Original Research

Characterization and Antibiogram Pattern of *Salmonella* Species Isolated from Cattle Meat and Abattoirs´ Environment

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ABSTRACT Hygienic measures in abattoir positively reflect on quality of meat. So, characterization of *Salmonella* species isolated from the environment of abattoirs as well as meat of slaughtered cattle was carried out in 5 municipal slaughterhouses located in different provinces, Egypt. A total of 300 samples were collected including slaughtered meat, water, and air samples beside swabs from floor, wall, and workers hand (50 samples/each). It was observed that the highest isolation rate was obtained from hand swabs (8%) followed by meat samples (6%) then floor and wall swabs, and water samples (4% for each) while *Salmonella* could not be isolated from air samples. Moreover, serotyping of the recovered isolates (n=13) clarified the presence of various serotypes including *S. Enteritidis, S. Typhimurium, S. Heidelberg,* and *S. Virchow* with different rates. In addition, PCR was employed successfully for confirmation of the obtained isolates through detection of *inv*A gene of *Salmonella* strains. Antibiogram pattern of the recovered *Salmonella* isolates (n=13) clarified that isolates were resistant to Amikacin and Ciprofloxacin (76.9%) then Vancomycin (53.8%), while it was found that 92.3% of isolates were sensitive to Gentamycin and Linezolid. Based on the recorded result, isolation of *Salmonella* from abattoirs environment as well as meat constitutes public hazard for consumers.

Keywords: Hygienic measures, *Salmonella*, Abattoir, Meat, Antibiogram pattern

INTRODUCTION

The primary goal of building an abattoir (slaughterhouse) is to produce hygienically prepared meat by treating animals humanely and employing hygienic methods for slaughtering and dressing **(FAO, 1992).** To produce safe, healthy, and wholesome halal meat under veterinary supervision and sanitary conditions and prevent exposure to a contaminated environment containing dangerous germs, an abattoir is necessary **(Zailani et al., 2016).** To ensure the fundamental environmental and operational conditions required to produce safe food, several obligatory programmes must be taken into account in abattoir operations. Appropriate animal handling techniques, good sanitary habits, and standard operating procedures are part of these necessary programmes **(Declan et al., 2004).**

If the product is not handled properly or is communicated to consumers, the intestinal contents will spread over the meat surface and start to penetrate tissues, causing the meat to degrade quickly **(Diyantoro and Wardhana, 2019).** Additionally, there are a variety of additional sources of external germs in abattoirs, such as inefficient worker handling, the usage of contaminated tools (such as tables, knives, and other equipment used in cutting operations), and dirty air **(Birhanu et al., 2017).** Additionally, using subpar water for equipment and carcasses during processing and slaughter might contribute to contamination **(Parvin et al., 2017).** Most workers are unaware of the value of maintaining good personal hygiene, which allows them to grossly contaminate items with their hands and clothing or to invite flies, insects, rodents, dust, and other pests into their workspace **(Pradhan et al., 2018).**

One of the main causes of foodborne illnesses and fatalities is contaminated meat, which is brought on by the consumption of substances (bacteria, microorganism cells, and toxins) that are not destroyed even after the meat is cooked **(Bersisa, 2019).** *Salmonella* is a gram-negative, motile member of the *Enterobacteriaceae* family with peritrichous flagella and rods that do not form spores. *Salmonella* is also an anaerobic facultative catalase positive and oxidase negative bacterium. *Salmonella*, however, is not a member of the class of

organisms known as coliforms **(Lawley et al., 2008).** There are more than 2,500 different forms of *Salmonella*, some of which can infect both people and animals **(Brands, 2006).** As a result, the current study also sought to identify the antibiogram of bacterial isolates while isolating *Salmonella* from meat and the abattoir environment.

MATERIALS AND METHODS

Study area: This investigation was carried out in five municipal slaughterhouses in various Egyptian districts. Manually operated slaughterhouses are known as abattoirs. They were well-built with a fence and had a condemnation chamber, an emergency slaughtering room, a quarantine divider, and eviscerated rooms. The daily cow slaughtering capacity is about 200 heads. Depending on how many heads were admitted for slaughter, the slaughter procedures typically began at 6:00 am and continued until 10:00 to 12:00. At the conclusion of each working day, the slaughtering area is regularly cleaned. **Sampling:** 300 samples total were taken, including swabs from the floor, wall, and employees' hands (50 samples each), as well as samples of water, air, and butchered meat. Samples were obtained during twice visits per week, labelled, and transported in an icebox to the Animal Health Research Institute's laboratory in Cairo, Dokki, Egypt. **Preparation of samples: 1. Meat samples:** Under strictly aseptic conditions, 25 g of each sample were transferred aseptically into a sterile blender flask containing 225 ml of sterile peptone water 0.1%, homogenized at 1400 rpm for 2–5 minutes to produce a homogenate 1/10 dilution, and then left to stand for approximately 6 minutes at room temperature **(APHA, 2002)**. **2. Water samples:** From the identified operable tanks and water taps, water samples were taken. Using sterile plastic screw-capped bottles, samples were taken (500 ml capacity). The contents of the sampling bottles were well mixed by shaking and one ml was transferred with a sterile pipette to a sterile tube containing sterile peptone water **(APHA, 1998)**. **3. Air samples:** Using an impinger that was loaded with 225-259 ml of peptone water, air samples were taken. The impinges outlet was attached to the top (inlet) of the trap, whereas the trap's outlet (side arm) was attached to the inlet of the pump. The external calibrator and calibrate were connected to the impedance inlet. After carefully mixing the contents of the sampling bottles, one ml was transferred using a sterile pipette to a sterile tube contained sterile peptone water **(Hamet et al., 1991). 4. Floor and Wall swabs:** Utilizing sterilized cotton swabs, samples were taken from floors and walls in an area of about 1 cm^2 each. The samples were then placed in sterile peptone water (Merck) and transported to the lab while being kept chilled. After carefully mixing the contents of the swab tubes, one milliliter was transferred using a sterile pipette into a sterile tube containing sterile peptone water. **5. Hand swabs of slaughterhouse workers:** A total of 50 hand swabs were taken from employees at the five abattoirs under investigation (10/each). They were collected using sterile swabs, put in sterile test tubes filled with buffer peptone water, and covered with sterile cotton plugs before being quickly transported to the lab in an ice box. After carefully mixing the contents of the swab tubes, one milliliter was transferred using a sterile pipette into a sterile tube containing sterile peptone water. **Isolation, identification, and molecular detection of *Salmonell*a**: **1. Isolation of *Salmonella* (ISO 6579-1/2017):** One ml of inoculated nutrient broth into 9 ml Rappaport Vassilidis broth tube, then the tube was incubated at 43° C for 24 hours. Loopfuls from the inoculated tubes were separately streaked onto Xylose lysine deoxycholate agar medium and incubated at 37º C for 24 hours. *Salmonellae* appeared as red colonies with or without black center. The suspected colonies were sub-cultured onto nutrient agar plate and incubated at 37º C for 24 hrs. The purified isolates were identified morphologically, biochemically, and serologically. Biochemically proved isolates to be *Salmonellae* were subjected to serological identification according to Kauffman white scheme **(Kauffman, 1974). 2. Molecular Identification of *Salmonella*: Oligonucleotide primers used in PCR for molecular identification of *Salmonella* according to Rahn et al., (1992):**

Gene	Primer Sequence	Amplified product (bp)
***Inv*A**	5´GTG AAA TTA TCG CCA CGT TCG GGCA –3´	**284 Rahn et al., (1992)**
	3´ TCATCG CAC CGT CAAAGG AAC C -5´	

Antibiotic resistance profiles in *Salmonella* strains: The susceptibility of *Salmonella* confirmed strains to eight antimicrobial agents were performed on Mueller-Hinton agar using disc diffusion method and were evaluated as described in Clinical and Laboratory **(CLSI, 2017).** The test was performed by applying the bacterial inoculum on the surface of Muller Hinton agar medium and streaked with swab sticks. Discs were placed on the inoculated agar plates which were then incubated at 37°C for 24 hours. The zone diameters of isolates were measured in millimeters with ruler. Isolates were classified as sensitive or resistant based on the criteria published by the Clinical and Laboratory Standards Institute **(CLSI, 2017)** as shown in the following table.

Antibiotics	Sensitivity disc content	Resistant (mm)	Sensitive (mm)
Amikacin (AK)	5 µg	≤ 15	≥15
Amoxiclav (AMC)	10 µg	≤ 11	≥12
Cefotaxime (Cfm)	30 µg	≤ 13	≥14
Ciprofloxacin (CIP)	10 IU	≤ 20	≥21
Gentamycin (Cn)	2 µg	≤ 13	≥14
Linezolid (LZD)	10 µg	≤ 12	≥13
Rifampin (RA)	30 µg	≤ 14	≥15
Vancomycin (VA)	5 µg	≤ 13	≥14

RESULTS

Table (1): Serotyping of *Salmonella* isolated from different examined samples (n=50):

Salmonella serotypes	Meat		Water		Air		Floor swabs		Wall swabs		Hand swabs	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S. Enteritidis	1	2.0	1	2.0	0	0.0	0	0.0	0	0.0	2	4.0
S. Typhimurium	1	2.0	1	2.0	0	0.0	1	2.0	1	2.0	1	2.0
S. Heidelberg	0	0.0	0	0.0	0	0.0	1	2.0	0	0.0	0	0.0
S. Virchow	1	2.0	0	0.0	0	0.0	0	0.0	1	2.0	1	2.0
Total	**3**	**6.0**	**2**	**4.0**	**0**	**0.0**	**2**	**4.0**	**2**	**4.0**	**4**	**8.0**

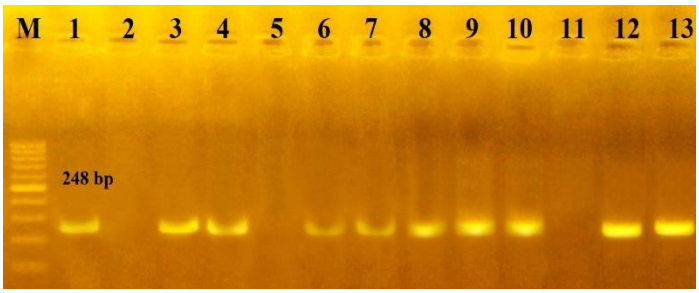

Fig. (1): PCR products of *inv*A gene of *Salmonella* strains (10/13) isolated from different examined samples

Table (2): Antibiogram pattern of *Salmonella* spp. (n =13 isolates) obtained from different examined samples

Antibiotics	Sensitive		Resistant	
	No.	%	No.	%
Amikacin (AK)	3	23.1	10	76.9
Amoxiclav (AMC)	11	84.6	2	15.4
Cefotaxime (Cfm)	10	76.9	3	23.1
Ciprofloxacin (CIP)	3	23.1	10	76.9
Gentamycin (Cn)	12	92.3	1	7.7
Linezolid (LZD)	12	92.3	1	7.7
Rifampin (RA)	9	69.2	4	30.8
Vancomycin (VA)	6	46.2	7	53.8

DISCUSSION

In cases of poor hygiene, the slaughterhouse may be the microbiological cause of meat contamination. Meat quality and the risk of food poisoning bacteria to the public health are estimated by studying the microbiological quality of the slaughterhouse and the meat.

The slaughterhouses should have enough clean water that doesn't contain chemicals or a lot of microbes **(Gracey et al., 1999).** Abattoir trash that is not properly disposed of could contaminate ground water **(Adebowale et al., 2010).** A fundamental prerequisite for good health is clean air. The air pollution from slaughterhouses is one of the environmental pollutants that abattoirs are regarded to be significant sources of. Lifting the blood from dead animals that has been left on the ground without cleaning it properly causes an offensive odour, poses health risks to those nearby due to respiratory manifestations, and spreads microorganisms to the surface of meat and the water in slaughterhouses, especially in hotter climates **(Magaj and Chup, 2012).**

Meat contamination and cross-contamination occur mostly due to inadequate hygienic conditions and improper handling in slaughterhouses **(Koo et al., 2013).** Since one major task of animal hygiene is to protect both meat and the meat handlers from cross-contamination, thus, this study was carried out to investigate an important food safety issue related to the control of hygienic measures in local abattoirs.

Salmonella is the most complex of all the *Enterobacteriaceae* and have more than 2200 serotypes **(Ewing, 1986)** and it could be considered as one of the most causes of food borne illness since the major source of human illness is the contaminated carcasses **(Small et al., 2006)**. Contamination of animal carcasses with *Salmonella* organisms may be via various routs including butcher's hands, knives, and tables (**Uche and Agbo, 1985)** feces of animals slaughtered in abattoirs **(Woldemarium et al., 2005)** excessive handling of the carcasses, cross contamination, retail shop floors, lack of drainage, lack of dressing facilities **(Bhandare et al., 2007).**

Isolation and identification of *Salmonellae* were tabulated in **Table (1)** and it was observed that the highest isolation rate was obtained from hand swabs (8%) followed by meat samples (6%) then floor and wall swabs, and water samples (4% for each) while *Salmonella* could not be isolated from air samples. Moreover, serotyping of the recovered isolates (n=13) clarified the presence of various serotypes including *S. Enteritidis, S. Typhimurium, S. Heidelberg,* and *S. Virchow* with different rates (**Table, 1)**.

The incidence of *Salmonella* in the current work (6.67%) was lower than that recorded by **Fahem, (1993)** who collected a total of 50 samples of fresh raw beef from different butcher shops in Cairo and Giza cities and found that the incidence of *Salmonella* was 16% and the isolated serovars were *S. Westhampton* and *S. Typhimurium* while it was higher than that recorded by **Samaha et al., (2015)** who found that the frequency of isolation of *Salmonella* species from examined meat samples of cattle was 4% and the identified serotypes were *S. Enteritidis* and *S. Typhi*. **Edris et al (2013)** found that the prevalence of *S. Typhimurium, S. Enteritidis* and *S. Virchow* was 6.67, 6.67 and 1.67%, respectively and **Bakhtiari et al. (2016)** who found that the incidence of *Salmonella* in cattle carcass samples in Iranian abattoirs were 30%.

Staff working with food must maintain a high degree of personal hygiene; wear suitable clean clothing, clean footwear, never eat or drink during work, don't wear jewelry or watches **(Slobodan et al., 2017)** to avoid contamination with *Salmonella*.

The *Inv*A protein is a putative inner membrane component of the *Salmonella* pathogenicity island 1 (SPI-1) type 3 secretion system (TTSS). It has been reported that invA is present only in *Salmonella* species and therefore is used as a golden marker in genetic diagnosis of *Salmonella* species (**O'Regan et al., 2008).**

In addition, PCR was employed successfully for confirmation of the obtained isolates through detection of *inv*A gene of *Salmonella* strains **(Fig., 1)**.

Isolation of *Salmonella* from meat is considered a bad indicator as **FAO, (2002)** stated that *Salmonella* was a common cause of enteric illness, which may be ranged from mild gastroenteritis to systemic illness such as septicemia and other longer-term conditions. In Egypt, *Salmonella* was widely recognized as one of the most important causes of food poisoning outbreaks occurring as a result of consumption of contaminated meat. *S. Enteritidis* and *S. Typhimurium* were the most frequent serotypes found in cases of human Salmonellosis.

Antibiogram pattern of the recovered *Salmonella* isolates (n=13) was illustrated in **Table (2)** clarified that isolates were resistant to Amikacin and Ciprofloxacin (76.9%) then Vancomycin (53.8%), while it was found that 92.3% of isolates were sensitive to Gentamycin and Linezolid.

The recorded results agreed with that of **El-Sharkawy et al., (2017)** who found that all *S. enterica serovars Typhimurium* were resistance to ampicillin, chloramphenicol, and tetracycline. All isolates were sensitive to gentamicin. S. enterica serovar Typhimurium was 89.7% and 94.8% susceptible to streptomycin and trimethoprim/sulphamethoxazole, respectively. All non-typable *Salmonella* bacteria were responsive to trimethoprim/sulphamethoxazole and streptomycin, whereas 10.3% displayed intermediate sensitivity to this drug. However, every isolate of S. enterica serovar Enteritidis examined was susceptible to every antimicrobial agent. In contrast, South African Chicken *Salmonella* isolates were resistant to gentamicin (48%), ampicillin (47%), chloramphenicol (31%), and streptomycin (12%). They were also resistant to tetracycline (93%), trimethoprim-sulfamethoxazole (84%), and trimethoprim-sulfamethoxazole (**Zishiri et al., 2016).**

In the present work, the microbiological examination revealed the presence of *Salmonella* in meat as well as abattoirs environment which have a negative influence on meat safety. The slaughtering of the animals on the ground and then skinning and evisceration in the same place under poor hygienic conditions are the major risk factors for bacterial contamination of carcasses. Therefore, it requires a serious attention from all relevant authorities to apply and maintain the basic hygienic slaughterhouse practices. To ensure a maximum safety and lowering the carcasses contamination with *Salmonella*, abattoirs should be constructed with high level of sanitation to minimizing the initial bacterial count with application of mechanical techniques in slaughtering to minimize human intervention and antibiotic treatment of animals should be applied only with veterinarian prescription and after sensitivity test with diagnosis of bacteria to minimize the risk of MDR bacteria.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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