolated from the salad samples in the current study and recorded the highest percentage in both Crushed and sliced green salad among the isolated strains, 89%, and 9.7 %, respectively. *E. faecalis* make up a significant component of the autochthonous bacteria found in the gastrointestinal system of mammals because of their amazing ability to resist or develop in hostile conditions [24,25].

However, worries regarding enteric pathogens introduced to the supply food chain have arisen as a residue of animal manure-based fertility inputs in organic fruit, vegetable, and salad crops. Furthermore, a recent report on different pathogens linked to vegetables in the United States emphasized that the pathogen-transfer is connected with outdoor/free-range cattle agriculture[26]. All these types of pathogenic bacteria originate from animal sources and become a major cause of contamination and diseases. Based on the above statement, many studies worldwide confirmed the spread of bacterial pathogens in different types of ready-to-eat (RET) salads. Also, many of these studies agreed with the results of our current study with variations in the levels of microbial contamination in different types of salads.

The current study also focused light on the mode of transmission of pathogens to humans through eating ready-to-eat salads sold in Makkah restaurants, which could cause food poisoning if these salads are prepared under unacceptable environmental conditions and non-adherence to health requirements. The occurrence of food poisoning, and preventing its spread requires strengthening health control over these restaurants to avoid potential risks as a result of these malpractices. Standard operating procedures should be applied to inhibit microbial contamination in ready-to-eat processing foods. We advise continuing research on this important topic to cover the parts that were not covered in the study, including other types of diets.

CONCLUSION

This study was conducted on 110 samples of two types of salad, crushed salad and sliced green salad collected from fifty-five restaurants in Makkah. The results revealed that most of these samples were contaminated with pathogenic bacteria such as *E. coli*, *E. faecalis*, and *S. aureus* which can spread some diseases and may acquire an epidemic situation and posing health risks to humans. The high pollution rates indicate that workers in this field are not sufficiently aware of the role of food in transmission of pathogens. Also, this pollution is due to the lack of personal hygiene of employees, the improper environment for preparing food, and the failure to implement health requirements in these restaurants. Hence, it is recommended that all restaurants and fast-food outlets must apply health standards of food safety and continuous monitoring by health authorities on these restaurants to ensure the application of health requirements and standards for this important commercial activity.

Competing interests

The author declares that they have no competing interests.

Declarations:

Ethics approval was obtained from the Ethics Committee of the Faculty of Medicine (approval number: HAPO-02-K-012-2022-02-946; Date: 11/02/2022)

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