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Evaluation of Some Pathogenic Microorganisms in Ready to Eat Meat Sandwiches in Menofia Governorate

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ABSTRACT

Meat meals can resolve the problem of lack of fresh meat at high prices but they can be a source of certain microorganisms of public health hazards. Therefore, 100 random samples of ready-to-eat (RTE) meat sandwiches of shawerma, kofta, hawawshi and kebda (25 of each) were collected from different restaurants and street vendors in Menofia governorate. The obtained results indicated that the incidence of Salmonellae in the examined samples of shawerma, kofta, hawawshi and kebda sandwiches were 16%, 8%, 8% and 8 %, respectively. The isolated serotypes of Salmonellae were S. Enteritidis, S. Enterica S. Montevideo and S. Typhimurium which were identified with different percentages in such examined RTE sandwiches. However, the incidences of S. aureus in examined samples of shawerma, kofta, hawawshi and kebda were 80%, 60%, 52% and 24% with average counts of Log 3.19 ± 0.18; 2.77 ± 0.14; 2.67 ± 0.09 and 2.09 ± 0.09, respectively. Eight enterotoxigenic strains of S. aureus were isolated from the examined samples, three enterotoxigenic strains were isolated from shawarma (A, B & A+C), two from kofta (A & A+C), two from hawawshi (A & B) and one enterotoxigenic from kebda sandwiches (A+D). The verification of isolated pathogens was carried out using the VITEK2 compact system which is a modern technique character with high accuracy results.

1. INTRODUCTION

Ready-to-eat (RTE) food or fast food is prepared and served very quickly by restaurants and street vendors without further preparation. Consumers take away or consume at the point of sale (Hussein et al, 2018). Accordingly, RTE food consumption increased significantly to be suitable for the lifestyle (Saghaian et al.,2018). Modern lifestyles lead to a wide variety of people depending on RTE food as a main meal during working, school days or even in transportation. RTE food satisfies consumers' demand as it is easy to get, has a low cost, and has a delicious taste that people are interested in (Samapundo et al.,2015). RTE foods provide consumers with nutrients containing protein with a high biological value, fatty acids, and minerals which are essential for human growth (Speedyn,2003). On the other hand, RTE food can be a source of certain pathogens arising from the food processing chain being exposed to many sources of contamination (Angelidis et al.,2006). There are many factors that affect the microbial quality of RTE meat as low quality of raw meat and other ingredients, inadequate heat treatment during cooking, bad personal hygiene, uncleaned utensils used in processing and serving as well as additives such as spices, sauce and vegetables (KayaardiS et al.,2006). Cross-contamination may occur during processing by many sources including using the same knives and cutting board for chicken, meat, and salad without interval cleaning (Petra,2009). The sanitary condition of RTE food should be recorded from point to point to evaluate its microbiological quality during production and distribution (Koutsoumanis et al.,2008). RTE food is

considered a high-risk food because it has no further thermal treatment or other processing before consumption (Fang et al.,2003). RTE food was incriminated in outbreaks of foodborne diseases all over the world (Gracey et al., 1999). Salmonellosis is primarily zoonotic disease related to food consumption especially of animal origin (Edward and Ewing.,1972). Fecal contamination of meat and meat products is the main source of Salmonella, Salmonellae result in diarrheal diseases to about 275 million humans all over the world (Cabedo et al.,2008). The symptoms of Salmonellosis accompanied by gastroenteritis may be prolonged to systemic illness such as septicemia and other longer-term conditions (Johnson et al., 2006). Staphylococcus aureus is considered as one of the most significant cause of food borne disease. Thus, food handlers are the major source of food contamination due to unhygienic practices during food processing and storage (Schelin et al., 2011). S.aureus is able to grow in different types of food and results in food poisoning by secreting enterotoxins (Balaban and Rasooly., 2000). The symptoms of staphylococcal food poisoning (SFP) include nausea, abdominal cramping, diarrhea, and vomiting, symptoms appear within 24 hours from ingestion of food contaminated with S. aureus enterotoxins (Abd Alrahman and Fakhr., 2015). Vitek2 Compact System is a modern technique that makes biochemical bacterial identification depend on an expanded identification database, the most automated plate forms available which provides rapid and accurate phenotypic identifications (Darbandi., 2010). RTE food consumption has increased in every part of the world, and severe health problems have been associated with them (Fang et al., 2003). Therefore, the present study was designed for monitoring of Salmonellae and S. aureus microorganisms contaminating ready to eat meat sandwiches in Menoufia governorate.

2. MATERIALS AND METHODS

2.1. Sampling (APHA, 2001):

100 random samples of ready to eat meat sandwiches represented by shawarma, Kofta, Hawawshi, and kebda sandwiches (25 of each) were collected from different restaurants and street vendors at Menofia governorate. Each sample was taken in a separate sterile plastic bag and put in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay for bacteriological examination.

2.2. Preparation of sample (APHA,2001):

Twenty-five grams of the samples under examination were taken under the aseptic condition to a sterile stomacher bag then 225 ml sterile peptone water 0.1% was added, the contents were homogenized at Stomacher device (Seward 400 circular) for 2 minutes, the mixture was allowed to stand for 5 minutes at room temperature. The contents were transferred into the sterile flask and thoroughly mixed by shaking then 1 ml was transferred into a separate tube each containing 9 ml of sterile peptone water 0.1%, from which ten-fold serial dilutions were prepared. The prepared samples were subjected to the following microbiological examination.

2.3. Detection of salmonellae was performed according to (ISO 6579, 2017).

From the prepared dilution, one ml was inculcated in to sterile peptone water at 37^{o}c for 18 hrs, then inoculated in Rappaport Vassilidis broth (RVB) and incubated at 43^{o}C/24hr. Loopfuls from the inoculated tubes were separately streaked on to xylos lysin desoxychocalate (XLD) agar and incubated at 37°C for 24 hours, plates were inspected for supposed Salmonella colonies which were then isolated for confirmation. Suspected purified salmonella was cultured on three biochemical media represented by TSI agar, Urea agar, and L-Lysine decarboxylation media and incubated at 37^{o}C for 24 hours.

2.3.1 Serological identification of *salmonella* (Kauffman, 1974):

Serological identification of salmonella was carried out according to Kauffman – White scheme. Purified salmonella colony was classified according to somatic (O-) and flagellar (H-) antisera.

2.4. Isolation and identification of S.aureus (MacFaddin., 2000).

Hundred microliters from previously prepared serial dilutions was spread over duplicate plates of Baired Parker ager using a sterile glass spreader. Suspected colonies were counted and isolated for microscopical and biochemical identification

2.4.1 Detection and typing of S.aureus enterotoxins.

Detection and typing of enterotoxins were done using serological tests by reversed passive latex agglutination technique (kit used for the detection of

staphylococcal enter0otoxins {A, B, C, and D} (Shingaki et al., 1981).

2.5. Identification and Confirmation of isolated strains:

were carried out by using the VITEK2 compact system according to the manufacturer's instructions (Pincus, 2006).

3. RESULTS

(Table 1) indicated that shawerma is the most contaminated sample with salmonellae (16%), followed by kofta, hawawshi, and kebda sandwiches (8% of each). (Table 2) declared serological identification of isolated Salmonella strains as ten strains isolated from the examined samples. Four strains isolated from shawerma as S. Enteritidis, S. Enterica, S. Montevido and S. Typhimurium, two strains isolated from kofta as S. Enteritidis and S. Typhimurium, two strains isolated from hawawshi as S. Enterica and S. Typhimurium and two strains from kebda sandwiches as S. Enteritidis and S. Typhimurium. (Table 3) revealed that shawarma had the highest incidence of S. aureus (20%), followed by kofta (15%), hawawshi (13%), and kebda sandwiches revealed the lowest incidence (6%). However, (Table 4) pointed out the mean values of S. aureus (log cfu/g) in the examined samples of shawarma 3.19 ± 0.18, kofta 2.67 ± 0.09, hawawshi 2.67 ± 0.09, and kebda sandwiches 2.09 ± 0.09. (Table 5) proved the occurrence of staphylococcal enterotoxins (SEs) in the examined samples as SEA, in shawarma, kofta, and hawawshi; SEB in shawarma and hawawshi; SEA+C in shawarma and kofta and, SEA+D in kebda sandwiches only. (Table 6) shows the confirmation of the isolated strains by using the VITEK2 compact system. The incidence of Salmonellae according to a number of examined samples shawarma, kofta, hawawshi and kebda sandwiches were 37.5%, 25%, 12.5%, and 25%, respectively. While, the incidences of S. aureus for the same sample were 23.2%, 30.7%, 30.7, % and 15.4%, respectively

Table (1): Incidence of isolated salmonellae from the examined samples of ready to eat meat sandwiches (No. of each=25).

Products	Shawerma	Kofta	Hawawshi	Kebda
No	4	2	2	2
%	16	8	8	8

Table (2): Incidence of Salmonella serotypes detected from examined ready to eat meat sandwiches (No. of each=25).

Products Salmonellae	Shawerma		Kofta		Hawawshi		Kebda		Group	Antigenic Structure	
	No	%	No	%	No	%	No	%		O	H
S.Enteritidis	1	4	1	4	-	-	1	4	D1	1,5,10	I:1,2
S. Enterica	1	4	-	-	1	4	-	-	C3	5,6	R:1,4
S. Montevideo	1	4	-	-	-	-	-	-	E1	6,7	g.m.s:1,2
S.Typhimurium	1	4	1	4	1	4	1	4	B	1,12	g.m.s:1,7
Total	4	16	2	8	2	8	2	8			

Table (3): Incidence of S. aureus in the examined ready to eat meat sandwiches (No. of each=25).

Products	Shawerma	Kofta	Hawawshi	Kebda
No	20	15	13	6
%	80	60	52	24

Table (4): Average counts of S. aureus (log cfu/g) in the examined ready to eat meat sandwiches (No. of each=25).

Products	Shawerma	Kofta	Hawawshi	Kebda
Min.	1.99	1.98	1.95	1.4
Max.	4.2	3.72	3.41	2.8
Mean ± S.E*	3.19 ± 0.18^{a}	2.77 ± 0.14^{b}	2.67 ± 0.09^{c}	2.09 ± 0.09^{d}

*Mean values with different superscripts in the same column are significantly different at (P<0.05).

Table (5): Occurrence of enterotoxins secreted by S.aureus isolated from the ready to eat meat sandwiches (n=25)**.**

Enterotoxins	Shawerma		Kofta		Hawawshi		Kebda	
	No	%	No	%	No	%	No	%
A	1	4	1	4	1	4	-	-
B	1	4	-	-	1	4	-	-
A + C	1	4	1	4	-	-	-	-
A + D	-	-	-	-	-	-	1	4
Total	3	12	2	8	2	8	1	4

Table (6): The incidence of salmonellae and S. aureus by using VITEK 2 compact system

Meat sandwiches		Shawerma	Kofta	Hawawshi	Kebda
Salmonellae	**No**	3	2	1	2
n (8)	**%**	37.5	25	12.5	25
S.aureus	**No**	3	4	4	2
n (13)	**%**	23.2	30.7	30.7	15.4

4. DISCUSSION

Salmonella species is one of the most important food-borne diseases causing symptoms of gastroenteritis to systematic illness as septicemia. Many types of food especially of animal origin can be a source of Salmonella (FAO/WHO). Last few years, Salmonellae occupied an advanced position in food-borne disease all over the world, especially through the consumption of RTE meat sandwiches, there are 275 million people around the world had salmonellosis (Cabedo et al., 2008).

(Table 1) shawarma samples recorded the highest incidence of salmonellae at 16%; this slightly agrees with Hassanin et al (2014). who recorded 20%, while higher than Sotohy et al (2019) who obtained 3.3%, and Rahman et al (2018) who obtained 8%. However, the obtained results were lower than Mustafa (2016) which recorded 30%. Moreover, kofta recorded 8% which somewhat agrees with Gaafar et al (2019a) who obtained 6.66%. However, higher thanRahman et al (2018) who obtained 4%. Even though, lower than Hassanin et al (2014) who recorded 33.3%. Hawawshi recorded 8% which slightly agrees with Younes et al (2017) who recorded 15%, but Hassanin et al (2014) recorded 40%, such a finding disagrees with Sotohy et al (2019) who failed to detect Salmonellae in hawawshi. Also, kebda sandwiches recorded 8%, which was nearly similar to Sotohy et al (2019) which recorded 6.6%. However, a lower result was recorded by Rahman et al (2018) who obtained 4%, and higher result was recorded by Shaltout et al (2017) who recorded 60%. The variation in the results between different authors is due to the differences in hygienic technique during manufacturing, handling and inadequate heat during cooking.

(Table 2) indicated that the isolated Salmonellae serovars were classified into S.Enteritidis, S. Enterica, S.Montevido, and S.Typhimurium. The four isolated serovars were recorded from shawerma, the results somewhat agree with Hassanin et al (2014) and Shaltout et al (2017) who isolated S.Typhimurium and S.Enteritidis from shawerma, and Sotohy et al.(2017) who isolated S.Enteritidis from a shawarma. Furthermore, two strains isolated from kofta (S.Enteritidis and S.Typhimurium), Hassanin et al.(2014) and Shaltout et al (2017) isolated S.Enteritidis and S.Typhimurium from kofta, and Gaafar et al (2019a) also isolated S.Typhimurium from kofta but failed in detecting S.Enteritidis from kofta samples. In addition, two strains were isolated from hawawshi (S. Enterica and S.Typhimurium), Hassanin et al (2014) detected S.Typhimurium, while Sotohy et al (2019) failed to detect salmonellae from hawawshi. Moreover, two strains isolated from kebda sandwiches (S. Enteritidis and S. Typhimurium), Shaltout et al (2017) isolated S. Enteritidis and S. Typhimurium, Sotohy et al (2019) and Gaafar et al (2019a) isolated S. Enteritidis, Ashraf (2014) isolated S. Typhimurium. It is noticeable that the most isolated serotypes were S. Typhimurium followed by S. Enteritidis. WHO (1988) states that S. Typhimurium is the most widespread serotype globally and this serotype causes severe salmonellosis in all kinds of animals and causes gastroenteritis in men. Also, Herikstad et al (2002) revealed that S. Typhimurium and S. Enteritidis

were the most common isolated serotypes that cause food-borne illness globally. Zhao et al (2002) reported that S. Typhimurium and S. Enteritidis caused illness for over 100 years.

Staphylococcus aureus is a widespread bacterium in the environment (Schelin et al., 2011). Its presence in food indicates bad hygienic conditions such as bad handling, contaminated equipment, improper heating during cooking, and cross-contamination from raw meat or fresh vegetables, S. aureus is one of the most public causes of food intoxication all over the world (Balaban and Rasooly.,2000).

(**Table 3)** shawerma was the highest contamination with S. aureus (80%) which typically agrees with Morshdy et al (2018) who recorded 80%, lower result was obtained by Sotohy et al (2019) 26.6%. followed by kofta (60%) whose result was nearly similar to Shaltout et al (2015) and Gaafar et al (2019b) who recorded 45% and 40%, respectively, while lower than Morshdy et al (2018) who recorded 90%, and higher than Hassan et al (2016) who recorded 25.7%. Then hawawshi recorded 52%, Shaltout et al (2015) recorded 70%, (Morshdy et al.,2018) recorded 65%, Hassan et al (2018) recorded 31.4%, and Sotohy et al (2019) recorded 20%. kebda sandwiches recorded the lowest occurrence of S. aureus 24%, such a result was nearly similar to Sotohy et al (2019) who recorded 23.3%, and Gaafar et al (2019b) 26.6 %, however lower than Morshdy et al (2018) who recorded 55%.

(Table 4) revealed that shawerma was the most contaminated RTE food with S. aureus with a mean value of 3.19±0.18 (log cfu/g), this result agrees withSotohy et al (2019) who acquired 3.55±0.13 log cfu/g andMorshdy et al (2018) who obtained 3.92±0.41 log cfu/g. Kofta showed 2.77±0.14 log cfu/g, whose result was slightly similar to Shaltout et al (2015 who found 8.13×102cfu/g, while a higher result was obtained by Gaafar et al (2019b) who recorded 0.78×103 cfu/g. Hawawshi recorded 2.67 ± 0.09 log cfu/g which somewhat agrees with Morshdy et al (2018) who found 2.52±0.11 log cfu/g, while lower than Shaltout et al (2015) who recorded 4.28×103 log cfu/g, and Sotohy et al(2019) who recorded 3.38±0.17 log cfu/g. Kebda sandwiches reported 2.09±0.09 log cfu/g, this result agrees with Ashraf (2014) whose result was 6×102 log cfu/g, while lower than Sotohy et al (2019) who reported 3.51±0.14 log cfu/g, and Morshdy et al (2018) who reported 3.64±0.39 log cfu/g. Statistically, there was a significant difference between samples due to the difference in handling statutes, such as hygienic conditions, processing temperature, additives added, and status of raw materials used.

(Table 5) Staphylococcus aureus secret enterotoxins responsible for staphylococcal food poisoning (SFP). S. aureus enterotoxins (SEA to SEE) are known to be responsible for 95% of (SFP) cases (Schlievert, and Case.,2007) From the obtained results SEA was the most common enterotoxin responsible for SFP outbreaks worldwide. SEA was incriminated in 77.8% of all SFD outbreaks in the United States, and also the highest frequently found enterotoxin among SFD outbreaks in France, Japan, and the UK. (Gaafar et al.,2019b). SEB is involved in SFP and is considered the most potential enterotoxin because a tiny amount of it causes severe intoxication, moreover, if the strain carries more than one toxin, it will cause potential intoxication (Greenfield et al.,2002 and Gill 1982). SEB has been known as a restrictive factor by the Centers for Disease Control and Prevention (CDC) (Poli et al., 2002) However, SEC and SED are also associated with SFP (Hassan et al., 2014). Further, S. aureus bacteria can be destroyed by heat and gastrointestinal enzymes but their toxin can't because it is a heat-stable toxin (100^{o}C for 1hr) and resists gastrointestinal enzymes (Garrote et al.,2002). Hassan et al. (2024) can detect SEA in Hawawshi and SED in Hawawshi and kofta but failed to detect SEB and SEC in examined samples. Sotohy et al (2019) isolated SEA and SEB from shawarma, SEC from hawawshi and kebda sandwiches.

(Table 6) Garrote et al (2000) evaluated the VITEK 2 system in a study. They stated that the VITEK 2 system is an easy-to-use system that delivers a fast (4 to 15 h) and sensibly perfect means for identifying the most commonly isolated species. Moreover, one of the most important benefits of the VITEK 2 system is the significant decrease in handling time, which will positively influence the workflow of the clinical microbiology laboratory. Moreover, Gherardi et al (2012) did a comparative study between the VITEK 2 system and other standard method for identifying Gram-negative and Gram-positive bacteria, VITEK2 showed an excellent performance. Also, Mellissa et al (2017) stated that the VITEK 2 system performed very well in microbial identification.

5. CONCLUSION

Shawerma is the most contaminated RTE meat product recorded with the highest incidence of Salmonellae and S. aureus this may be due to the cooking method of shawerma as it is not exposed to enough heat. RTE food can be a source of some pathogenic microorganisms which have a public health hazard to humans. Accordingly, fast food at restaurants and street vendors should be subjected to strict laws from the Ministry of Health and continuous inspection to ensure that hygienic standards are applied to obtain maximum food safety. VITEK2 compact system is an advanced biochemical bacterial identification system that provides rapid and accurate phenotypic identifications.

Authors' declarations

Publication consent

Each author has demonstrated their consent for the publication of the current manuscript.

Data and material availability:

All data of this study is provided.

Conflict of interests.

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A.K: Data Curation, Formal Analysis, Investigation, Resources, Writing – original draft.

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