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Prevalence of Antimicrobial Resistance of *Salmonella* Species Isolated from Chicken Meat in Riyadh City

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Abstract: ***The global emergence of antimicrobial resistance has become a pre-eminent concern in medicine, veterinary medicine and public health. Antimicrobial resistance is of particular concern because the problem is widespread, the causative factors are uncontrolled, and national strategies to address the problem had been lacked. The persisting burden of infectious diseases makes elimination of antibiotic use unethical, but dramatic overuse and misuse of antimicrobial agents around the world must be reduced to extend the useful lifetimes of these drugs. Salmonella is one of the most prevalent causes of food borne illness worldwide. The aim of this study was to determine the prevalence of Salmonella species in chicken meat in Riyadh city using standard culture method (ISO 6579: 2002) and confirm the results using VITEK 2 COMPACT and the protein finger print of Salmonella using MALDI TOF BIOTYPER then determine the antimicrobial resistance of the isolated strains of Salmonella using the AST cards and VITEK 2 COMPACT. A total of 100 samples of chicken meat comprising of 30 raw chicken meat (whole carcasses), 30 chicken cuts, 20 chicken fillets, 10 chicken livers and 10 chicken kidneys were purchased from different slaughterhouses and supermarkets in Riyadh city. The obtained results indicated that the total number of positive samples of raw chicken meat for Salmonella species was 13 out of 30 examined samples representing 43.33%, 18 out of 30 examined chicken cuts representing 60%, 16 out of 20 examined chicken fillets representing 80%, 7 out of 10 examined chicken livers representing 70% and 4 out of 10 examined chicken kidneys representing 40%. The Salmonella isolates were resistant to Ciprofloxacin, Levofloxacin, Gentamicin, Trimethoprim / Sulfamethaxazole, Minocycline, Ampicillin / Sulbactam, Tobramycin and Aztreonam. The present study indicates high prevalence of Salmonella in raw chicken meat due to poor hygienic practices and therefore emphasizes the need for adopting these hygienic practices.***

Keywords: *Salmonella*, chicken meat, antimicrobial resistance, ISO 10272-1:2006, protein finger print

1. Introduction

Antimicrobial resistance (AMR) is a major public health issue. It leads to therapeutic failure and to increased morbidity and mortality of those affected with infections caused by resistant pathogens. Drug-resistant pathogens are estimated to be responsible for 25,000 deaths every year in Europe **(ECDC, EMEA. 2009).** The epidemiology of AMR is complex; humans can become exposed through varied pathways such as; hospital-acquired, environmental, direct contact with pets, wildlife, food-producing animals or humans, but also through water and food. Antimicrobial use (AMU) is one of the major factors associated with the emergence and spread of AMR **(Davies and Davies 2010).** Antimicrobials are widely used in agriculture to prevent and treat infectious diseases in livestock and plants and, in some countries outside the EU they are also used as growth promoters (AGPs) in food-producing animals (4, 5). In EU Member States, the use of AGPs has been progressively prohibited since 2006 **(Anon. Regulation (EC) no 1831/2003).** AMU is regulated in most European countries but in many countries outside the EU, antimicrobials can be purchased over the counter or are counterfeit and their use occurs often without veterinary supervision. This could pose a serious risk to consumers, as individuals could later become exposed through food to drug-resistant bacteria, resistance determinants (i.e. genes) or antimicrobial drug residues that could result in selective pressure in the gut flora. Drug-resistant food borne pathogens such as fluoroquinolone-resistant Campylobacter spp. and extended-spectrum β-lactamase (ESBLs)-producing bacteria have been isolated with increasing frequency in food, food-producing animals and humans in Europe **(EFSA, ECDC.2015).** Food animals and their production environments are reservoirs of both resistant bacteria and resistance genes that could be transferred to humans either by direct contact between animals and humans or indirectly via the food production chain **(Marshall and Levy,2011).**Although E. coli is a normal inhabitant of the intestinal tract ofwarm blooded animals, commensal E. coli from humans and animals can cause extra intestinal infections and are a potential reservoir of antimicrobial resistance genes **(Guerra et. al. 2003).**The use of antimicrobials combined with improvements in sanitation, nutrition and immunization has led to a dramatic decrease in deaths and a major gain in human life expectancy **(WHO, 2002).**The presence of AMR bacteria in primary animal production represents a high risk for humans since AMR bacteria of animal origin can be transmitted from animals to humans through the food supply (food-borne pathogens), water or direct contact with animals**(Funk et. al., 2006).** In farms, factors that can influence bacterial resistance vary depending on herd or flock health status, farm management and environment. These practices include over-prescription of broad spectrum drugs by veterinarians instead of narrow-spectrum drugs**) Sarkar andGould,2006)** also the use of non-approved drugs or drugs used in extra-label manner are believed to contribute to the development of antimicrobial resistance**(Sharma et. al., 2005).**

It was stated by well-established evidence that antibiotics can

lead to the emergence and dissemination of resistant E. coli which can then be passed into people via food or direct contact with infected animals. These resistant microbes may function as a potential source in the transportation of antimicrobial resistance to human pathogens **(Van and Stobberingh, 2000).**

At butchery/ slaughter, resistant strains from the gut readily contaminate poultry carcasses which often cause contamination of poultry meats and eggs during lay with multi resistant E. coli **(Turtura et. al., 1990).** Due to enormous exploitation of antibiotics in the field of veterinary medicine, an increased number of resistant bacterial strains were developed in recent years. The transmission of plasmid mediated resistance between different bacterial species and genera are now widely occurred **(Davies, 1994).**In different parts of the world, multi drug resistant strains of E. coli are ubiquitous in both human and animal isolates**(Amara et. al., 1995).** Acquired multi drug resistance to antimicrobial agents creates an extensive trouble in case of the management of intra and extra intestinal infections caused by E. coli, which are a major source of illness, death, and increased healthcare costs **(Gupta et. Al., 2001).**

A wide range of foods has been implicated in human salmonellosis. However, contaminated foods of animal origin, especially meat, milk and dairy products, poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human salmonellosis. To date, over 2579 different Salmonella serotypes have been identified. All Salmonella serotypes are considered potentially pathogenic and various serotypes are implicated in foodborne infections **(FDA, 2012 and WHO, 2013).**

Several studies that evaluated the performance of MALDITOF MS in microorganism identification demonstrated that these systems are highly descriptive, accurate and reproducible. In a retrospective investigation carried out by **Eigner et al.(2009).**

2. Material and Methods

a) Sample collection

A total of 100 samples of chicken meat comprising of 30 raw chicken meat (whole carcasses); 30 chicken cuts ; 20 chicken fillets ; 10 chicken livers and 10 chicken kidneys in their original packs were purchased from different supermarkets in Riyadh city. The collected samples were rapidly transported to the laboratory of the national center for agriculture and animal wealth researches in chilled containers. All samples were stored at 4°C prior to testing and were bacteriologically analyzed within 24 h of receipt at the laboratory.

b) Isolation of Salmonella species

Isolation and identification of Salmonella species was performed according to the standard method of **(ISO 6579: 2002).**Briefly, 25g of each chicken meat sample was added to225ml of Buffered Peptone Water and homogenized for 2 min. using a stomacher, then incubated at 37°C for 18h followed by transferring of 1ml of the pre-enrichment culture to 10ml of Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn) and 0.1ml to 10ml of Rappaport Vassiliadissoya peptone broth (RVS Broth), respectively with incubation for 24h at 37°C MKTTn and 41.5°C RVS broth. From each of selective enrichment cultures one loopful was sub-cultured on two selective plating agars, xylose lysine deoxycholate (XLD) and brilliant green agar (BGA). The plating agars were inoculated at 37°C for 24-48h. The selective agar plates were examined for typical colonies of Salmonella, red with black centers colonies on XLD and red colonies on BGA. Finally, 5 presumptive Salmonella colonies from each selective agar plate were picked, purified and sub-cultured onto nutrient agar (NA) plates. NA plates were incubated at37°C for24h.

2.1 Identification and confirmation of Salmonella species:

a) Identification and antibiotic susceptibility

The Vitek 2 compact automated system (Biomérieux) will be used for the identification and the antibiotic susceptibility testing of the collected isolates. By using the Vitek 2 ID-GNB card (Biomérieux), identification of bacterial pathogen occurs through testing the organism's metabolic activity in 41 fluorescent biochemical tests including 18 enzymatic tests, 18 fermentation tests, two decarboxylase tests and three other miscellaneous tests. Antibiotic susceptibility testing is based on kinetic analysis of the bacterial growth in the presence of selected antibiotics (20 antibiotics representing all antibiotic families) and the antibiotic susceptibility profile is then analysed in order to predict the underlying resistance mechanisms present in each isolate. The antibiotic panel (Vitek 2 AST-292) will be selected, as it covers the commonly used antibiotics and it is one of the standard antibiotic panels used in the KSA. It consists of the following antibiotics: Ampicillin / Sulbactam, Ticarcillin /Clavulanic acid,, Piperacillin/Tazobactam, Ceftazidime, Cefepime, Aztreonam, Ertapenem, Imipenem, Meropenem, Tobramycin, Amikacin, Gentamicin, Ciprofloxacin, Levofloxacin, Minocycline, Tigecycline, Colistin and Trimethoprime/ Sulfamethoxazole.

b) Identification by MALDI-TOF MS fingerprinting

Briefly, after overnight culture, a fresh colony incubated for 18-24 h at 37°C was inoculated onto two spots of target plate and then enclosed with one µl of matrix solution (saturated α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid). The spectra were directly produced by applying new software, namely, Compass Satellite software and the identification was conducted with a Microflex LT device.

2.2 Data analysis

According to the instructions of Bruker Daltonics, the score value of unidentified spectrum in the range from zero to three was determined by matching the unidentified spectrum with the stored spectrum in the Bruker database. The accurate identification of the field isolates is carried out when the score value ranges from 2.30 to 3.00. Nevertheless, species and genus levels are detected when the score value ranges from 2.00 to 2.29 and 1.700 to 1.999, respectively. In contrast, the identification is not reliable when the score value ranges from 0.00 to 1.69. The spectra created by

compass software were measured in a m/z range between 3000 and 20000 Daltons (Da).

3. Results

Table 1: Prevalence of Salmonella species in examined chicken meat samples:

Type of samples	Number of examined samples	Positive samples for salmonella	
		No	%
Whole chicken	30	13	43.33%
Chicken cuts	30	18	60%
Chicken fillets	20	16	80%
Chicken livers	10	7	70%
Chicken kidneys	10	4	40%
Total	100	58	58%

Table 2: Resistance % of the isolated Salmonella from the examined samples:

Antimicrobial used	Number of resistant isolates	Percentage
Ampicillin/Sulbactam	10	17.24 %
Aztreonam	9	15.51%
Gentamicin	6	10.34 %
Tobramycin	4	6.89 %
Ciprofloxacin	3	5.17 %
Levofloxacin	5	8.62%
Minocycline	17	29.31%
Trimethoprime/ Sulfamethoxazole	9	15.51 %

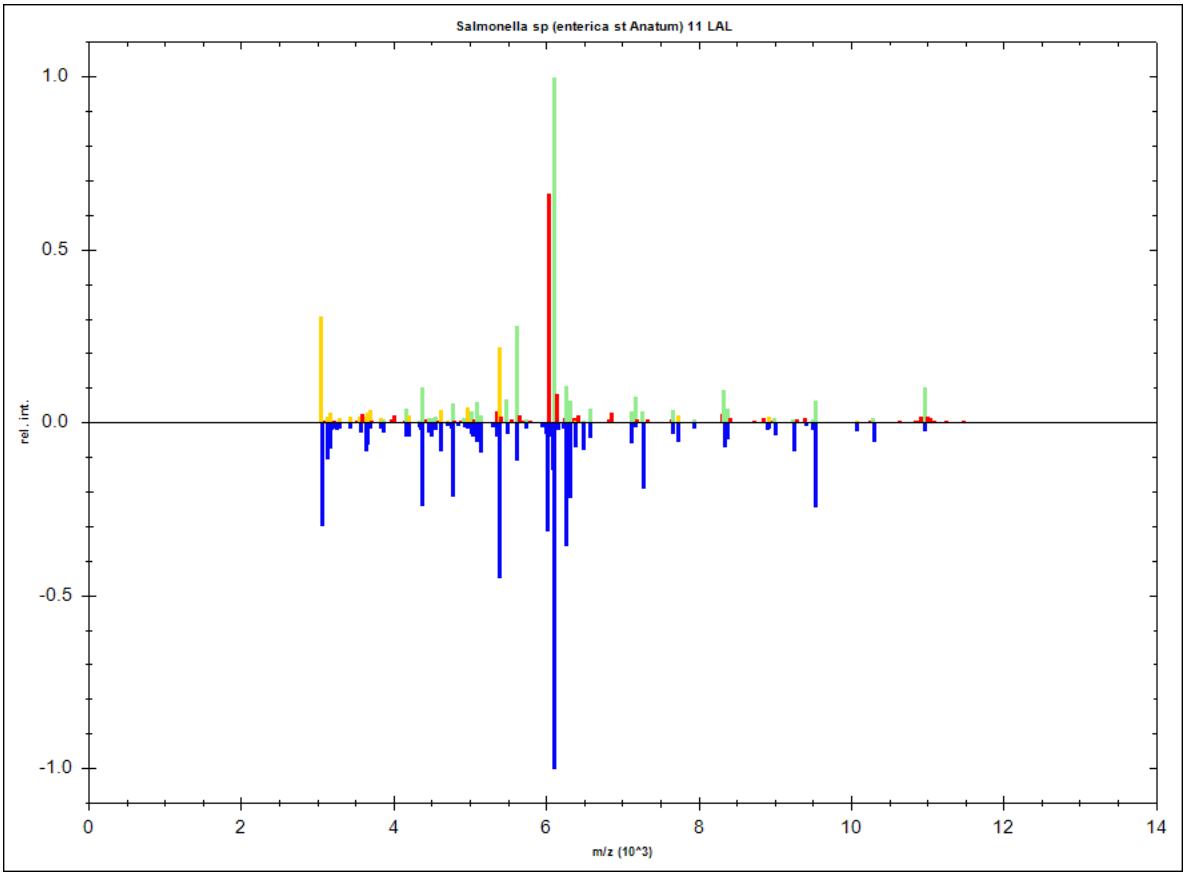

Figure 1: Protein finger print of *Salmonella* isolate from chicken meat

4. Discussion

Antimicrobial resistance (AMR) particularly that observed to antimicrobials used to treat infections in humans and animals is a major public health problem. This is due to the risk of treatment failure that may lead to an increase in duration of illness and, even death, of individuals and animals with infections caused by antimicrobial-resistant bacteria. People may become exposed to such organisms through a number of routes such as direct contact with animals, and the environment and also through the food chain. There was lack of AMR prevalence data for Saudi Arabia-produced food. Food-producing animals are reservoirs of pathogens with the potential to transfer resistance to humans. Resistance can transfer from animals to humans by transfer of antibiotic resistant zoonotic or commensalistic bacteria, or by transfer of resistance genes in the human gastro-intestinal tract following ingestion of contaminated animal products **(WHO, 1984).** Chickens can be reservoirs for several food-borne pathogens including Campylobacter and Salmonella **(Kazwala et. al., 1990).** Therefore, this study was performed to determine the prevalence of Salmonella and its resistance to the antimicrobials in chicken meat in Riyadh city.

As shown in table (1) Salmonella was isolated from 13out of 30 examined raw chicken meat (whole chicken) samples representing 43.33%. Regarding chicken cuts Salmonella was isolated from 18 out of 30 examined samples representing 60%. Concerning chicken fillets, Salmonella was isolated from 16 out of 20 examined samples representing 80%.On the other hand in chicken livers, Salmonella was isolated from 7 out of 10 examined samples

representing 70% while in chicken kidneys, Salmonella was isolated from 4 out of 10 examined samples representing 40%.The total number of positive samples for Salmonella was 58 out of 100 examined samples representing 58%.

Cutting boards and knives may cause cross contamination of the chicken cuts and fillets by Salmonella, and this may be the reason of the high percentage of Salmonella positive samples in chicken cuts and fillets.

Nearly similar results were detected by **El-Sharkawy et al. (2017).** Also our results are in agreement with **Ramya, et al. (2012)** who reported that *Salmonella* positive samples were 64% of chicken meat. While lower results were obtained by **Lobna M.A. Salem et al. (2016)** who detected the Salmonella in 15.5% of chicken meat samples.

Regarding the antimicrobial sensitivity in table (2), the numbers of resistant isolates to Ampicillin/ Sulbactam, Aztreonam, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Minocycline and Trimethoprime/ Sulfamethoxazole were 10 (17.24 %), 9 (15.51%), 6 (10.34 %), 4 (6.89 %), 3 (5.17 %), 5 (8.62 %), 17 (29.31 %) and 9 (15.51 %), respectively. These results did not agree with **Lobna M.A. Salem et al. (2016)** who reported that all Salmonella strains were sensitive to levofloxacin and amikacin (100%), while all isolates were resistant to erythromycin (100%). In contrast, ampicillin had the basic effect on viability of Salmonella strains followed by cefexime and tetracycline.

Proteomic identification of foodborne pathogens: In the present study, all Salmonella isolates isolated from various chicken meat samples were identified by MALDI-TOF-MS fingerprinting and the spectra obtained were compared with the spectra stored in the Bruker database. All Salmonella isolates (58) were 100% correctly identified by MALDI-TOF-MS fingerprinting with a score value ≥2.00. Our results using MALDI-TOF-MS fingerprinting were similar to those obtained by (**Dieckmann and Malorny, 2011; Sparbier et al., 2012).**

The antibiotic susceptibility test revealed the presence of multiple drug resistant Salmonella in chicken meat. The present study indicates high prevalence of Salmonella in raw chicken meat due to poor hygienic practices and therefore emphasizes the need for adopting these hygienic practices.

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