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Prevalence and Antimicrobial Resistance Profile of Different *Salmonella* serovars Isolated from Food Products of Animal Origin

Azza S. El-Demerdash[1], Mervat A. Said[2*], Hazaa M.M.[2], Amer M.M. [2] and Ahmed G. Abdelhamid[2]

1- Agriculture Research Center (ARC), Animal Health Research Institute (AHRI), Zagazig, Egypt

2-Department of Microbiology, Faculty of Science, Banha University, Egypt

*E.Mail: mervat_amer@fnur.bu.edu.eg

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ABSTRACT

Salmonella is the major cause of foodborne diseases and a serious public health problem in the world, with an increasing concern for the emergence and spread of antimicrobial-resistant strains. Our study was conducted to assess the prevalence and antimicrobial resistance profiles of Salmonella isolates using standard bacteriological methods. The overall prevalence rate of 11.4% was recorded from the total analyzed food items of animal origin. Salmonella isolates were detected from 5.7% of minced meat, 1.4% of kofta, 1.4% of luncheon, and 2.8% of burger. All *Salmonella* species recovered were resistant to amoxicillin-clavulanic acid with 100% sensitivity to ciprofloxacin and meropenem. Findings on the multidrug-resistant (MDR) profile showed that a total of 6/8 (75%) of Salmonella Enteritidis were resistant to 3 or more antibiotics. Therefore, our findings provide the prevalence and drug resistance of Salmonella from foods of animal origin and contribute information to scientists as well as public health researchers to minimize the prevalent and resistant foodborne *Salmonella* species in Egypt.

INTRODUCTION

Salmonella is a gram-negative bacterium that belongs to the Enterobacteriaceae family (Boyle et al. 2007). Salmonella spp. are the most important bacterial pathogens among other foodborne pathogens and are responsible for causing gastroenteritis in humans (Ahmed & Shimamoto 2014). Salmonella enterica subsp. enterica includes more than 2600 serotypes and is capable of infecting animals and humans (Crump *et al.*, 2015). Infections caused by *Salmonella* spp. in farm animals have been documented as the leading cause of considerable economic losses worldwide (Jajere 2019).

Salmonellosis is responsible for a variety of clinical syndromes, including enteric fever, which is usually caused by typhoid or paratyphoid species, enterocolitis, bacteremia, and severe local infections (De LeBlanc *et al.*, 2010).

The worldwide increase of foodborne infections linked with antimicrobial-resistant pathogenic microorganisms and the dissemination of antimicrobial resistance (AR) is one of the key concerns in all countries (Prasertsee *et al.*, 2019). To date, the emergence and spread of antimicrobial resistance among zoonotic Salmonella have become a public health threat (Jajere 2019). Importantly, Salmonella strains having "clinically important resistance" to some agents like extended-spectrum cephalosporins and fluoroquinolones, have been isolated from livestock (Li *et al.,* 2013). In most developing countries, misuse and overuse of antibiotics have contributed to the increasing trend of multi-resistance in Salmonella (Borah *et al.*, 2021). Furthermore, Salmonella with antibiotic resistance in contaminated products could infect humans directly or transmit their resistance genes to human pathogens through the food chain, leading to the failure of antibiotic treatment and may pose a serious threat to human health. Therefore, the study aimed to assess the prevalence and antimicrobial resistance of Salmonella serovars isolated from retail beef meat in the Qalyubia governorate in Egypt.

MATERIALS AND METHODS

Sampling:

A total of 70 meat products were purchased from different supermarkets at Benha city, Qalyubia Governorate including minced meat, sausage, burger, luncheon, kofta, raw meat, and canned beef (10 each). Samples were collected under hygienic conditions using sterile polyethylene bags, labeled, and transported immediately to the laboratory for microbiological investigation.

Isolation and Identification:

The procedures for isolation of Salmonella were carried out according to the techniques recommended by the International Organization for Standardization (ISO 6579, 2002). Briefly, 25 g of bacterial sample was pre-enriched in buffered peptone water (BPW) at 37 ◦C overnight. The enriched samples were then inoculated on modified semi-solid Rappaport–Vassiliadis (MSRV) and incubated at 42 ◦C for 24 h. A loopful of the positive growth taken from the MRSV colony was further inoculated onto MaCconkey′s agar and xylose lysine deoxycholate (XLD) and was kept in an incubator overnight. Among the suspected colonies, one colony was seeded in Luria–Bertani (LB) for DNA extraction and validated by polymerase chain reaction (PCR). Distinctive round red colonies with black centers on xylose lysine deoxycholate media were subjected to biochemical identification including triple sugar iron agar, Urea hydrolysis test, Lysine decarboxylase test, Indole production test and Citrate utilization test (Qunin *et al.*, 2002).

Salmonella isolates were then serotyped in the Serology Unit Animal Health Research Institute, Dokki, Giza Egypt using commercial antisera (Difco, Detroit, MIUSA) according to the manufacturer's instructions.

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility testing of the Salmonella isolates to various antibiotics was determined by the Kirby-Bauer disc diffusion method (Bauer *et al.,* 1966) on Trypticase soy agar (TSA) using commercially available discs. The panel of antimicrobials included were amikacin (AK, 30 μg), amoxicillin-clavulanic acid (AMC, 30 μg), ceftriaxone (CRO,30 μg), ceftazidime (CAZ, 30 μg), doxycycline (DO, 30 μg), meropenem (MEM, 30 μg), gentamicin (CN,10 μg), novobiocin (NV, μg), ciprofloxacin (CIP, 5 μg), chloramphenicol (C, 30 μg), sulfamethoxazole + trimethoprim (SXT, 25 μg).

Plates are incubated for 16–24 h at 37°C The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using the criteria published by the (CLSI (Clinical and Laboratory Standards Institute) 2013).

RESULTS

Isolation and Identification of Salmonella Isolates:

1. Colonial Appearance:

On MacConkey agar, salmonella colonies appear colorless and transparent (though they sometimes have dark centers) due to the lack of lactose fermentation which is of great importance in differentiating *Salmonella* from other bacteria present in the specimen.

On XLD medium the majorities of Salmonella serotypes produce hydrogen sulfide and have red colonies with a black (H2S) center

2. Biochemical Identification:

Salmonella isolates recovered from different sources were positive for lysine decarboxylase, TSI agar where Typical Salmonella cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90 % of the cases) formation of hydrogen sulfide (blackening of the agar) where after inoculation fermentation of dextrose by the organisms leads to acid production which causes a subsequent color change of the bromocresol purple indicator to yellow, citrate (blue color). Salmonella isolates were negative for tryptophan utilization (indole test) (yellow-brown ring) and urease production (yellow color) giving an overall prevalence of 11.4%. (8/70) as shown in Table 1

3. Serotyping of Salmonellae Isolates:

Serotyping of eight Salmonella isolates was applied by slide agglutination test using specific polyvalent "O" I, II, III and "H" Salmonella sera. four different serotypes were identified among selected Salmonella isolates; *S. typhimurium* was predominated with a higher percentage (50%) followed by *S. enteritidis* (25%), *S. Kentucky* and *S. anatum* (12.5% each).

Antibiotic Susceptibility Testing:

The antibiotic resistance rates for each source and the whole set of isolates are represented in Figure 1. Of the 8 isolates, 100% showed resistance to amoxicillin-clavulanic acid, followed by novobiocin and sulfamethoxazole–trimethoprim (87.5% for each). All isolates are susceptible to meropenem and ciprofloxacin. Of the 4 serovars, S. Kentucky showed resistance to most antibiotics under test. Interestingly, 75% (6/8) of the obtained Salmonella serovars tested are multidrug=resistant (resistant to three or more antibiotics).

Table 1 Salmonella prevalence from animal-origin food items.

Type of products	Samples tested no.	No. of Samples positive for Salmonella (%)
Minced meat	10	4 (5.7)
Kofta	10	1 (1.4)
Luncheon	10	1 (1.4)
Burger	10	2 (2.8)
Sausage	10	0(0)
Raw meat	10	0(0)
Corned beef	10	0 (0)
Total	70	8 (11.4)

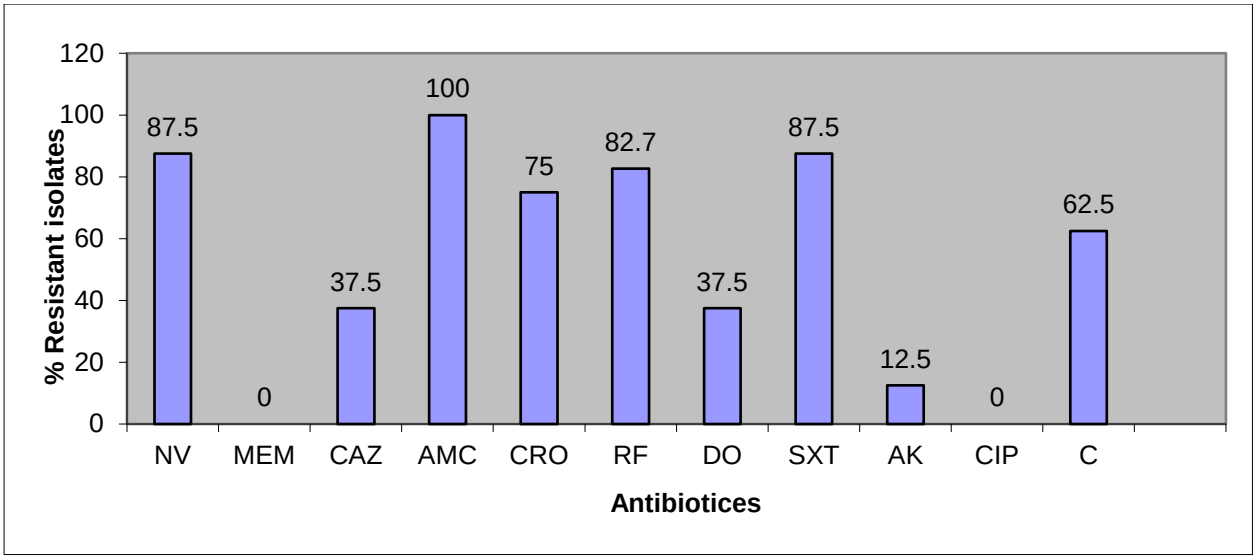

Fig. 1. Percentage of antimicrobial resistance of Enterobacteriaceae isolates in the present study.

DISCUSSION

Contaminated meat products are well established as the main sources of transmission for pathogenic bacteria. It is the major cause of several diseases in developing countries resulting in many cases of mortality and morbidity.

In the present study, the prevalence and the antimicrobial resistance patterns of *Salmonella* spp. isolated from foods of animal origin were evaluated. The study revealed an overall prevalence rate of 11.4% (8 of 70) in the studied food items. This can seriously affect the quality and safety of the processed meat and possess a potential risk to the consumer.

The recovery rate of Salmonella from meat products varied among countries such as in Vietnam, the prevalence of *Salmonella* spp was 60% (Caniça *et al.,* 2019), 1% in Northwestern Greece (Gousia e*t al.,* 2011) and 3.2% in Ahvaz (Enayat *et al.,* 2012). The difference might be the sample type, sample procedures, the detection methods employed for different studies. in handling, manufacturing practices, time of exposure and the different geographic locations of the sampling sites.

Serotyping results revealed that *S. Typhimurium* was the most predominant serotype as previously mentioned by (El-Demerdash *et al.,* 2018), (Ammar *et al.,* 2016) and (Abbassi-Ghozzi *et al.,* 2012).

Detection of four Salmonella serovars in this study reflects the possibility of cross-contamination from various sources in slaughterhouses and poor hygiene during the butchering and processing of meat. During the last decade, antimicrobial resistance and multi-drug resistance of *Salmonella* spp. has increased to a great extent, especially in the developing countries commensuration with increased and indiscriminate use of antimicrobial agents in the treatment of humans and animal diseases

Hither, most isolates could be identified according to antimicrobial susceptibility patterns in addition to variations in the size of inhibition zones. Most isolates that had variable antibiogram and few isolates that had the same antibiogram were differentiated through the differences in the size of the inhibition zone.

All isolates were susceptible to ciprofloxacin and meropenem and absolute resistance was obtained among the isolates against amoxicillin-clavulanic acid (100%) followed by novobiocin and sulfamethoxazole-trimethoprim (87.5%), ceftriaxone (75%), chloramphenicol (62.5%), gentamicin (50 %), ceftazidime and doxycycline (37.5%) and amikacin (12.5%). This finding is exactly in conformity with that recorded by Aslam et al. (2012) who demonstrated the absolute susceptibility to ciprofloxacin and more than 20% resistance

to ceftriaxone, amoxicillin-clavulanic acid, streptomycin and doxycycline.

Furthermore, many studies have reported almost the same results as Zhao et al. (2008) who reported that all Salmonella isolates were susceptible to ceftriaxone and ciprofloxacin and exhibited resistance to streptomycin (37.8%), sulfamethoxazole-trimethoprim (27.7%) and gentamicin (25.7%) and Al-Sultan *et al.*, (2012) who found that susceptibility of their Salmonella isolates to gentamicin, ciprofloxacin and chloramphenicol was 95%, 90% and 80%, respectively and high level of resistance was observed against amoxicillin-clavulanic acid (100%) and erythromycin (80%).

In the present investigation**,** it was noted an incidence of multidrug resistance among 75%of Salmonella isolates which was higher than that obtained previously by Shen et al. (2008) (28.5%) and Ahmed et al. (2009) (14.4%).

Obolski *et al.* (2015) and Elbakry et al. (2020) attributed the exacerbation of this MDR to the diminishing of new antibiotics and considered as a danger to public health.

Conclusion

From the above-mentioned results, it is important to note that Salmonella can easily acquire multiple resistances to most antimicrobials and transform them into humans especially through the food chain.

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