Received: 14 April 2023, Accepted: 13 May 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 6, 910-914

Prevalence of Multidrug Resistant *Salmonella* Among Fresh and Heat-treated Meat Products

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Abstract

The emergence of multidrug-resistant (MDR) *Salmonella enterica* along the food chain has posed a public health concern worldwide. In the current study 180 samples of sausage, beef burger, cattle minced meat, buffalo minced meat, luncheon, and hot dog 30 of each type were chosen at random from supermarkets and butcher shops in Egypt's EL-Sharkia Governorate. The aerobic plate count (APC) mean values were 5.58±0.26, 6.28±0.35, 6.36±0.45, 6.23±0.41, 3.22±0.28 and 3.08±0.22 log10 CFU/g in examined sausage, beef burger, cattle minced meat, buffalo minced meat, luncheon and hot dog, respectively. The *Salmonella* detected in was 3/30 (10%), 5/30(16.66%), 6/30(20%), 5/30(16.66%) and 1/30(3.33%) in examined sausage, beef burger, cattle minced meat, buffalo minced meat, and luncheon respectively. The *S.* Typhi was the predominant among isolates (30%) followed by *S.* Kentucky (20%) then *S.* Typhimurium (15%) and finally *S.* Anatum (10%). The resistance was 100% for ampicillin, 80% for Kanamycin, 65% for erythromycin, 60% for amoxicillin and penicillin, and 55% for sulfamethoxazole. On the other hand, the sensitivity was 90% for gentamycin, 85% for norfloxacin, 75% for ciprofloxacin and chloramphenicol. To reduce the risk of *Salmonella* infection in the consumer population, a food safety program should be implemented during the processing of meat products.

KEYWORDS
Salmonella, Meat products, Antibiotic resistance, Aerobic plate count

INTRODUCTION

Meat products are good source of concentrated nutrients because they contain protein with a high digestibility score as well as vitamins, minerals, essential amino acids, and fatty acids that are thought to be necessary for optimum human growth in young and adult. Also, offer customers a source for quick, affordable, and nutrient-dense meals, which they value highly for their flavor, affordability, and ease of preparation (Hussein *et al*., 2018). According to the statistics, consumers' demand for meat products is rising. Despite increased efforts in meat and processed meat hygiene, there has been growing concern about the presence of pathogenic microorganisms in meat products in recent years (Bae *et al*., 2011). In food safety programs, monitoring the microorganisms in meat products is a crucial step, and preventing the contamination of meat by spoilage organisms requires proper meat product storage (Kim and Yim, 2017). It has been proposed that food safety policies at meat processing facilities should be based on microbiological information, with estimates of the numbers of indicator organisms on meat products at different processing stages (Gill *et al*., 2000). At various points along the meat chain, *Salmonella* contamination of meat products takes place. (Processing, distribution, wholesale, manipulation, and preparation). Due to cross-contamination between equipment, utensils, and personnel in the abattoir, the *Salmonella* spreads during the evisceration procedure or removal of intestinal content (Rincón-Gamboa *et al*., 2021). Additionally, due to their careless use in production systems, *Salmonella* spp. resistance to one or more antimicrobial agents has dramatically increased. (i.e., the prevention, control, and treatment of infectious diseases). Additionally, its prophylactic use as growth promoters in livestock has led to therapeutic failures in the management of disease in both humans and animals, creating an even bigger public health issue (Arslan and Eyi, 2010). Additionally, meat products may serve as a vehicle for the spread of additional antibiotic-resistant bacteria and antibiotic resistance genes to humans (Verraes *et al*., 2013). Through contact with animals, the food supply (such as meat, fish, eggs, and dairy products), or more subtly through environmental pathways, multidrug-resistant (MDR) bacteria can spread to humans (Angulo *et al*., 2004). The current study aimed to identify *Salmonella* and aerobic plate count in the examined fresh and heat-treated meat products. Furthermore, identification of the isolated *Salmonella*'s antibiogram.

MATERIALS AND METHODS

Samples collection and preparation

A total of 180 samples of sausage, beef burger, cattle minced meat, buffalo minced meat, luncheon, and hot dog 30 of each

ISSN: 2090-6277/2090-6269/

type were chosen randomly from supermarkets and butchers shops collected from EL-Sharkia Governorate. The collected samples were transferred in an insulated icebox under complete aseptic conditions, without undue delay to the laboratory. According to ISO 6887-2 (2003) the sample and serial dilution were prepared.

Aerobic plate count

Approximately 15 ml of previously melted and adjusted (45 1°C) standard plate count agar Oxiod (CM325) were thoroughly mixed with one milliliter of each previously prepared serial dilution in separate, duplicate, appropriately marked Petri dishes. The inoculated plates and the control plate were immediately inverted and incubated for two days at 30°C after solidification. According to ISO 4833-1: 2013, plates with between 30 and 300 colonies were counted, and the total colony count per gram was computed and recorded.

Isolation and identification of Salmonella species

A 25-gram sample of meat was pre-enriched in 225 milliliters of buffered peptone water at 1% before being incubated at 37°C for 18–2 hours. A tube containing 10 ml of Rappaport Vassiliadis with soya (RVS broth) and 0.1 ml of pre-enrichment broth were combined. The tube was then incubated at 41.5+1°C for 24 hours plus three hours. A loopful of Xylose Lysine Desoxycholate Agar (XLD Agar) was streaked on the plates of (XLD) Agar from the culture obtained in the previous step, and the selective solid media was inoculated (ISO 6579-1:2017). According to MacFaddin (2000) morphological and biochemical identification. Using *Salmonella* antiserum (DENKA SEIKEN Co., Japan), the serological identification of *Salmonella*e was performed in accordance with the Kauffmann-White system (Kauffmann, 1974) for the determination of somatic (O) and flagellar (H) antigens.

Antimicrobial susceptibility

The antimicrobial discs were employed in line with Clinical and Laboratory Standards Institute (CLSI, 2021), as stated in Table 1. An inoculum of each strain was streaked on Mueller-Hinton agar (Himedia, Mumbai, India), and the impregnated discs were put on the agar surface. The multiple antibiotic resistance (MAR) index was calculated for each isolate using the formula MAR = a/b, where "a" stands for the number of antibiotics to which the test isolate displayed resistance and "b" stands for the total number of antibiotics to which the test isolate has been assessed for susceptibility (Krumperman, 1983). For the MAR indicator, intermediate-level isolates were thought to be sensitive. When isolate resist at least three antibiotics from different groups is known as multi drug resistance (MDR) Singh *et al*. (2010).

Statistical analysis

Every number in bacteriology has existed as a mean with standard error. (S.E). One-way analysis of variance and SPSS were used to evaluate all statistics with a 95% degree of confidence. (ANOVA). The DUNCAN test revealed significant variations between the means. Statistics were deemed significant for P-values below 0.05.

RESULTS

Aerobic plate count

The presented data in Table 2, declared that APC mean values were 5.58±0.26, 6.28±0.35, 6.36±0.45, 6.23±0.41, 3.22±0.28 and

Table 1. Zone size interpretation of disc diffusion antimicrobial susceptibility test of *Salmonella*.

Antimicrobial agent	Sensitivity disc content (µg)	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Methicillin	5	10 or less	13-Nov	14 or more
Amoxicillin+ clavulanic acid	20/10	19 or less	-	20 or more
Clindamycin	2	14 or less	15- 20	21 or more
Ciprofloxacin	5	15 or less	16 - 20	21 or more
Chloramphenicol	30	12 or less	13 -17	18 or more
Sulphamethoxazol + trimethoprim	25	10 or less	15-Nov	16 or more
Doxycycline	30	10 or less	13-Nov	14 or more
Cefotaxime	30	22 or less	23-25	26 or more
Gentamicin	10	12 or less	13 - 14	15or more
Streptomycin	10	11 or less	14-Dec	15 or more
Doxycycline	30	9 or less	12-Oct	13 or more
Chloramphenicol	30	12 or less	13 - 17	18 or more
Erythromycin	15	12 or less	13- 21	22 or more

Table 2. Aerobic plate count (APC) log10CFU/g in fresh and heat-treated meat products n.= 30 for each).

	Product	Minimum	Maximum	Mean±SE	Accepted	Refused
Fresh meat products	Sausage	4.21	6.24	5.58 ± 0.26^{b}	22 (73.33%)	8 (26.66%)
	Beef burger	5.26	7.39	6.28 ± 0.35^{ab}	18 (60%)	12 (40%)
	Cattle minced meat	5.68	8.42	6.36 ± 0.45^{a}	16(53.33%)	14 (46.66%)
	Buffalo minced meat	5.24	7.89	6.23 ± 0.41^{ab}	19 (63.33%)	11(36.66%)
Heat treated products	Luncheon	2.36	4.36	3.22 ± 0.28^{c}	25 (83.33%)	5 (16.66%)
	Hot dog	2.27	4.25	3.08 ± 0.22^{c}	27 (90%)	3 (10%)

$^{(a,b,c)}$ different superscript letters in the same column indicate significant differences ($p < 0.05$). Accepted and refused samples according Egyptian standard for meat products 106 and 104 for raw and heat-treated products, respectively.

3.08±0.22 log_{10} CFU/g in examined sausage, beef burger, cattle minced meat, buffalo minced meat, luncheon and hot dog, respectively.

Salmonella detection and antimicrobial resistance

The *Salmonella* prevalence rates were 3/30 (10%), 5/30(16.66%), 6/30(20%), 5/30(16.66%) and 1/30 (3.33%) in examined sausage, beef burger, cattle minced meat, buffalo minced meat, and luncheon respectively (Fig. 1). The *S.* Typhi was the predominant among isolates (30%) followed by *S.* Kentucky (20%) then *S.* Typhimurium (15%) and finally *S.* Anatum (10%) as shown in (Table. 3). The resistance was 100% for ampicillin, 80% for Kanamycin, 65% for erythromycin, 60% for amoxicillin and penicillin, and 55% for sulfamethoxazole. On the other hand, the sensitivity was 90% for gentamycin, 85% for norfloxacin, 75% for ciprofloxacin and chloramphenicol (Tables 4, 5).

DISCUSSION

The extended oral or parenteral administration of antibiotics results in the formation of resistant strains of microorganisms. Drug tolerance in bacteria is a result of transduction, conjugation, and mutation. Retail meat and meat products have the ability to disseminate zoonotic foodborne pathogens and bacteria that are resistant to antibiotics.

Aerobic plate counts (APC) are frequently used to evaluate the microbial load of fresh meat and meat products in order to assess overall product quality, examine how the product

Table 3. Serotyping of *Salmonella* in fresh and heat-treated meat products (n.= 30 for each).

	Product	*S.* Typhimurium	*S.* Typhi	S. Enteritidis	*S.* Anatum	*S.* Kentucky
Fresh meat products	Sausage	1	1	1	0	0
	Beef burger	0	2	1	0	2
	Cattle minced meat	2	0	2	1	1
	Buffalo minced meat	0	2	1	1	1
Heat treated products	Luncheon	0	1	0	0	0
	Hot dog	0	0	0	0	0

Table 4. Antimicrobial susceptibility of *Salmonella* species isolated fresh and heat-treated meat products (n.=20).

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
Ampicillin (AM)	-	-	-	-	20	100
Kanamycin (K)	3	15	1	5	16	80
Erythromycin (E)	5	25	2	10	13	65
Amoxicillin (AMX)	5	25	3	15	12	60
Penicillin (P)	6	30	2	10	12	60
Sulphamethoxazol (SXT)	7	35	2	10	11	55
Nalidixic acid (NA)	8	40	4	20	8	40
Oxytetracycline (T)	9	45	3	15	8	40
Streptomycin (S)	11	55	2	10	7	35
Neomycin (N)	13	65	1	5	6	30
Ciprofloxacin (CP)	15	75	1	5	4	20
Chloramphenicol (C)	15	75	1	5	4	20
Norfloxacin (NOR)	17	85	0	0	3	15
Gentamycin (G)	18	90	1	5	1	5

Table 5. Multi antibiotic resistant MAR index of *Salmonella* species isolated from fresh and heat-treated meat products.

Resistance pattern	Resistance profile	Number of isolates	Number of antibiotics	MAR
I.	AM,K,E,AMX,P,SXT,NA,T,S,N,CP,C,NOR,G	1	14	1
II.	AM,K,E,AMX,P,SXT,NA,T,S,N,CP,C,NOR	2	13	0.93
III.	AM,K,E,AMX,P,SXT,NA,T,S,N,CP,C	1	12	0.86
IV.	AM,K,E,AMX,P,SXT,NA,T,S,N	2	10	0.71
V.	AM,K,E,AMX,P,SXT,NA,T,S	1	9	0.64
VI.	AM,K,E,AMX,P,SXT,NA,T	1	8	0.57
VII.	AM,K,E,AMX,P,SXT	3	6	0.43
VIII.	AM,K,E,AMX,P	1	5	0.36
IX.	AM,K,E	1	3	0.21
X.	AM,K	3	2	0.14
XI.	AM	4	1	0.07
Average				0.54

has been handled and stored, and possibly provide information on product safety and shelf life. The APC obtained in this study comparable to Shaltout *et al.* (2016) who recorded APC in sausage 6.32±0.42 $\log_{10}$ CFU/g and luncheon 5.3±0.41 $\log_{10}$ CFU/g from Qalyobia governorate, Egypt. Salem *et al.* (2018) examined samples from Menoufia governorate, Egypt and the counts for minced meat, sausage and burger were 6.46±0.74, 5.08±0.51 and 5.32±0.56, respectively. In addition, Zayed *et al.* (2022) detected APC in sausage and Luncheon as 22.69×10^4 CFU/g and 2.06×10^4 CFU/g. Higher APC for sausage 8.33±7.32$\log_{10}$ CFU/g $\log_{10}$ CFU/g (Morshdy *et al*., 2018). Meanwhile, lower counts in frozen beef burger 2.93±0.21 $\log_{10}$ CFU/g and sausages 2.92±0.27 $\log_{10}$ CFU/g collected from Alexandria province, Egypt (Mousa *et al*., 2014). There were significant differences ($p< 0.05$) between examined products, where minced meat higher contaminated thus attributed to sanitary level during mincing and preparation. The lower counts in luncheon and hot dog samples indicate the role of thermal processing but also refer to post processing contamination during retail and slicing process. The acceptability rate was 73.33%, 60%, 53.33%, 63.33%, 83.33% and 90% of sausage, beef burger, cattle minced meat, and buffalo minced meat, luncheon and hot dog, respectively. According to Egyptian standard (ES, 2005) which permitted the maximum value for APC in raw and heated products were 10^6 and 10^7CFU/g.

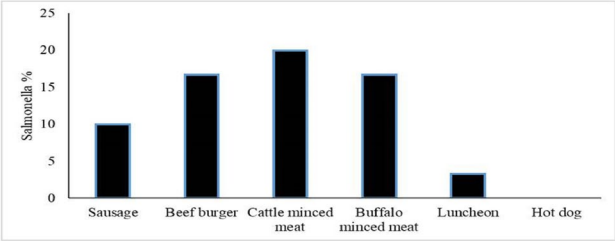

Fig. 1. Prevalence of *Salmonella* in fresh and heat-treated meat products.

The presence of *Salmonella* in meat products and its transmission to humans is a serious problem at the level of developing and developed countries. In the United States, *Salmonella* causes 26.500 hospitalization, 1.35 million illnesses and 420 death, each year (CDC, 2022). In South Korea, *Salmonella* illness ranks third in terms of frequency of food poisoning, behind EPEC and norovirus (MFDS, 2022). Nearly similar isolation rate 15.8% in minced meat (Stock and Stolle, 2001), 16 and 8% in minced meat and sausage (Eleiwa, 2003), 12% in sausage samples (Hassanien, 2004), 11.1% in dried meat (Cabedo *et al*., 2008), 19.43% of examined samples in Algeria (Mezali and Hamdi, 2012), 8% and 4% in minced meat and sausage from Egypt (Ibrahim *et al*., 2015). Meanwhile, higher isolation rate 69.5% in minced meat from Brazil (Fritzen *et al*., 2006), 26% in sausage samples from Botswana (Mrema *et al*., 2006), 40% of minced meat from Egypt (Mohamed, 2013), 40% in minced meat from Egypt (Shaltout *et al*., 2016) and 18.3% minced meat (El-Aboudy, 2018). The lower isolation rate was 4.2% minced meat from the USA (Bosilevac *et al*., 2009) and 3.14% minced meat from Istanbul (Çetin *et al*., 2010). Nearly similar isolate *S.* Typhimurium (36.3%), *S.* Enteritidis (36.3%) and S. Infantis (27.3%) obtained from meat products (El-Aboudy, 2018), also, *Salmonella* serologically typed to *S.* Typhimurium, S. Enteritidis and S. Heidelberg by (Mohammed, 2018).

The antimicrobial resistance of *Salmonella* spp. is currently one of the most significant health issues in the globe. According to data from the EU, *Salmonella* resistance in pigs, cattle, and broiler chickens largely mimics *Salmonella* resistance found in various foodstuffs and in humans (EFSA, 2014). Additionally, there is proof that commercial chicken and red meat in Egypt contain antibiotic residues, which serve as a subnormal dose and hasten the development of antibiotic resistance (Hussein and Khalil, 2013; Morshdy *et al*., 2013). The resistance achieved in this investigation was partially different from that obtained in Egyptian meat and milk samples food. According to Ahmed *et al.* (2014), found that ampicillin had the highest percentage of resistance (95.7%), followed by kanamycin (93.6%) and, sulfamethoxazole/ trimethoprim (91.5%). In Italy the resistance was 35.9%, 38.46% and 7.69% to ampicillin, amoxicillin, and amoxicillin and clavulanic acid. In the case of aminoglycosides, 10.25% were resistant to neomycin and 25.64% were resistant to tetracycline (Pławińska-Czarnak *et al*., 2022). The multiple antibiotic resistances (MAR) index was 0.54 for examined *Salmonella* spp. Moreover, 13/20 (65%) isolates identified as multiple antibiotic resistance (Table. 4). The resulting (MAR) index was in agreement within *Salmonella* isolates from Iran, the MAR index varied from 0.45 to 0.81, with 0.63 as the average (Mir *et al*., 2022). Meanwhile, the lower resistant pattern MAR index 0.37 ranged from 0.06 to 0.56 (Khan *et al*., 2015). There have been numerous papers published on the risk factors connected to the occurrence of MDR *Salmonella* isolates. According to Kayode *et al*. (2010), the indiscriminate use of antibiotics at prescribed levels or at sub-therapeutic doses as feed additives in chicken farms is positively correlated with the emergence of MDR *Salmonella* isolates. Additionally, genetic and pharmacological factors may have played a crucial role in the formation of MDR *Salmonella* strains, preserving their drug resistance genes and boosting their ability to survive.

CONCLUSION

In this research, isolated *Salmonella* from raw meat and heat-treated meat products have a high rate of resistance and considered as MDR. This finding indicates that the situation is alarming when irrational antibiotic use is coupled with insufficient surveillance and facilities to detect MDR. In order to successfully compare antimicrobial resistance from various origins, ongoing surveillance of antimicrobial resistance *Salmonella* strain from different steps in food processing chain.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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