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ORIGINAL ARTICLE

Detection of *Salmonella* spp in Chicken Shawerma and Antimicrobial susceptibility of *Salmonella* isolate to some antibiotics

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ABSTRACT

The objectives of this study was to isolate, identify and characterize Salmonella spp from Shawerma product prepared from chicken meat, and to test the antibiotic susceptibility of the isolated Salmonella spp. Samples were collected in sterile plastic bags from local markets: Alsug Alkabeer (AK), Alsug Ashabi (ASH) and Alsug Alsageer (AS) at Wad-Medani, central Sudan during 2016-2017. The results showed that, the highest Salmonella spp count were found in Shawerma samples that collected from AB (mean of 6.4 cfu/g), followed by AK (mean of 3.9 cfu/g) and at last AS (mean of 2.3 cfu/g). Six isolates of Salmonella species were distributed and found within the food samples collected from all locations. These species included: Salmenella typhi, S. enteritids, S. arizona, S. paratyphi, S. pullorum and S. gallinarum. The antimicrobial susceptibility of Salmonella isolates to12 different common antibiotics showed varied responses towards these antibiotics. S. pulorum was the only isolate that showed resistance to some antibiotics. More studies should be conducted to evaluate the food safety within Gezira State.

Key words: Poultry, biochemical tests, hygienic practices, multi-drug resistant.

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INTRODUCTION

Food safety is one of the most important concerns of humans, and closely related to health, economic development, and social stability [43]. The most important food safety problem is microbial foodborne illness. All those who handle food, including farmers, food producers, individuals who work in markets and food service establishments, and other food preparers, have a responsibility to keep food as safe as possible.

Poultry meat utilization is relentlessly expanding around the world; the last information accessible demonstrate it achieved 14.2 kg per capita per year (Meat Consumption 2017). Among poultry meat products, chicken carcasses, cuts, and processed products are the most consumed (~75% of total poultry meat) followed by turkey (~25%) and, to a lesser extent, duck. Chicken is generally recognized to be a significant reservoir for Salmonella (3, 9) Chicken is broadly recognized to be a noteworthy repository for Salmonella [18, 19].

Utilization of contaminated ready to eat foods including red meat, eggs, cheese & vegetables have been reported to fill in as vehicles for transmission of a few bacterial pathogens and food-borne outbreaks [5].

Infectious microbial disease constitute a major cause of death in many parts of the world, particularly in developing countries. *Salmonella* has been identified as an important food and water-borne pathogen that can infect humans and animals, resulting in significant morbidity and mortality. Much human

salmonellosis is directly related to human association with animals, both wild and domestic. The *Salmonella* group is presumably the best Known the best Known food poisoning organism and although associated with poultry and eggs can be found in a wide variety of foods although associated with poultry and eggs can be found in a wide assortment of foods. Foods of animal origin are vehicles for salmonellosis. *Salmonella* was isolated in 19–54% of cattle carcasses, 1.9% of beef samples at retail and 4.2% of retail chicken samples [42, 4].

The huge number of publications devoted to poultry meat microbiology and the assortment of the outcomes feature the wide diversity of the microbiological status of poultry meat products. The bacterial loads can vary by several log CFU/g for comparative cuts, put away under comparable condition. Until now, the microbial environment of poultry meat products has been basically through social strategies, which can an inclination due to the general selectivity of the media utilized [2].

Analyzing the antibiotic susceptibility patterns of pathogens is significant toward fitting treatment to the regularly changing resistance patterns and appropriation of pathogenic bacteria. During the most recent decade, antibiotic resistance and multiresistance of Salmonella spp. have expanded a great deal, especially in developing countries with an increased and unpredictable utilization of antibiotics in the treatment of humans and animals. The objectives of the present study include : isolation and identification of *Salmonella* spp. in shawerma product prepared from chicken meat at WadMedani City, Gezira State, Sudan. Another objective was the detection of antibiotic susceptibility of the isolated *Salmonella* spp.

MATERIALS AND METHODS

Study area

This study was carried out in Wad Madani city, centre of Sudan. The city is located between 14◦24‘N-14.4◦N longitude and 33◦31‘E- 33.517◦E latitude, 136 Km southeast to Khartoum on the west bank of the Blue Nile.

Collection of samples:

Samples of raw and cooked Shawerma prepared from chicken meat were collected from Alsug Alkabeer (AK), Alsug Ashabi (ASH) and Alsug Alsageer (AS) at Wad-medani city during the period 2016-2017. All samples were kept in sterile ice bags and transported to the Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum.

Determination of *Salmonella*

For preparation of serial dilution,9 ml of sterile distilled water was poured aseptically into five tubes each and 1 ml of the sample was added to the first tube giving 1:10 dilution. Again, 1 ml was transferred from the first tube, added to the second tube, and thoroughly mixed well. Procedure continued until the fifth test tube. Each sample was diluted from 10^{-1} to 10^{-5}. The samples were then taken for further analysis [18].

Twenty- five grams sample were weighed aseptically and mixed well with 225 ml of sterile Nutrient broth and was incubated at 37 C° for 24 hours. Then 10 ml were drawn aseptically and added to 100 ml of sterile Selenite cysteine broth. The broth was incubated at 37C° for 24 hours; a decimal dilution series was prepared in 0.1% peptone solution in the usual way by surface inoculated 0.1 ml amount of dilution onto pre-poured pre-dried plate of Bismuth Sulphite Agar. The plates were incubated at 37 C° for 72 hours. Black metallic sheen discrete colonies indicated the presence of Salmonella. A colony counter was used to count the viable colonies of Salmonella after incubation and the results were expressed as colony forming unit per gram (cfu/g).

Purification and identification of isolates

Typical colonies were streaked onto sterile Nutrient agar plates. The plates were incubated at 37° C for 24 hours. The pure colonies of isolates of *Salmonella* were sub- cultured in slopes of Nutrient Agar and incubated at 37° C for 24 hours and then the culture were kept in the refrigerator at 4°C until used for biochemical test. The identification of purified isolates was carried out according to [11].

Identification and characterization of salmonella isolates

Biochemical testes were employed for the identification and characterization of salmonella isolates according to Harrigan [18]. These tests included: Gram stain, Catalase test, Nitrate reduction test, Vogs-Proskauer (VP) test (acetone production), Utilization of citrate, Urease test, Indole test, Motility test, Sugar fermentation, Casein hydrolysis, Starch hydrolysis and Methyl Red Test.

Antimicrobial susceptibility testing

The *Salmonella*, serotypes were tested against 12 antimicrobial agents namely: Ampicillin/Sulbactam, Co-Trimoxazole, Cefotaxime, Tazobactam/piperacillin Chloramphenicol, Ciprofloxacin, Ceftriaxone, Tetracycline, Ofloxacin, Gentamicin, Amikacin and Levofloxacin. The antimicrobial susceptibility test was

performed by the disk diffusion method with standard antibiotic disks (Oxoid, Basingstoke and Hampshire, England) using Muller-Hinton agar plates as per the National Committee for Clinical Laboratory standards [27]. 20 ml of medium was poured in to sterile Petri dishes to a depth of 4mm and left at 37C° overnight to check for sterility.5 ml of Nutrient broth was inoculated with test isolates and incubated at 35C° for 24 hours. Isolates were inoculated on Mueller. Hintor agar using swabs and inoculated plates were left at room temperature for 30 minutes to allow drying. *Salmonella* isolates were tested for susceptibility to 12 antibiotics by using multidisc. The multi-disk was transferred to the surface of culture of Muller-Hinton agar and incubated at 35C° for 20 hours. The diameters of the zones of inhibition were recorded to the nearest **mm** as resistant, intermediate or susceptible.

Statistical analysis

Microsoft office, Excel 2007, was used to analyze the data obtained. Two factors ANOVA test was used to describe the observed between the obtained data.

RESULTS AND DISCSSION

Enumeration of *Salmonella*

The present study investigated *Salmonella* as foodborne pathogen in shawerma product prepared from chicken meat at Wad Medani, Gezira State, Sudan. Investigation of salmonella was accomplished using conventional bacteriological methods. *Salmonella* count within the samples
(raw and cooked Shawrma) that were collected from three locations (Alsoug Alkabeer, Alsoug Alshabiand Alsoug Alsageer) is presented in Table (1).The highest *Salmonella spp* count was recorded in Shawerma samples collected from Alsoug Alshabi (mean of 6.4 cfu/g), followed by Alsoug Alkabeer (mean of 3.9 cfu/g) and finally Alsoug Alsageer (mean of 2.3 cfu/g). Table (1) also showed that there was also significant differences between salmonella counts at the different locations (F= 169.35; F-crit= 4.46. The high count of *Salmonella* in this study demonstrates poor food preparation and handling practices for example, deficient cooking or cross contamination. These procedures allow cross contamination from diseased bird or contaminated carcass to healthy and clean ones. Besides, the lack of veterinary supervision inside these markets may lead to slaughtering of diseased birds. Thought may likewise given to researching the health status of food handlers on the premises who may have been experiencing salmonellosis or a symptomatic bearers of the organism [16]. The presence of such elevated amounts of Salmonellae in analyzed examples was a lot higher than 9.2%, 6.2%, 3.4% reported by El Hussein *et al*. [14], Yagoub [41]; Elsafi *et al*., [15], respectively, moreover, the present findings were likewise much higher than reports from other countries, such as 14.5% from Nepal [23], 14% from Canada [2], 19.2% from South Africa [28], and 12% from Turkey [29]. A few investigations in other developing countries have reported a higher overall prevalence of *Salmonella* (human, food, and animal) such as 73.3% from Egypt [1], 68.2% in Ethiopia, 51.2% in Argentina, 25.9% in Korea, and 72% in Thailand [9].

Table (1) *Salmonella* count (cfu/g) of Shawerma samples collected from different locations

Location	Raw Shawerma	Cooked Shawerma
Alsoug Alkabeer	3.9	2.7
Alsoug Alshabi	4.1	4.3
Alsoug Alsageer	4.3	2.1

Isolation and identification of *Salmonella* spp. isolated from Shawerma

The biochemical identification tests revealed that six *Salmonella* species were isolated from Shawerma including: *Salmenella typhi, S. enteritids, S. arizona, S. paratyphi, S. pullorum* and *S. gallinarum*. However, these species were distributed and found in Alsug Alkabeer and Alsug Alshabi samples, whereas, all isolates, except *S. gallinarum* was noticed in Alsug Alsageer samples (Table 2).

It is essential to perceive that, the pervasiveness and distribution of *Salmonella* serovars varies from region to region [12, 36] and isolation rates rely on the country where the study was carried out, the testing plan and the detection limit of the methodology [30, 31, 36]. Therefore, it is hard to make comparison between *Salmonella* surveillance surveys conducted in different countries. Anyway the contrasts between Salmonella occurrence starting with one country then onto the next might be because of various hazard elements of transmission of Salmonella to food that can be happened, including contaminated of raw food by animal faeces, contact with animals or their environment, contaminated water and personal hygiene [19].

Various studies have been attempted in Sudan on Salmonella and salmonellosis strikingly in animals. Mamoun *et al*., [24] isolated 21 *Salmonella* strains from several poultry farms in three different States in the Sudan. *S. eneritidis* was found in 1.43% of raw milk samples [38, 39]. Yagoub *et al*. [41] isolated *S. paratyphi* A and *S. paratyphi* B from 6% of the white cheese samples collected from retailer shops and restaurants in Khartoum and Omdurman cities. Yagoub [39] detected *Salmonella sp*. in 6.2% of fish samples and Hag Elsafi *et al*. [17] detected *Salmonella sp*. in 3.4% of faecal samples collected from in and around Khartoum State. *S. enterica* subspecies was recovered from 9.2% of different raw and cooked food items [14, 38]; Saeed and Hamid [39] affirmed the role of food handlers in the spread and transmission of food borne transmittable maladies which includes salmonellosis as they recognized pathogens in 30.1% of the food handlers.

Table 2. *Salmonella* species isolated from Shawerma

Salmonella species	Samples collection sites		
	Alsug Alkabeer	Alsug Alshabi	Alsug Alsageer
S. typhi	+	+	+
S. enteritidis	+	+	+
S. Arizona	+	+	+
S. paratyphi	+	+	+
S. pulorum	+	+	+
S. gallinarum	+	+	-

Antimicrobial susceptibility of *Salmonella* isolate to some antibiotics

Comprehensively, Salmonella has been a standout amongst the most commonly reported causes of food-borne pathogens from far off and recent times [37, 32, 31]. In sub-Saharan Africa, non-typhoidal salmonellae are the most common causes of Bacterial blood streaming infections in both adults and children represented by fever and are associated with case fatality rate of 20-25% [26]. Infections can happen frequently by means of ingestion of debased meat, eggs, rawmilk, fruits, and vegetables [3,6,9]..

An expanding extent of Salmonella isolates is resistant to generally utilized antibiotics in both developing and developed countries [35], and this expansion is seen in both veterinary and public health sectors [21, 8, 9]. Salmonella infections are one of the major worldwide general medical issues. During the most recent decade, antibiotic resistance and multiresistance of Salmonella spp. have expanded a lot, particularly in, developing countries with an increased and aimless utilization of antibiotics in the treatment of humans and animals.

The antimicrobial susceptibility (or resistance) of *Salmonella* isolate to 12 different common antibiotics were presented in Table (2). The different isolates showed varied responses towards these antibiotics.

Ampicillin/Sulbactam (AS) antibiotic showed intermediate sensitivity in three of the salmonella isolates (*S. enteritids, S. arizon,* and *S. pullorum*), but it showed high sensitivity in *S. gallinarum.* Co-Trimoxazole (BA) antibiotic showed intermediate sensitivity in two of the salmonella isolates (*S. typhi* and *S. paratyphi*), but it showed high sensitivity in *S. pulorum.* Cefotaxime (CF) antibiotic showed intermediate sensitivity in all salmonella isolates, except *S. pulorum*, that showed high sensitivity towards it. Tazobactam/Piperacllin (TZA) antibiotic showed intermediate sensitivity in three isolates (*S. arizona, S. pulorum* and *S. gallinarum*), and it showed high sensitivity in *S. paratyphi.*Chloramphenicol (CH) antibiotic showed intermediate sensitivity in three isolates (*S. typhi, S. enteritids,* and *S. pullorum*), and it showed high sensitivity in the rest of the isolate. Ofloxacin (OF) antibiotic showed moderate sensitivity in three isolates (*S. typhi, S. arizona,* and *S. paratyphi*), and it showed high sensitivity in the rest of the isolates.

Gentamicin (GM) antibiotic showed intermediate sensitivity in four isolates (*S. typhi, S. arizona, S. paratyphi* and *S. pulorum*), and it showed high sensitivity in *S. enteritidis* and *S. gal+linarum* isolates. Ciprofloxacin (CP) antibiotic showed intermediate sensitivity in *S. enteritidis* and *S. arizona*, and it showed high sensitivity in *S. typhi, S. paratyphi* and *S. gallinarum,* but *S. pulorum* showed resistance to this antibiotic.Ceftriaxone (CR) antibiotic showed intermediate sensitivity in *S. paratyphi* and high sensitivity in the rest of the isolate and it did not showed resistance in any isolate.Levofloxacin (LE) antibiotic showed high sensitivity in all isolates except, *S. pulorum* as same as Amikacin (AK) antibiotics (but it showed resistance in *S. pulorum*).Tetracycline (TE) antibiotic showed intermediate sensitivity in *S. paratyphi* isolate and high sensitivity in *S. typhi, S. enteritidis* and *S. arizona,* but *S. pulorum* showed resistance to this antibiotic.

The emergence of antimicrobial resistant Salmonella has become a major public health concern [10]. Antimicrobial drug resistance is an available consequence of the extensive use of antimicrobial drugs in

human medicine and in the treatment of food producing animals [35]. The most common recorded resistance were to Ampicillin, Chloramphenicol, Streptomycin, Sulfonamides and Tetracycline.

In the present study, 83.33% of Salmonella isolates were high sensitive to Levofloxacin and Amikacin, this result was comparable with that reported by Munawwar *et al.* [25], who found that 87.88% of *Salmonella* isolate from meat chicken were sensitive to Amikacin and levofloxacin, and100% of *Salmonella* isolate from chicken and human sensitive to Levofloxacin and Amikacin reported by Lobna *et al.* [22]. 83.33% of *Salmonella* in this study showed moderate sensitive to Cefotaxime and Gentamicin (33.33%) of *Salmonella* isolate were showed low sensitive to (Co-Trimoxazole and Ampicillin),while only 1(16.66%) isolate was resistant to Tetracycline, Ceftriaxone and Amikacin. Shereen *et al*., [33] reported that 4 (33.3%) isolate from chicken showed resistance to Tetracycline

In Sudan Elabbas [13] screened the antibiotics sensitivity test of 15 (*S. enteritidis*) isolated from fecal samples of chickens suffering from diarrhea in Khartoum State, Each isolate was tested against ten antimicrobial agents amikacin, ceftizoxime, ciprofloxacin, gentamicin, ampicillin, sulbactam, piperacillin, tazobactam, cefotaxime, chloramphenicol, tetracycline and cotrimexazole, all of the isolates were sensitive to the tested drugs, except tetracycline and cotrimexazole. In Ethiopia, a significant proportion of Salmonella isolates have developed resistance for a number of anti-salmonella drugs, 32.7% of isolates were found resistant to one or more of the tested antimicrobial agents. The most common resistance was reported to streptomycin (75%), ampicillin (59.4%), tetracycline (46.9%), spectinomycin (40.6%) and sulfisoxazole (40.6%) [42]. In USA, 18.0% isolates from all sources were found resistant to two or more antimicrobials. Resistance to sulfisoxazole, streptomycin, and tetracycline was the most prevalent, whereas resistance to ciprofloxacin was the least. 27.7% isolates from animal feed, dog and environmental swabs were resistant to two or more antimicrobials.

From these result it is clear that there is a correlation between usage of Tetracycline as feed additive and prevalence of chicken salmonellosis as probable cause of heightened resistance to the drug due to continued misuse. In addition, because Tetracycline is the most widely used drug in both Veterinary and Human medicine practices [34]. Therefore Levofloxacin and Amikacin may continue to be the drugs of a choice for treatment of human salmonellosis in Sudan.

To control the antimicrobial resistance in Sudan, Many factors contribute to the high prevalence of antimicrobial resistance in Sudan, the most significant of which is the antibiotic formulary. Measures to control resistance, therefore, start with correcting the formulary. Other measures include education of healthcare workers, implementation of infection control policies and procedures.

Multidrug-resistant (MDR) strains of Salmonella are currently experienced frequently worldwide and the rates of multidrug-resistance have expanded impressively lately. Far more atrocious, a few variations of Salmonella have developed multidrug-resistance as a basic part of the genetic material of the organism, and are along these lines prone to hold their drug-resistant genes even when antimicrobial drugs are never again utilized, a circumstance where other resistant strains would ordinarily lose their resistance [37].

In Sudan, the issue of antibiotics transferred resistance is relied upon to be declined because of the expanded interests in poultry monstrous cultivating, especially without settled guidelines and rules that administer, direct, and control the utilization of veterinary arrangements. In addition, the poor economical status of a sizeable segment of the Sudanese population drives them to look for conservative or less expensive sources of animal proteins. Consequently, poultry products are relied upon to represent the significant alternative in such manner.

Table (2) Antimicrobial susceptibility of *Salmonella* to some antibiotics

Salmonella spp	Antibiotic Susceptibility		
	R	I	S
S. typhi		BA, CF, CH, OF, GM	CP, CR, TE, AK, LE
S. enteritidis		AS, CF, CH,CP	TE, OF, GM, AK, LE
S. Arizona		AS,CF, TZA, CP, OF,GM	CH, CR, TE, AK, LE
S. paratyphi		BA, CF, CR, TE, OF, GM	TZA, CH, CP, AK, LE
S. pulorum	CP, TE, AK	AS, TZA, CH, GM	BA, CF, CR, OF
S. gallinarum		CF, TZA	AS, CH, CP, CR, OF, GM, AK, LE

Symbol: R= Resistant, I= Intermediate, S= Sensetive, AS = Ampicillin/Sulbactam, BA= Co-Trimoxazole,CF= Cefotaxime, OF= Ofloxacin,TZA= Tazobactam/Piperacllin,CH = Chloramphenicol,CP = Ciprofloxacin,CR= Ceftriaxone,TE = Tetracycline,GM= Gentamicin,AK = Amikacin,LE = Levofloxacin.

CONCLUSION

The present study indicated detection of Salmonella from shawerma products at Wad-Medani city. The results of the present study indicate poor hygienic practices of workers, which could result in the contamination of the poultry meat and cross contamination from positive animals. This study also revealed high resistance of Salmonella to generally utilized antibiotics.

The outcome of the present study are indicative of Salmonella risk in poultry meat products in Wad-Medani. from the abattoir, but more detailed studies should be conducted including possible routes of transmission of Salmonella as well as amplification of antibiotic resistance transfer.

The surveillance of antimicrobial resistance in Salmonella spp. is significant. Likewise, it is essential to keep up salmonella active surveillance of resistance on a global and intersectoral level. It is highly recommended to use an adequate temperature in food cooking to avoid the risks of contamination by foodborne pathogens. Control during transportation of animals and animals products, and hygienic practices in abattoris and further processing is required. Good hygienic practices (GHP) and Hazard Analysis Critical Control Points (HACCP)application are needed to ensure quality and safety of food products.

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