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Quality assessment of ready-to-eat foods served in some cafeterias of Makkah City, Saudi Arabia

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Abstract. Ready-to-eat foods (RTEs) are items prepared in advance and ready for consumption only after heating without further processing or preparations. This study aimed to assess the microbiological incidence of ready-to-eat (RTE) foods served in some cafeterias located in Makkah city. A total of 108 samples of six different types of ready-to-eat food (foul, falafel, boiled egg, fried egg, shakshuka, and sheep liver slices) were collected randomly from six different cafeterias located in Makkah city. Samples were taken in sterile containers and transmitted immediately to the microbiology lab for analysis. Different media types were used to isolate and identify bacterial and fungal species. The results revealed that the highest aerobic plate counts were detected in sheep liver slices samples with the value of 8.4×10^3 CFU/mL, while the lowest was found in foul samples with a value of 0.5×10^3 CFU/mL. The highest count of *Staphylococcus aureus* was found in shakshuka samples with a value of 9.8×10^3 CFU/mL, and the lowest count was observed in falafel samples with a count of 1.2×10^3 CFU/mL. The current study's findings revealed that the total aerobic counts for *S. aureus* were high, and 42% of the samples were contaminated with *Escherichia coli* and exceeded the acceptable limit (0-10^3 CFU) ready-to-eat foods.

***Keywords**: Ready-to-eat, food, microbial contamination, food safety, Makkah.*

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1. Introduction

Ready-to-eat foods (RTEs) are items prepared in advance and ready for consumption only after heating without further processing or preparations. It could be raw or cooked meals. RTE food items consumption has recently increased in the Kingdom of Saudi Arabia, mainly in holly Makkah city, due to the rapid demographic change characterized by a change in lifestyle and a large number of itinerant workers, visitors, and pilgrims resulting in a large percentage of the population taking ready-to-eat food especially as breakfast's meal. In addition, cafeterias that serve RTE foods provide cheap, economical, and easily accessed items. Some RTE foods items contain raw materials of animal origin, such as eggs, fish, meat, and poultry, which can easily get contaminated by different microorganisms, including bacteria and fungi (Ashenafi, 1995).

The cross-contamination of RTE foods with pathogenic microorganisms could occur during the processing and preparation of fillings and sandwiches (Oranusi et al., 2013; Rahman et al., 2014; Sharma et al., 2014). Contamination of RTE food by pathogenic microorganisms like *Staphylococcus aureus*, *Salmonella* species, *Bacillus* species, and *Escherichia coli* leads to remarkable changes in food quality. Moreover, consequently causing food-borne illnesses and food poisoning are considered

major public health issues that negatively affect the socioeconomic development and output of many fields such as tourism and trading (Newman et al., 2015).

Food-borne diseases and food poisoning problems are becoming an important issue worldwide (Al-Mazrous, 2004). In the Kingdom of Saudi Arabia in general and holly Makkah city in particular, food-borne illness and food poisoning are emerging as significant public health threats that render a high burden of diseases and negatively affect the socioeconomic development and productivity of many sectors. Several studies have reported that foods served by catering services were the primary source of many food-borne outbreaks (Osimani and Clementi, 2016). In Makkah city, increasing food poisoning cases were reported, especially during the Hajj and Umrah seasons. It was mainly due to the high consumption of RTE meals, especially those from cafeterias (Shirah et al., 2017).

We think minimizing fungal and bacterial loads in RTE foods served in cafeterias within Makkah city by following good hygiene practices and continuous microbiological assessment programs is necessary. Moreover, using microbiological testing results of RTE foods in Makkah city will provide an example for other cities in Saudi Arabia for dealing with ready food. However, very little research has been carried out on assessing microbial quality and safety of RTE foods in Saudi Arabia, especially those distributed within Makkah city. Therefore, this study aimed to determine the microbial safety of RTE food items that are served in some cafeterias in Makkah city, Saudi Arabia.

2. Materials and Methods

2.1. Sample Collection

Ready-to-eat food samples were collected randomly from different cafeterias located in different sites of Makkah City during the period March-April, 2021. A total of 108 samples represented six different types of ready-to-eat food (foul, falafel, boiled egg, fried egg, shakshuka, and sheep liver slices) collected from six different cafeterias and replicated three times. Collected samples were handled in sterile plastic bags and directly transferred to the microbiology lab for investigation.

2.2. Microbiological Analysis

Isolation and enumeration of bacteria. Ten grams of each experimental sample was mixed with 90 mL Nutrient Broth (Biotech, UK), and serial dilutions of each food sample homogenate were prepared to reach 10^{-3} dilutions. A 1 mL aliquot portions of the prepared dilutions were precisely distributed onto duplicate sterile plates of Nutrient Agar (Himedia, India), Eosin Methylene Blue (EMB) Agar (Titan Biotech, India), and Mannitol Salt Agar (MSA) (India mart, India) for total microbial load, *Escherichia coli*, and *Staphylococcus aureus*, respectively. Counting of bacterial colonies was performed after incubation of agar plates at 37 °C for 24 to 48 h, by using the colony counter (Gallenkamp, England). Bacterial colonies were expressed as colony-forming units per mL of sample homogenate (CFU/mL).

Isolation and enumeration of fungi. For fungal isolation, 10 grams of each sample were mixed in Sabouraud broth (India mart, India), and the same dilutions were prepared. A 1 mL portion of the dilution (10^{-3}) was poured onto Sabouraud agar (India mart, India) medium supplemented with 0.005 gram/liter of rose Bengal (Sigma Aldrich, USA) for suppressing bacterial growth. The agar plates were incubated at 28 °C for 5-7 days; then, the developed colonies were counted and identified.

Identification of Microbial Isolates. The developed bacterial colonies were counted using colony counter, purified, and stored on nutrient agar slants at 4 °C for identification. Identification based on cultural morphology and biochemical tests, including carbohydrate utilization on Tri-sugar Iron (TSI) medium, IMViC test, starch hydrolysis, gelatin liquefaction, nitrate reduction, oxidase urease activity, and motility test were employed to confirm the purity of the isolates. The fungal morphology was studied macroscopically by observing the colony features (color, shape, size, and hyphae) and microscopically by a compound microscope according to the following references Gaddeyya et al. (2012), Domsch et al. (1980), and Barnett & Hunte (1972).

3. Results and Discussion

3.1. Bacterial species isolated from ready-to-eat foods samples

A total of 108 food samples, including 6 types (foul, falafel, boiled eggs, fried eggs, shakshuka, and sheep liver slices), were evaluated. The results showed in Table 1 that the highest aerobic plate counts were found in sheep liver slices sold by Cafeteria

No 5 with the value of 8.4 × 10^3 CFU/mL, while the lowest value was detected in foul sold by Cafeteria No.2 with a value of 0.5 × 10^3 CFU/mL. *S. aureus* appeared in all food samples collected from all cafeterias. The highest count (9.8 × 10^3 CFU/mL) for *S. aureus* was detected in shakshuka sold by Cafeteria No.6, while the lowest count (1.2 × 10^3 CFU/mL) was observed in falafel sold by Cafeteria 1. Generally, it could be reported that all the tested samples were highly contaminated with *S. aureus*, and 42% of the samples were contaminated with *E. coli*. The total counts for *S. aureus* and *E. coli* (in shakshuka and sheep's liver slices) exceeded the recommended levels by the International Commission on Microbiological Specifications for Foods (ICMSF, 1996). The ICMSF (1996) recommends that ready-to-eat foods between 0-10^3 CFU are acceptable, 10^4-10^5 CFU is tolerable, and 10^6 CFU and above are unacceptable.

Table 1. Mean total counts (CFU/mL) of each bacterial species isolated from ready-to-eat foods samples sold in Makkah city.

Vendors samples station	Food types	Bacteria		
		Aerobic plate count	*S. aureus* count	*E. coli* count
Cafeteria 1	Foul	2.4 ×10^3	3.6 ×10^3	ND
	Falafel	3.5 ×10^3	1.2 ×10^3	ND
	Boiled egg	1.2 ×10^3	4.6 ×10^3	ND
	Fried egg	2.5 ×10^3	1.3 ×10^3	ND
	Shakshuka	5.7 ×10^3	4.3 ×10^3	0.4 ×10^3
	Sheep liver slices	6.3 ×10^3	3.1 ×10^3	0.5 ×10^3
Cafeteria 2	Foul	0.5 ×10^3	3.4 ×10^3	1.2 ×10^3
	Falafel	3.4 ×10^3	5.2 ×10^3	ND
	Boiled egg	3.2 ×10^3	2.5 ×10^3	ND
	Fried egg	1.5 ×10^3	3.3 ×10^3	ND
	Shakshuka	4.3 ×10^3	6.4 ×10^3	2.3 ×10^3
	Sheep liver slices	5.9 ×10^3	3.6 ×10^3	0.6 ×10^3
Cafeteria 3	Foul	1.5 ×10^3	3.5 ×10^3	ND
	Falafel	3.2 ×10^3	4.1 ×10^3	ND
	Boiled egg	1.1 ×10^3	3.2 ×10^3	ND
	Fried egg	0.9 ×10^3	3.7 ×10^3	1.1 ×10^3
	Shakshuka	5.9 ×10^3	7.5 ×10^3	2.4 ×10^3
	Sheep liver slices	4.5 ×10^3	7.5 ×10^3	1.6 ×10^3
Cafeteria 4	Foul	4.2 ×10^3	8.7 ×10^3	ND
	Falafel	3.4 ×10^3	4.3 ×10^3	ND
	Boiled egg	2.8 ×10^3	4.9 ×10^3	ND
	Fried egg	2.1 ×10^3	3.5 ×10^3	ND
	Shakshuka	4.8 ×10^3	6.7 ×10^3	ND
	Sheep liver slices	5.6 ×10^3	6.8 ×10^3	2.3 ×10^3
Cafeteria 5	Foul	5.2 ×10^3	5.2 ×10^3	ND
	Falafel	2.8 ×10^3	5.5 ×10^3	ND
	Boiled egg	2.1 ×10^3	4.3 ×10^3	0.3 ×10^3
	Fried egg	1.1 ×10^3	4.2 ×10^3	ND
	Shakshuka	4.7 ×10^3	6.4 ×10^3	4.2 ×10^3
	Sheep liver slices	8.4 ×10^3	7.9 ×10^3	2.3 ×10^3
Cafeteria 6	Foul	2.8 ×10^3	4.2 ×10^3	ND
	Falafel	4.4 ×10^3	9.1 ×10^3	ND
	Boiled egg	1.4 ×10^3	4.2 ×10^3	0.2 ×10^3
	Fried egg	0.9 ×10^3	2.2 ×10^3	ND
	Shakshuka	5.9 ×10^3	9.8 ×10^3	1.7 ×10^3
	Sheep liver slices	4.8 ×10^3	7.5 ×10^3	0.7 ×10^3
% of contaminated samples			100%	42%

Note. ND - Not detected, APC - Aerobic plate count.

The consumption of cooked RTE foods analyzed herein might increase the risk of food-borne illness caused by various microorganisms, especially *S. aureus* and *E. coli*. The highest aerobic plate counts recorded in this study might be caused by different environmental factors, such as contaminated air, water, and utensils used in the meal preparation. Poor personal hygiene practices in the cafeterias could have also led to the contamination of these pathogens. Bezirtzoglou et al. (2000) reported that

RTE food exposure to air or dust at the vending point is likely to increase loads of the bacteria as it appears that most bacteria are carried in aerosols by dust and wind. Contamination by the food producer or handlers is also the most common means of transmitting this germ. *S. aureus* could be transmitted through the dirty hands and mouths of the producers and customers. Similarly, Burt et al. (2003) stated that food contamination by *S. aureus* originates from man's respiratory passages, skin, and uncovered wounds. Thus, its persistence in cooked RTE foods might cause many health risks to consumers. Several reports indicate that most strains of *S. aureus* are known to be pathogenic due to their excreted heat-stable enterotoxins in direct relationship to their inoculum level (Adebayo-Tayo et al., 2012). *S. aureus* produces enzymes incorporated with staphylococcal invasiveness and many extracellular substances, which are enterotoxins that are stable at high temperatures and turn the food riskier even though it appears normal (Prescott et al., 2019). The appearance of the symptoms may differ with the quantity of the contaminated food consumed and the susceptibility of the people to the toxin. Some symptoms of staphylococcal food poisoning include vomiting, nausea, diarrhea, and abdominal pain (Amusan et al., 2010).

E. coli was not detected in most tested food samples, but it appeared in shakshuka and sheep's liver slices from most cafeterias. The highest count was 4.2×10^3 recorded in shakshuka samples vended in cafeteria No. 5—a report by Idowu and Rowland (2006) showed that the highest aerobic plate numbers were found in a fang soup sold by street Vendors with a count of 2.80×10^6 CFU/mL while the lowest count was observed in stew sold by stationary food vendors with shade (SVWS) with several 1.20×10^6 CFU/mL. Moreover, the highest value of *S. aureus* was obtained in moimoi sold by a street vendor with the count of 4.30×10^6 CFU/mL, and the lowest count was observed in stew sold by SVWS with the count of 1.20×10^3 CFU/mL. *E. coli* counts were detected most in moimoi sold by MV with the value of 2.20×10^6 CFU/mL. The presence of *E. coli* in cooked RTE foods indicates secondary contamination, as *E. coli* is known to be correlated with the gastrointestinal tract of warm-blooded animals and not found in the environment as a natural flora (Amusan et al., 2010). However, contamination can take place by the use of contaminated water. *E. coli* has been detected in foods sold at fast foods in a report by Fowoyo and Baba-Ali (2015). In this study, direct or indirect fecal contamination may be the leading cause of *E. coli.*, and *E. coli* belong to the Enterobacteriaceae genus, the leading causal agent for diarrhea, gastroenteritis, urinary tract infections, meningitis, nosocomial pneumonia, and dysentery. The strain of *E. coli* named Enterohaemorrhagic *E. coli* can cause food-borne diseases similar to the *E. coli* O157H7 strain, which causes a severe and potentially fatal illness called Hemorrhagic colitis which is characterized by bloody diarrhea and severe abdominal pain (Evans & Evans, 1996). Following the Public Health Laboratory Service (UK) criteria, the count of *E. coli* in RTE food was defined as <20 CFU/mL (satisfactory), 20– <100 CFU/ML (acceptable), ≥100 CFU/mL (Health Protection Agency, 2009). In our findings, the counts of *E. coli* in the samples of shakshuka and sheep's liver slices can be considered unacceptable.

3.2. Fungal species isolated from ready-to-eat foods samples

Data shown in Table 2 indicated that 21 fungal species belonging to 7 genera were identified. The number of isolated fungi varied according to different meals. The least fungal number (9 species) appeared in fried egg sandwiches followed by 12, 14, 15, 18, and 20 species in shakshuka, boiled egg, falafel, sheep's liver slices, and foul samples, respectively. *Aspergillus* appeared in most samples collected from cafeterias, represented by six species. Among *Aspergillus*, *A. flavus* was the highest, and its occurrence ranged between 50-83% of samples. Also, *A. niger* and *A. parasiticus* were among the common *Aspergillus* species found in the samples. Among samples analyzed herein, shakshuka samples were highly contaminated by *Aspergillus* species (50% by *A. flavus*, 83% by *A. niger*, 100% by *A. ochraceus*, and 50% by *A. parasiticus*). *Mucor racemosus* emerged in remarkable count in falafel samples (50%) and did not emerge in Shakshuka.

Four species of *Penicillium* were isolated, and among them, *P. aurantiogresum* was the most commonly isolated. It encountered 72%, 50%, 67%, and 83% in foul, falafel, boiled egg, and sheep's liver slices, respectively. *P. cyclopium* appeared in 5 types of samples with remarkable accounts. *Rhizopus nigricans* were found in high occurrence in foul (72%) and falafel (52%). About 100% of foul and 89% of falafel samples were contaminated by *Stachybotrys chartarum*. *Trichoderma* sp. was found in five samples, mostly foul (83%) and fried egg (50%). Uzeh et al. (2009) revealed that microorganisms found in salad constitute raw vegetables include *Mucor* sp., *Aspergillus fumigatus*, *Trichoderma*, *Neurospora crassa*, and *Aspergillus niger*. Barnett et al. (2000) reported several mycotoxins isolated from various foods. For instance, *Aspergillus* and *Penicillium* produced aflatoxins. The aflatoxins have been found in legumes, grains, fruits, meats, spices, cheeses, milk, rice, corn, cotton seeds. Other toxins with carcinogenic, hemorrhagic, hepatotoxic, neurotic uterotrophic effects have been found in foodstuff and recorded as metabolites of fungi (Jeswal & Kumar, 2015). Ochratoxins are toxins excreted by *Aspergillus ochraceus*, *A. sulphureus*, *A. m*alleus, and *A.*

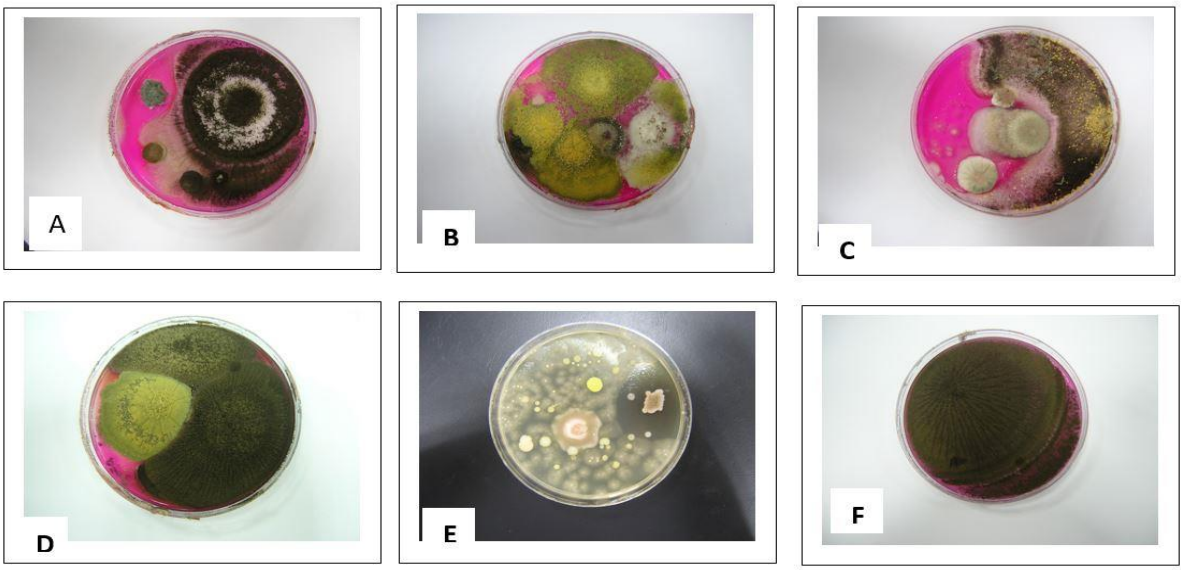
ochraceus can be found in soils and decaying vegetation, grains, wheat, corn, cotton seeds, legumes, peppers, onions, and pears (Adams & Moss, 2002).

Table 2. Mean total count and occurrence remarks (O.R.) of each fungal species (CFU/mL) isolated from ready-to-eat food samples sold in Makkah city.

Fungal genera & species	CFU/mL	O.R. out of 6 sites	Foul	Falafel	Boiled egg	Fried egg	Shakshuka	Sheep liver slices
			(% count of each isolate out of 18 samples)					
Alternaria alternata	11	H	00	00	3(17%)	3(17%)	3(17%)	2(11%)
Aspergillus								
A. flavus	56	VH	9(50%)	4(22%)	7(39%)	15(83%)	9(50%)	12(67%)
A. niger	42	VH	11(61%)	00	6 (33%)	9(50%)	15(83%)	1(5.6%)
A. ochraceus	32	H	3(17%)	00	3(17%)	00	18(100%)	8 (44%)
A. parasitus	27	VH	6 (33%)	3(17%)	14(78%)	00	9(50%)	4(22%)
A. sydowi	26	H	10(56%)	00	7(39%)	00	4(22%)	5(28%)
A. ustus	14	L	7(39%)	3(17%)	00	00	00	4(22%)
Cladosporium sp.	12	L	6(33%)	00	00	00	10(56%)	6(33%)
Mucor racemosus	36	VH	4(22%)	9(50%)	5(28%)	4(22%)	00	4(22%)
Fusarium								
F. culmorum	16	M	2(11%)	5(28%)	6(33%)	00	00	3(17%)
F. solani	3	R	3(17%)	00	00	00	7(39%)	00
Penicillium								
P. aurantiogriseum	62	VH	13(72%)	9(50%)	12(67%)	6(33%)	00	15(83%)
P. digitatum	29	H	5(28%)	8(44%)	7(39%)	00	5(28%)	9(50%)
P. citrinum	21	H	8(44%)	4(22%)	4(22%)	00	00	00
P. cyclopium	33	VH	10(56%)	4 (22%)	7(39%)	7(39%)	00	9(50%)
Rhizopus nigricans	38	VH	13(72%)	5(52%)	6(33%)	4(22%)	4(22%)	6(33%)
Stachybotys chartarum	40	VH	18(100%)	16(89%)	7(39%)	6(33%)	00	5(28%)
Trichothicium roseum	17	M	8(45%)	4(22%)	00	00	00	00
Phoma sp.	8	R	4(22%)	4(22%)	00	00	00	00
Trichoderma sp.	44	VH	15(83%)	6(33%)	00	9(50%)	5(28%)	2(11%)
Verticillium sp.	7	L	2(11%)	4(22%)	00	00	00	1(0.06%)
No. of isolated fungi			20	15	14	9	12	18

Note. Rare (R) = Appeared in 1 site, Low (L) = Appeared in 2 sites, Medium (M) = Appeared in 3 sites, High (H) = Appeared in 4 sites, and Very High (VH) = Appeared in 5 -6 sites.

Patulin is the primary toxic metabolite produced by *Penicillium* (Bennett & Klich, 2003). *Fusarium culmorum* and *Trichothecium roseum* appeared moderately, and the least encountered fungal species were *A. ustus, Cladosporium* sp., and *Verticillium* sp. (Table 2). The isolation of *S. aureus, Aspergillus niger, Aspergillus fumigatus*, *Mucor* sp, and *Penicillium* sp, are consistent with the findings of Attiya et al. (2015), Taulo et al. (2008) and Oranusi, et al. (2013), in which these microorganisms were found in RTE foods. Molds like *Mucor sp* and *Aspergillus sp.* Contaminated food samples through dust and soil as they disperse in the form of spores abundant in the environment (Apinis, 2003). Molds in food samples are a severe health threat due to mycotoxin production (Makun et al., 2009).



Picture 1. Most common isolated fungi and bacteria. **A).** *Aspergillus niger (black)*, *A. fumigatus* (olive colony), and *A. ustus* (brown colony); **B).** *A. flavus* (yellow-green) mixed with *Stachybotrys chartarum* (black); **C).** *Curvularia lanata* (black color) and *A. ustus* (grey colonies); **D).** *Alternaria alternate* (black) and *Trichoderma* SP. (green); **E).** Mixed cultures of *S. aureus* (golden) and *E. coli* (white); **F).** Pure culture of *Stachybotrys chartarum.*

4. Conclusion

Ready-to-eat foods (RTEs), especially of animal origin, could be considered as potential sources for microbial infections in humans. This study revealed that RTE foods served in cafeterias in Makkah city had unsatisfactory microbiological contamination; thus, it may increase the potential risk of food-borne poisoning and diseases among consumers. Accordingly, it is a priority for relevant public health and food safety agencies to establish training programs on food safety and sanitary in cafeterias, especially following the regulations of hazard analysis and critical control points (HACCP) principles during all food processing steps. All staff members, including administrators and the people who work in the kitchen, must be trained.

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Conflict of interest. The authors declare that there is no conflict of interest regarding the publication of this paper**.**

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