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Original Research Article

PREVALENCE OF MULTIDRUG-RESISTANT *Staphylococcus aureus* AND *Salmonella* Enteritidis IN MEAT PRODUCTS RETAILED IN ZAGAZIG CITY, EGYPT

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Abstract: This study aimed to monitor the hygienic status of fresh minced meat, smoked sausage and fresh beef burger (50 samples, each) retailed in Zagazig city, Egypt. Aerobic plate count, total *Staphylococcus aureus* count and most probable number of coliforms have been conducted. The prevalence, antibiotic susceptibility as well as detection of the drug resistance associated virulence genes of *S. aureus* (*mec*A, *bla*Z, *and aac (6') aph (2")*) and *Salmonella* species (*bla*TEM, *tet*A(A), and *flo*R) in the examined meat products have been carried out. The highest mean (log_{10} cfu/g) of aerobic plate counts (5.44±0.11) and most probable number (4.15±0.10-log10 MPN/g) were recorded in minced meat. However, the highest mean of *S. aureus* counts (3.47±0.12-log10 cfu/g) was recorded in beef burger. Aerobic plate counts, most probable number and *S. aureus* counts exceeded the recommendations of Egypt Organization for Standardization by (20, 4 and 16%), (14, 12 and 20%) and (50, 10 and 20%) in minced meat, sausage and beef burger, respectively. *Salmonella* Enteritidis was detected in 4 (8%) beef burger. However, *S. aureus* was isolated from minced meat and beef burger (5 samples, each, 10%) and 4 sausage samples (8%). *mec*A, *bla*Z and *aac(6')aph(2")* were detected in all *S. aureus isolates. bla*TEM, *tetA*(A) *and flor*R were detected in the all *S.* Enteritidis isolates. In conclusion, the achieved results revealed inadequate hygienic measures adopted during preparation of such meat products. Therefore, strict hygienic practices should be followed before serving such products to consumers.

Key words: *S. aureus; Salmonella* Enteritidis; drug resistance; meat products

Introduction

Meat products such as minced meat, sausage and beef burger are considered rich sources for animal derived proteins, essential fatty acids, fat soluble vitamins and minerals such as iron and phosphorus. In addition, such meat products have their unique aroma and flavour which make them highly attractive, especially for children (1). However, such meat products may be on responsible for human illnesses by food-borne pathogens such as *Staphylococcus aureus (S. aureus)* and *Salmonella* species.

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Microbial contamination of meat products may arise from the raw ingredients used in their manufacture, improper handling during transportation, processing, storage and distribution (2). Therefore, evaluation of the hygienic status of meat products is a major task for meat hygiene and food safety sectors in Egypt.

S. aureus enterotoxigenic strains are responsible for foodborne intoxication due to the production of heat-stable enterotoxins (3). *Salmonella* spp. is a leading cause of foodborne infection (4,5). The abuse of antimicrobials in the veterinary field and the use of the same drugs for treatment of both humans and animals had resulted in development of antimicrobial resistant organi-sms. Such organisms may harbour some virulence attributes, which are positively contribute to the development of this multidrug resistance phenomenon (6). However, there is a clear lack of information about multidrug resistant foodborne pathogens in Egypt, in particular among strains isolated from meat products.

Therefore, this study was conducted to evaluate the microbiological quality (aerobic plate count (APC), total *S. aureus* count and most probable number (MPN) of coliforms) of meat products including minced meat, sausage and beef burger retailed in Egypt. Additionally, the prevalence of some foodborne organisms including *S. aureus* and *Salmonella spp.* was investigated. Furthermore, the multidrug-resistance profiles of the identified strains were examined. Finally, the expression of drug resistance-related genes in the isolated organisms was detected using PCR assay.

Material and methods

Collection of samples

One hundred and fifty samples of fresh minced meat, smoked sausage and fresh beef burger (50 samples each) were randomly collected from butcher shops and stores in Zagazig city, Egypt. Samples were kept in an ice tank and then immediately transferred to Food Control Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt for bacterial isolation and identification.

Preparation of samples, enumeration and isolation procedures

From each sample, 25g were aseptically homogenized in 225 ml of 1% sterile peptone water (Oxoid CM9) to make a dilution of 10^{-1} and then serial dilutions were followed up to 10^{-7} dilution (7). For aerobic plate count, 1ml of each dilution was pipetted into separate duplicate petri dishes, and then overlaid by 12-15ml of nutrient agar (CM003, Oxoid, England), mixed well by alternate rotation and then let to solidify. Solidified petri dishes were inverted and incubated at 37ºC for 24 h. All colony-forming units (pinpoint size) were counted (8).

For *S. aureus*, isolation and count were done on Baird Parker agar (Biolife, Italy) supplemented with egg yolk-tellurite emulsion (Himedia, India). After incubation at 37ºC for 48 h, colonies (black, shiny, convex, 1–1.5 mm in diameter, and surrounded by a clear halo zone) and/or atypical colonies (black with no zones) presumptive colonies were counted and five colonies were selected and sub-cultured on blood agar plates (Difco Laboratories, Detroit, MI) and incubated for 24 h at 37ºC (8). Gram's stain and biochemical tests were performed on suspected colonies for identification of *S. aureus* (9). For the most probable number (MPN) of coliforms; 1ml of each dilution was inoculated separately into 3 MacConkey broth tubes with inverted Durham's tubes. Then, tubes were incubated at 37ºC and examined after 24 and 48h. Positive tubes showing acid and gas productions in inverted Durham's tubes were recorded as MPN of coliforms (10).

Regarding *Salmonella* spp., original homogenate was pre-enriched in buffered peptone water 1% at 37ºC for 24h. Then 1 ml of pre-enriched peptone water was enriched in Rappaport Vassiliadis broth with soya broth at 41.5ºC. A loopful was streaked on XLD agar, incubated at 37ºC for 24h and red colonies with black centre were enumerated (11). The obtained purified isolates were identified biochemically and serologically (12).

Genomic DNA extraction and PCR analysis

Genomic DNA extraction was done using QIAamp DNA kit according to the manufacturer's instructions. Primer sequences for identification of antibiotic resistance genes were described in Table 1. The target genes of *S. aureus* included *mec*A (encoded for methicillin-resistance) (13)**,** *bla*Z (encoded for β-lactamase-resistance) (13) and *aac (6') aph (2'')* (encoded for aminoglycoside-resistance) (13). For *Salmonella* spp., the targets genes were *bla*TEM (encoded for ampicillin-resistance) (14), *tet*A(A) (tetracycline resistance gene) (15) and *flo*R (florfenicol/ chloramphenicol resistance gene) (16). Uniplex PCR assays were carried out according to Darwish et al. (17). The thermal cycle of the reaction was started with a single 1 min cycle at 94°C, followed by 35 cycles of 10 sec denaturation at 94°C, 1 min annealing (annealing temperatures are indicated in Table 1) and 1 min extension at 72°C and then a final cycle of extension for 7 min was carried out at 72°C. The amplified products were then electrophoresed in 2% agarose gel and stained with ethidium bromide (18).

Table 1: Primers' sequences of the investigated drug resistance associated genes in *S. aureus* and *S.* Enteritidis isolated from different meat products

Gene	Primer sequence (5'-3')	Amplicon size (bp)	Annealing (°C)	Reference
***mec*A**	F-GTAGAAATGACTGAACGTCCGATAA R-CCAATTCCACATTGTTTCGGTCTA A	310	50	(13)
***bla*Z**	F-ACTTCAACACCTGCTGCTTTC R-TGACCACTTTTATCAGCAACC	173	54	(13)
aac(6')aph (2'')	F-GAAGTACGCAGAAGAGA R-ACATGGCAAGCTCTAGGA	491	54	(13)
***bla*TEM**	F-ATCAGCAATAAACCAGC R-CCCCGAAGAACGTTTTC	516	54	(14)
***Tet*A(A)**	F-GGTTCACTCGAACGACGTCA R-CTGTCCGACAAGTTGCATGA	576	50	(15)
***flo*R**	F-TTTGGWCCGCTMTCRGAC R-SGAGAARAAGACGAAGAAG	494	50	(16)

Antibiogram

Antibiotic sensitivity testing of *S. aureus* and *Salmonella spp.,* was performed using single diffusion assay against 11 commercially prepared antibiotic discs (6 mm) with variable concentrations (19).

Statistical analysis

Statistical significance was tested using One way analysis of variance (ANOVA) followed by Tukey-Kramer HSD test (JMP statistical package, SAS Institute Inc., Cary, NC, USA) (P <0.05).

Results and discussion

The microbiological quality of meat products examined reflects the hygienic measures adopted during the preparation and post-processing handling of such products. In the present study, the results revealed the average aerobic plate counts (log_{10} cfu/g) were 5.44±0.11, 5.41±0.08 and 4.07±0.11 in the examined minced meat, beef burger and sausage, respectively (Table 2). Comparing the recorded values with the permissible limits set ensured by Egypt Organization for Standardization (EOS) (20), it was clear that, 20%, 4% and 16% of minced meat, sausage, and beef burger, respectively exceeded that limits. *S. aureus* counts expressed as log_{10} cfu/g was found to be; 3.45±0.20 in minced meat and 3.47±0.12 in beef burger that was significantly (p<0.05) higher than in sausage (2.31±0.19). Moreover, it was found that, 14, 12, and 20% of minced meat, sausage, and beef burger exceeded EOS recommendations (20). The Most Probable Number values (log_{10} MPN/g) of coliforms were higher in minced meat (4.15±0.10), followed by beef burger (2.99±0.12) and sausage (2.12±0.12) that exceeded EOS limits by 50, 20 and 10%, respectively.

Table 2: Hygienic indicators in the examined meat product samples

	Minced meat	Sausage	Beef burger
Aerobic plate count			
Mean ± SE	5.44±0.11[a]	4.07±0.11[b]	5.41±0.08[a]
Range	4.45–6.85	4.00–6.18	4.30–6.60
Exceed PL (%)	20%	4%	16%
S. aureus count			
Mean ± SE	3.45±0.20[a]	2.31±0.19[b]	3.47±0.12[a]
Range	1.80–4.18	1.50–3.78	1.80–3.90
Exceed PL (%)	14%	12%	20%
MPN of coliforms			
Mean ± SE	4.15±0.10[a]	2.12±0.12[c]	2.99±0.12[b]
Range	3.00–5.30	1.00–3.15	1.00–4.15
Exceed PL (%)	50%	10%	20%

Means and ranges of the examined samples are expressed as $\log_{10}$ cfu/g in case of aerobic plate count and *S. aureus* counts and expressed as $\log_{10}$ MPN/g in most probable number count.
Means carrying different superscript letters within the same row were significantly different at $p < 0.05$.
SE: standard error of mean. PL: is the permissible limits of aerobic plate count (5 $\log_{10}$ cfu/g); *S. aureus* count (2 $\log_{10}$ cfu/g) and MPN of coliforms (3 $\log_{10}$ MPN/g) according to Egyptian Organization for Standardization (EOS 2005).

Lower values of hygienic indicators were recorded in sausage compared to minced meat and beef burger that agreed with those recorded in Greece (21). This may be attributed to composition of sausage (minced meat packed in the intestine of animals). These intestines may be insufficiently cleaned, hence, lower the hygienic indicators.

In general, meat products had relatively high microbial contamination indicating inadequate measures adopted during manufacturing of such products. High contamination of meat products was reported in catering establishments in Hay Hassani district-Casablanca, Morocco (22). High microbial loads in the final products may arise from contamination of the contact surfaces of the meat products (23).

Meat products are responsible for a significant number of foodborne illnesses due to ingestion of foodborne pathogens such as *S. aureus* and *Salmonella spp. S. aureus* is considered one of the most important causes of food poisoning worldwide that is responsible for food borne intoxication due to the production of heat-stable enterotoxin.

In the current study, *S. aureus* was detected in 5(10%), 4(8%) and 5(10%) out of the examined minced meat, sausage and beef burger, respectively. This reflects unsatisfactory hygiene measures during handling and processing of meat. Food handlers may be responsible for meat contamination by *S. aureus* as a result of cross contamination from their hands (3). *Salmonella spp.* is a natural inhabitant in the intestinal tract of animals and can contaminate animal carcasses via cross contamination by meat contact surfaces, meat handlers, low hygienic standards, inadequate storage, dust and insects (23). *Salmonella spp*. was isolated only from 2 beef burger samples (4%), the isolated strains were identified as *Salmonella* Enteritidis. Similarly, *S. aureus* and *Salmonella Enteritidis* were isolated from meat products in Greece, Morocco, Algeria and China (21,22,24,25).

Emergence of multidrug-resistance among foodborne pathogens had a worldwide concern due to its public health and economic impacts. For instances, United States Centre for Disease Control and Prevention (CDC) reported that more than two millions of US population is suffered annually from drug resistant organisms (26). In addition, this number was estimated to be 400000 in Europe (27). Development of drug resistance among foodborne pathogens is mainly due to the abuse of antibiotics in the veterinary field including improper use, lack of adherence to treatment guidelines, inadequate dosing and using of therapeutic agents as feed additives (28). Several pathogenic organisms had

evolved some genetic traits to resist antibiotics as an evolutionary protection; such organisms include *S. aureus* and *Salmonella* spp.

In the current investigation, *S. aureus* isolates showed multidrug resistance profiles for AMC, CTX, DA, E, G, ME and S as indicated in Table 3. All isolated strains harboured drug resistance-related virulence attributes including *mecA, blaZ* and *aac(6')aph(2'')*. This result agreed with previous reports on *S. aureus* strains isolated from chicken meat and giblets and ready-to-eat meat products from Egypt and China (3,25). Globally, the proportions of multidrug resistant *S. aureus* especially for methicillin-resistant *S. aureus* (MRSA) combined with one or more antibiotics ranged from 20% to 80% in all WHO regions (29).

Table 3: Antibiotic susceptibility of the isolated 14 *S. aureus* and 2 *Salmonella* Enteritidis strains from meat products examined

		S. aureus			*Salmonella* Enteritidis		
	Disc concentration	**S**	**I**	**R**	**S**	**I**	**R**
Amoxicillin-clavulanic acid (AMC)	30 µg	6 (42.9)	0 (0)	8 (57.1)	0 (0)	0 (0)	2 (100)
Cefotaxime (CTX)	30 µg	6 (42.9)	3 (21.4)	5 (35.7)	1 (50)	0 (0)	1 (50)
Chloramphenicol (C)	30 µg	13 (92.9)	0 (0)	1 (7.1)	0 (0)	0 (0)	2 (100)
Ciprofloxacin (CIP)	5 µg	12 (85.7)	1 (7.1)	1 (7.1)	2 (100)	0 (0)	0 (0)
Clindamycin (DA)	2 µg	10 (71.4)	1 (7.1)	3 (21.4)	1 (50)	0 (0)	1 (50)
Doxycycline (DO)	30 µg	13 (92.9)	0 (0)	1 (7.1)	0 (0)	0 (0)	2 (100)
Erythromycin (E)	15 µg	11 (78.6)	0 (0)	3 (21.4)	1 (50)	0 (0)	1 (50)
Gentamicin (G)	10 µg	5 (35.7)	0 (0)	9 (64.3)	1 (50)	0 (0)	1 (50)
Methicillin (ME)	10 µg	0 (0)	0 (0)	14 (100)	0 (0)	0 (0)	2 (100)
Streptomycin (S)	10 µg	0 (0)	0 (0)	14 (100)	2 (100)	0 (0)	0 (0)
Sulfamethoxazole-Trimethoprim (SXT)	25 µg	13 (92.9)	0 (0)	1 (7.1)	2 (100)	0 (0)	0 (0)

The 14 *S. aureus* isolates were 5 from each of minced meat and beef burger and 4 from sausage.
Values between brackets are the percentages of the isolates showed susceptibility (S), intermediate (I) or resistance (R) to the tested antimicrobials. *mecA, blaZ and aac(6')aph(2'')* were detected in all *S. aureus isolates (n=14).blaTEM, TetA(A) and florR* were detected in the 2 *S.* Enteritidis isolates from beef burger.

All *Salmonella* Enteritidis strains were resistant to AMC, C, DO, and ME. However, only 50% were resistant to each of CTX, DA, E, and G, These strains harboured *blaTEM*, *tetA(A)* and *floR* associated resistance genes. Similarly, multidrug resistant *Salmonella* Spp. were isolated from red meat, poultry meat and processed meat products from Algeria and South Korea (24,29). Multidrug resistant *Salmonella* spp. is associated with invasive infections and increased risk of hospitalization and deaths. Recently, several studies have shown a decreased susceptibility of *Salmonella spp.* to fluoroquinolones, drugs of choice for treatment of *Salmonella*-related gastrointestinal infections. According to WHO statistics (30), the resistance percentage of *Salmonella* to fluoroquinolones had been raised to reach 35% in Africa, 49% in Middle East and 50% in Europe. Therefore, it is highly recommended to reduce the abuse of antibiotics in veterinary field and to find alternatives to antibiotics to be used as feed additives.

Conclusions

The results of this study revealed improper hygienic measures adopted during processing of meat products marketed in Zagazig city, Egypt. Furthermore, some of these meat products were contaminated with *S. aureus* and *Salmonella* Enteritidis. The isolated strains showed multidrug resistance profile. Therefore, strict hygienic measures should be followed during processing of these meat products. In addition, strong legislations should be taken in order to produce meat products of high keeping qualities.

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Conflict of interest

None of the authors have any conflict of interest to declare.

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