

Occurrence and antimicrobial susceptibility of *Salmonella* isolates from grilled chicken meat sold at retail outlets in Erbil City, Kurdistan region, Iraq

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Abstract

Food borne salmonellosis is a major public health problem worldwide. This study aimed to detect the occurrence and antibiotics sensitivity of *Salmonella* species in grilled chicken meat sold at retail outlets in Erbil City, Kurdistan, Iraq. Two hundred and twenty-five (225) samples were aseptically collected from central and sub-urban retail outlets. For isolation of salmonellae, samples were cultured on selective media and tested for their susceptibility to common antibiotics by disk diffusion assay. The results revealed that the overall prevalence of *Salmonella* among grilled chicken meat samples was 7.1%. The isolates belonged to eight different serotypes of *Salmonella*. These include *S. Typhimurium*, *S. Tennessee*, *S. Newport*, *S. Enteritidis*, *S. Anatum*, *S. Arizona*, *S. Muenchen*, and *S. Montevideo*. The antibiotic resistance profile revealed a total resistance to Levofloxacin and total sensitivity to Cefotaxime, Amoxicillin, and Cefadroxil. This resistance among *Salmonella* may pose a public health hazard that requires effective precautions and response.

Introduction

Salmonella genus is one of the most significant food poisoning agents all over the world. The pathogenic potential of *Salmonella* was recognized in 1885 in pigs by two American pioneer scientists; Daniel E. Salmon and Theobald Smith (de Freitas Neto *et al.*, 2010; Octavia & Lan, 2014). *Salmonella* is a ubiquitous bacterium that can survive several weeks in a dry environment and several months in water. Within only two species (*S. enterica* and *S. bongori*), more than 2,600 serotypes of *Salmonella* are dispersed in nature and often found in the intestinal tract of a wide range of mammalian hosts including

humans, animals, and also found in birds. For practical purposes, *Salmonella* is generally classified into two categories; the first category is Typhoidal salmonellae, which include strains causing typhoid fever (TF) or paratyphoid fever (*Salmonella* Typhi, Paratyphi A, Paratyphi B, and Paratyphi C). These strains are carried only by humans. The second category called Non-typhoidal salmonellae (NTS), which include all other *Salmonella* strains. Non-typhoidal *Salmonella* is the most common and is an important cause of food poisoning. Strains of this category are carried by humans, different types of animals, poultry, wild birds, and flies (Octavia & Lan, 2014).

Salmonellosis in human is a serious foodborne disease (FBD). Globally, it is estimated that the annual deaths by salmonellosis reach 500,000 and the incidence rate of non-typhoidal *Salmonella* gastroenteritis is 94 million cases, with approximately 85% of foodborne cases (WHO, 2018). Indeed, the World Health Organization (WHO) declared that FBDs is a major public health problem that increased in the last decade in both developed and developing countries (Ullah *et al.*, 2019; WHO, 2008). Salmonellosis is most common in children and reaches its peak during summer and fall (Katiyo *et al.*, 2019). Contaminated ready-to-eat food and fast food are a major source for human salmonellosis (WHO, 2008, 2018). Such contamination may occur during food processing, due to poor hygienic conditions of raw materials, or during handling characterized by poor hygiene (Adesetan *et al.*, 2017; Bukar *et al.*, 2010). The sources of contamination vary greatly but are mostly represented by fecal material from livestock, pets, birds, reptiles, asymptomatic human carriers (Abd El-Aziz, 2013; de Freitas Neto *et al.*, 2010). Recently, there have been several salmonellosis cases linked to consumption of raw fruits and vegetables contaminated by manure from farmhouses or by insect vectors (Barreiro *et al.*, 2013; Eni *et al.*, 2010; de Freitas Neto *et al.*, 2010). Despite the fact that freezing of meat greatly reduces viability of food borne pathogens, *Salmonella* tend to tolerate low temperature for long periods of time (Almashhadany, 2008; Shafini *et al.*, 2017).

In Kurdistan region (Iraq), grilled chicken meats are most commonly served in food outlets especially in restaurants, retail outlets, street vendors, hotels, schools, canteens and even small outlets which involved different styles of preparations. Therefore, the aim of the study was to determine the occurrence and antimicrobial resistance of *Salmonella* among grilled chicken meats sold at retail outlets in Erbil City.

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Materials and Methods

Study design and sampling

A total of 225 grilled chicken meat samples (75 Shawarma, 75 Thigh, and 75 Breast) were collected under sterile hygienic conditions during July to December 2018 in Erbil city according to previously published method (Sharaf and Sabra, 2012). One hundred eighteen (118) samples were collected from retail outlets at city center, whereas 107 samples were obtained from city outskirts.

Isolation and identification of *Salmonella*

Isolation procedures were carried out according to WHO enrichment method (WHO, 2010). Briefly, 10 g of each sample were added to 90 ml of Buffered peptone water (Oxoid, UK) and incubated at 37°C for 24 h in ambient air. One mL of this culture was inoculated into Tetrathionate broth (9 mL) and incubated aerobically at 37°C. After 24 h, a loopful of broth was streaked on Brilliant Green Agar (BGA) and Xylose Lysine Deoxycholate agar (XLD) plates (Oxoid, UK) and aerobically incubated at 37°C for 24-48h. Colonies suspected to belong to *Salmonella* were picked and submitted for biochemical tests according to Todar (2012) and Strockbine and colleagues (2015).

Serotyping of isolates

Serotyping of the isolates was carried out by slide agglutination using Remel® kit according to manufacturer instructions (Remel Europe Ltd., UK).

Antibiotics susceptibility testing

Disk diffusion assay on Mueller-Hinton agar (Oxoid, UK) was employed to evaluate the susceptibility of *Salmonella* isolates to a panel of ten commonly used antibiotics (Mast diagnostics, UK). The guidelines of CLSI were followed to perform the Modified Kirby-Bauer method and to interpret the inhibition zones diameters around antibiotic disks (CLSI, 2015). The following antimicrobials were tested: amikacin (AK₁₀), amoxicillin/Clavulanic acid (AMC₃₀), cefadroxil (CFR₃₀), cefotaxime (CTX₃₀), imipenem (IMP₁₀), kanamycin (KAN₃₀), levofloxacin (LEV₅), streptomycin (STR₁₀), and tetracycline (TEC₃₀).

Statistical analysis

Data were analyzed using SPSS software version 21. Confidence intervals are exact Clopper-Pearson confidence intervals. Two-sample proportion test was used to assess the difference in prevalence of *Salmonella* between city center and suburbs. Alpha level was set to 0.05. Association between seasons and prevalence was calculated by Fisher's exact test.

Results

Out of 225 grilled chicken meat samples, 16 (7.1%) were positive for the presence of *Salmonella*. Of note, 9.3% of thigh samples harbored *Salmonella*. The distribution of positive samples is summarized in Table 1. Statistically, it is estimated that 4.12-11.29% (95% confidence interval) of grilled chicken meat in Erbil retail outlets are contaminated by *Salmonella* species.

Grilled chicken meat samples from city center showed less contamination rate (5.9%) in comparison to suburbs samples (8.4%). Despite the apparent difference in prevalence between samples from city center and those from outskirts, the difference between the two groups is not statistically significant ($Z_c=0.72$; $P=0.471$). On the contrary, summer months (July and August) were significantly associated with increase in *Salmonella* prevalence ($P=0.033$) (Table 2). Regarding *Salmonella* serotypes, sixteen isolates belonged to eight different serotypes. *S. Typhimurium* and *S. Anatum* were the most commonly detected serotypes (25% and 19%, respectively), while other detected serotypes and their iso-

lation rate are summarized in Figure 1.

Salmonella isolates were tested against ten commonly used antibiotics. The results of susceptibility testing are depicted graphically in Figure 2. Strikingly, penicillins and cephalosporins antibiotics showed a good activity against isolated salmonellae, while higher number of resistant strains were observed to tested aminoglycosides and levofloxacin and imipenem.

Discussion

Salmonellosis is a major cause of human bacterial gastroenteritis that represents a growing public health concern in both developing and developed countries. It has been declared by the Food Agriculture Organization (FAO) and the Pan American Health Organization (PAHO) as the most common and significant food-borne disease since 1950 (FAO & PAHO, 2017). In the present study, the prevalence of *Salmonella* in grilled chicken meat samples was 16/225 (7.1%, CI; 4.12% - 11.29%) (Table 1). These results are consistent with an Egyptian study in Luxor city which found that the isolation rate of *Salmonella* in ready-to-eat meat (RTE) samples was 6.67% (Younis *et al.*, 2019). However, a slightly lower rates were reported in Bangladesh (5%) and in Egypt (4.4%) where *Salmonella* prevalence in street food was 5% (Abd-El-Malek, 2017; Khalif *et al.*, 2018). In contrast, higher rates were reported from Korea (22.4%), Australia (38.8%), and China (54%) (Kim *et al.*,

2012; Fearnley *et al.*, 2011; Yang *et al.*, 2010). Such variations may be attributed to difference in geographical location, season, sanitation practices, and laboratory detection methods (Lee *et al.*, 2015). A higher proportion of contaminated chicken meat samples was found in suburb areas (9.3%). This may be a direct result of exposure to many potential contaminating sources (such as farms, waste treatment facilities, wild animals) existing in the outskirts of cities. In community-based studies, FAO & WHO found a link between the increase prevalence of *Salmonella* in poultry and increase of human salmonellosis cases (WHO, 2018). Consequently, reducing poultry contamination would reduce salmonellosis in human.

In terms of detected serotypes, various reports showed different serotypes to be the most common. However, *S. Typhimurium* and *S. Enteritidis* tend to have the highest prevalence in different regions. For instance, a Korean study found *S. Enteritidis* to be the dominant serovar (57%). Furthermore, a recent Egyptian study found *S. Typhimurium* and *S. Enteritidis* serovars to constitute 62.5% of detected salmonellae in ready-to-eat meat samples (Younis *et al.*, 2019). It is well-known that ecological distribution of *Salmonella* serovars are subjected to various environmental factors (Chousalkar & Gole, 2016).

Regarding *Salmonella* serovars isolated in this study (Figure 1), *S. Typhimurium* was the most prevalent (25.0%) which is in good agreement with the results obtained in reports from Egypt (25%) and China

Table 1. Occurrence of *Salmonella* among grilled chicken meat samples.

Collection site	No. examined	No. positive (%)	95% CI
Shawarma	75	4 (5.3)	1.47-13.10
Thigh	75	7 (9.3)	3.84-18.29
Breast	75	5 (6.7)	2.20-14.88
Total	225	16 (7.1)	4.12-11.29

Table 2. Prevalence of *Salmonella* among grilled chicken meat during study period.

Month	No. of examined (no. of positive)			Total examined (total positive)	% (95% CI)
	Shawarma	Thigh	Breast		
July	12 (1)	12 (2)	12 (1)	36 (4)	11.1 (3.11-26.06)
August	12 (2)	12 (3)	13 (2)	37 (7)	18.4 (7.74-34.33)
September	13 (1)	12 (1)	12 (1)	37 (3)	8.1 (1.7-21.91)
October	13 (0)	13 (1)	13 (1)	39 (2)	5.1 (0.63-17.32)
November	12 (0)	13 (0)	13 (0)	38 (0)	0 (0.00-9.49)
December	13 (0)	12 (0)	12 (0)	37 (0)	0 (0.00-9.25)
Total	75 (4)	75 (7)	75 (5)	225 (16)	7.1 (4.12-11.29)

(27.6% for *S. Typhimurium* and *S. Enteritidis*) (Yang *et al.*, 2010; Younis *et al.*, 2019). On the contrary, lower prevalence of salmonellae in chicken meat samples was documented in other reports from Brazil and Egypt ranging from 4% to 5% (Giombelli & Gloria, 2014; Shaltout *et al.*, 2013). Antibiotics resistance in *Salmonella* is increasing constantly. Surveillance reports demonstrated a two-fold increase in *Salmonella* resistant phenotypes (from 20%-30% to ca. 70%) since early 1990s to 2000s (Su *et al.*, 2004). In this study, an intriguing observation is the complete resistance to levofloxacin (Figure 2). In general, fluoroquinolones resistance mechanisms in *Salmonella* can be mediated by point mutations that protect DNA gyrases or efflux pumps that extrude antibiotics out of the bacterial cell or plasmid mediated resistance determinants (Eng *et al.*, 2015). The latter is of public health importance due to its inter-species potential spread. Development of such high level of fluoroquinolones resistance in *Salmonella* is usually rare (CDC, 2010). This observation is a serious alarm owing to the fact that fluoroquinolones are central drugs in treatment of severe human salmonellosis.

Indeed, increase use of antibiotics in food animals' industry for therapeutic, prophylactic, and growth promoting purposes increases and selects for resistant phenotypes for employed antibiotics. Unfortunately, to the best of our knowledge, the status of usage of fluoroquinolones in poultry and resistance rate in Iraq are still unknown. Recently, multidrug resistant *Salmonella* isolates were recovered from poultry meat samples and showed resistance to streptomycin (30.9%), tetracycline (13.9%), gentamicin (12.6%) (Liljebjelke *et al.*, 2017). However, the susceptibility pattern to ceftriaxone (75% resistant) was higher than the findings of the current study (12.5%). A close rate of ceftriaxone resistance (16%) was found in a large Chinese study (Yang *et al.*, 2010). Additionally, recent report from Pakistan Kohat district found salmonellae resistant to ampicillin (80%), tetracycline (78%), and poor efficacy of fluoroquinolones (ciprofloxacin and levofloxacin) (Ullah *et al.*, 2019).

Generally, resistance to carbapenems, including imipenem, in Enterobacteriaceae is usually mediated by either decreased outer membrane permeability (loss/modification of porins channels) or by carbapenems-hydrolyzing enzymes (best reviewed in Nordmann *et al.*, 2012). Carbapenemases are three different types of β -lactamases, designated as Amber classes (A, B, & C). These enzymes are either chromosomally-encoded or plasmid-mediated with poten-

tials to be transferred between related bacterial species (Patel & Bonomo, 2013). Imipenem resistance in *Salmonella* is very rare. However, a resistant clinical strain had been recovered in 2003 and found to harbor KPC-2 β -lactamase gene (Miriagou *et al.*, 2003). Moreover, imipenem resistance in *Salmonella* has been reported to be conferred by both mechanisms (porin loss and β -lactamase) simultaneously (Armand-Lefevre *et al.*, 2003).

Conclusions

A slightly alarming prevalence of *Salmonella* in grilled chicken meat samples has been found in the present study. Salmonellosis is an important food borne

disease that needs to be further investigated in Erbil city. The findings should be interpreted with caution due to small recovery rate and short study period. Recovery of different *Salmonella* serovars from chicken meat samples is of concern, especially because of their high resistance level to important antimicrobials. Epidemiological and molecular investigations should follow this previous study to assess the risk for consumers. Random antibiotics usage should be restricted to minimize public health hazards of spreading multi-drug resistance pathogens. A four-season study is recommended to further investigate the distribution of salmonellae isolates in various meat samples accompanied by antimicrobial susceptibility testing to reduce burden and morbidity of salmonellosis in Erbil city.

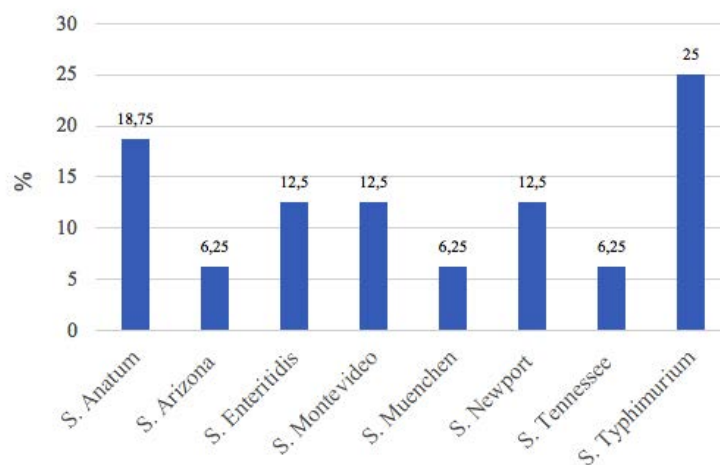


Figure 1. Percentage of different detected *Salmonella* serotypes in positive samples (n=16).

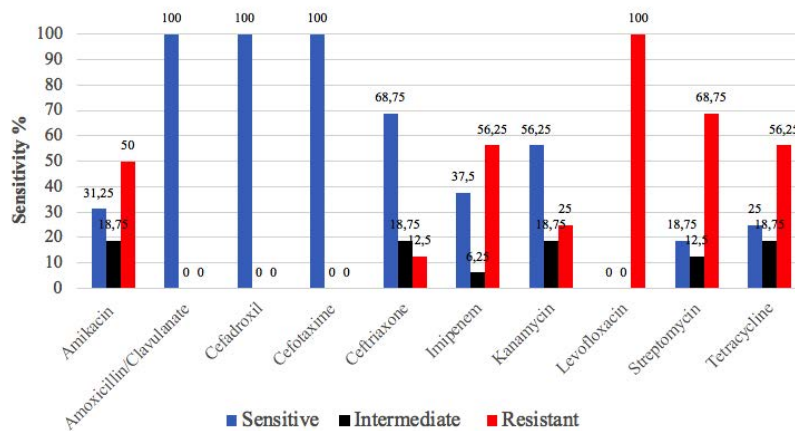


Figure 2. Antibiogram profile of *Salmonella* isolates from grilled chicken meat samples.

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