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*Full Length Research Paper*

# Prevalence and anti-microbial susceptibility of *Salmonella* isolates from chicken carcasses and giblets in Meknès, Morocco

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**A study was made of *Salmonella* contamination in chicken carcasses and giblets sampled from retail outlets in Meknès, Morocco. The serotypes as well as antibiotic-resistance patterns of the *Salmonella* isolates were determined. A total of 576 samples (144 from popular market, 144 from artisanal slaughterhouses, 144 from poulterers' shops and 144 from a supermarket) were tested. Among them, 57 (9.90%) were positive for *Salmonella*, 20.83% (30/57) from popular market, 16.66% (24/57) from artisanal slaughterhouses and 2.08% (3/57) from poulterers' shops. The 57 *Salmonella* isolates were divided into 4 serotypes. The most prevalent serotypes were *Salmonella typhimurium* (40.35%) and S. *newport* (26.31%). All *Salmonella* isolates were tested for their susceptibility to 12 selected antimicrobial agents by the agar diffusion method. 43 (75.43%) isolates were resistant to one or more antimicrobials. Out of 43 resistant *Salmonella* isolates, 17 (39.5%) showed multiple resistance to tow or more different antimicrobials. Resistance to tetracycline, sulfamides, trimethoprim and streptomycin was the most frequent. We found 17 different patterns of multiresistant strains. The high level of antibiotic resistance of *Salmonella* isolates in the present study showed the possible significance of chicken meat as a source of multiple antimicrobial-resistant *Salmonella* for human infections and suggest more restrict-tions on the irrational use of antibiotics.**

**Key words:** *Salmonella*, antibiotic-resistance, chicken, retail outlets, Mèknès, Morocco.

## INTRODUCTION

Microbial food safety is an increasing public health concern worldwide. Epidemiological reports suggest that poultry meat is still the primary cause of human food poisoning (Mulder, 1999). Poultry meat is more popular in the consumer market because of advantages such as easy digestibility and acceptance by the majority of people (Yashoda et al., 2001). However, the presence of pathogenic and spoilage microorganisms in poultry meat and its by-products remains a significant concern for suppliers, consumers and public health officials worldwide. *Salmonella* has been consistently associated with foodborne illnesses in most countries of the world. Poultry and eggs are frequent vehicles in outbreaks involving this organism (Todd, 1994). Bacterial contamination of these foods depends on the bacterial level of the poultry carcasses used as the raw product, the hygienic practices during manipulation and on the time and temperature of storage (El-Leithy and Rashad, 1989).

The utilisation of antimicrobial drugs has played an important role in animal husbandry, since they are used in prophylaxis, treatment and growth promotion. However, the extensive use of those in human and animals has led to an increase in bacterial multidrug resistant among several bacterial strains including *Salmonella*. The husbandry practices used in the poultry industry and the widespread use of medicated feeds in broiler and layer operations made poultry a major reservoir of antimicrobial-resistant *Salmonella* (D'Aoust et al., 1992).

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**Table 1.** *Salmonella* isolated from retail outlets.

| Sample From | Number of samples | | % | Serotypes | | | |
|---|---|---|---|---|---|---|---|
| | Examined | Positive | | *S. typhimurium* | *S. newport* | *S. montevideo* | *S. heidelberg* |
| Popular market | 144 | 30 | 20.83 | 10 | 9 | 6 | 5 |
| Artisanal slaughterhouses | 144 | 24 | 16.66 | 11 | 5 | 4 | 4 |
| Poulterers' shops | 144 | 3 | 2.08 | 2 | 1 | 0 | 0 |
| Supermarket | 144 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 2.** *Salmonella* isolated from chicken meat and giblets.

| Sample From | Number of samples | | % |
|---|---|---|---|
| | Examined | Positive | |
| Breast | 144 | 9 | 6.25 |
| Legs | 144 | 12 | 8.33 |
| Liver | 144 | 16 | 11.11 |
| Gizzard | 144 | 20 | 13.88 |

Concern about poultry, meats and other foodstuffs contaminated with foodborne pathogens has gained considerable attention (Seyfarth et al., 1997 and Breuil et al., 2000).

To satisfy the requirements of consumers in protein animal, the production of poultry meat shows an upward trend in Morocco. However, the control and inspection during production, storage and distribution are generally rare. Therefore, it is important to prevent the hazards and to provide a safe and wholesome product for human consumption (Singh et al., 1984). This study was designed to investigate the frequency of occurrence of *salmonella* from chicken carcasses and giblets and their microbial resistance profile in Meknès, Morocco.

## MATERIALS AND METHODS

### Samples

Between November 2005 and November 2006, a total of 576 samples (included 144 breast, 144 legs, 144 gizzards and 144 livers) were collected every 10 days from retail outlets in Meknès. Of these, 144 were from popular market, 144 from artisanal slaughterhouses, 144 from poulterers' shops and 144 from a supermarket at Meknes, Morocco. Each sample was placed in a separate sterile plastic bag. Samples were transported to the laboratory immediately after collection in an ice chest and microbiological analysis was carried out immediately.

### Isolation and identification of *Salmonella*

25 g of each sample were put into a stomacher bag containing 225 ml buffered peptone water (AES Laboratoire, Combourg, France) and homogenised using a stomacher (Colworth 400, London). The homogenate was incubated at 37°C for 16 to 20 h. 2 and 0.1 ml of the pre-enrichment were then respectively transferred in 20 ml of selenite cystine broth (Biorad/356 - 4074/Biorad/Marnes la coquette/France) and 10 ml of Rappaport–Vassiliadis broth (Biorad/356 - 4324/Biorad/Marnes la coquette/France), and incubated for 18 - 24 h at 37°C (Selenite Cystine) and at 42°C (Rappaport Vassiliadis). Afterwards, one *Salmonella-Shigella* (SS) agar plates per tube was inoculated and incubated at 37°C for 18 - 24 h. Presumptive *Salmonella* colonies were confirmed by biochemical assays on Kligler Hajna medium, ONPG medium and lysine decarboxylase, and then serotyped by slide agglutination test using *Salmonella* polyvalent O and H antisera (Diagnostic Pasteur, Paris, France).

### Antimicrobial resistance test

The antimicrobial resistance of the isolates was determined by the agar diffusion method with Mueller Hinton agar and bio- rad disks (Marnes-La-Coquette, France). The 12 antimicrobials tested were those commonly used in poultry herds or in human. There are: streptomycin (10 µg), gentamicin (15 µg), neomycin (30 UI), tetracyclin (30 µg), nalidixic acid (30 µg), flumequin (30 µg), sulphonamides (200 µg), trimethoprim-sulphamethoxazole (1.25 µg/25.75 µg), trimethoprim (5 µg), chloramphenicol (30 µg), colistin (50 µg) and amoxicillin-clavulanic acid (20/10 µg). The categories susceptible or resistant were assigned on the basis of the critical points recommended by the French committee on guidelines for susceptibility testing (Comité de l'Antibiogramme de la Société Française de Microbiologie, C.A.-S.F.M., 2006).

## RESULTS

Of the total of 576 samples examined, 9.90% (57/576) were contaminated with *Salmonella* (Table 1). Out of the total 144 samples (n = 576) analysed from popular market, 30 (20.83%) proved to be *Salmonella* positive whereas from 144 samples obtained from traditional slaughterhouses 24 (16.66%) contained *Salmonella*. A low level of *Salmonella* contamination was found in samples obtained from poulterers' shops 3 (2.08%). However, *Salmonella* was not detected in any of the samples purchased from supermarket. Among the 57 *Salmonella* isolates, 4 different serotypes were identified of which *S.* Typhimurium (40.35%) was the most frequent, followed by *S. newport* (26.31%). *S. montevideo* (17.54 %) and *S. heidelberg* (15.78%). *S. montevideo* and *S. heidelberg* were detected only from samples taken at popular market and at traditional slaughterhouses.

As shown in Table 2, a high level of *Salmonella* contamination was found in chicken gizzard (13.88%) and liver (11.11%), followed by legs (8.33%) and breast (6.25%). Contamination rates of chicken parts (12.50%) were higher than those of chicken carcasses (7.29%).

Antibiotic resistance in *Salmonella* strains to 12 antimi-

**Table3.** Resistance of *Salmonella* serotypes isolated from chicken carcasses and giblets to antimicrobial agents.

| Serotypes | Antibiotics | | | | | | | | | | | | | | Recapitulatory | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | n r | S | GM | N | TE | NA | UB | SSS | SXT | TMP | C | CL | AMC | 1 | 1 | 1 |
| *S. typhimurium* | 23 | 19 | | | | | | | | | | | | | 11 | 11 | 11 |
| *S. newport* | 15 | 14 | 5 | 0 | 2 | 9 | 2 | 0 | 5 | 5 | 5 | 0 | 2 | 0 | 12 | 12 | 12 |
| *S. montevideo* | 10 | 6 | 2 | 1 | 0 | 5 | 1 | 0 | 5 | 1 | 1 | 0 | 1 | 0 | 2 | 2 | 2 |
| *S. heidelberg* | 9 | 4 | 2 | 0 | 1 | 3 | 0 | 0 | 3 | 4 | 3 | 0 | 0 | 1 | 1 | 1 | 1 |
| Total | 57 | 43 | 1 | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 2 | 0 | 1 | 0 | 26 | 26 | 26 |

S = streptomycin, GM = gentamycin, N = neomycin, TE = tetracyclin, NA = nalidixic acid, CIP = ciprofloxacin, UB = flumequin, SSS = sulphonamides, SXT = trimethprim-sulphamethoxazole, TMP = trimethoprim, C = choramphenicol, CL = colistin, AMC = amoxicillin-clavulanic acid. n = number of isolates, 0 = susceptible; 1 = resistance to one antibiotic; 2 - 4 = resistant to 2 - 4 antibiotics; 4 + = resistance up to 4 antibiotics.

**Table 4.** Multiple antimicrobial resistance patterns of 4 *Salmonella* serotypes isolated from chicken carcasses and giblets.

| Serotypes | Number of Isolates testes | Resistant isolates | Resistance pattern (Resistance to two or more) | | | | | Number of Multiresistant isolates |
|---|---|---|---|---|---|---|---|---|
| *S. typhimurium* | 23 | 19 | S | N | SXT | TMP | | 1 |
| | | | S | TE | NA | SXT | TMP | 1 |
| | | | N | TE | SXT | | | 1 |
| | | | TE | SSS | TMP | | | 1 |
| | | | TE | SSS | | | | 1 |
| | | | TE | SXT | | | | 1 |
| | | | S | TE | TMP | | | 1 |
| | | | SSS | SXT | | | | 1 |
| *S. newport* | 15 | 14 | S | TE | | | | 1 |
| | | | SXT | TMP | | | | 1 |
| *S. montevideo* | 10 | 6 | S | TE | TMP | | | 1 |
| | | | N | SXT | SSS | TMP | | 1 |
| | | | S | SXT | SSS | TMP | | 1 |
| | | | SXT | SSS | AMC | | | 1 |
| *S. heidelberg* | 9 | 4 | S | TE | CL | | | 1 |
| | | | TE | NA | TMP | | | 1 |
| | | | SSS | TMP | | | | 1 |

S = streptomycin, GM = gentamycin, N = neomycin, TE = tetracyclin, NA = nalidixic acid, CIP = ciprofloxacin, UB = flumequin, SSS = sulphonamides, SXT = trimethprim-sulphamethoxazole, TMP = trimethoprim, C = choramphenicol, CL = colistin, AMC = amoxicillin-clavulanic acid. n = number of isolates, 0 = susceptible; 1 = resistance to one antibiotic; 2 - 5 = resistant to 2 - 4 antibiotics; 4 + = resistance up to 4 antibiotics.

antimicrobial agents is shown in Table 3. Overall, the highest percentage of resistance was found to the following antimicrobial agents: tetracycline (44.18%), sulfamides (34.88%), trimethoprim (25.58%) and streptomycin (23.25%). Low resistance rates were returned for amoxicilline-clavulanic acid, gentamycin, neomycin, naildixic acid and colistin, while zero resistance were recorded against flumequin or choramphenicol. On the other hand 75.43% of isolates were found to be resistant to one or more of the antibiotics tested. Multiple resistance was observed in 17 strains (39.5%). Therefore, a high prevalence of multiresistance among foodborne *Salmonella* strains was observed. A total of 17 different patterns of resistance were observed among *Salmonella* strains (Table 4). *S. Typhimurium* showed the highest percenttages of resistance to the tested drugs. It is worthy remarking that one strain of this serotype was resistant to 5 antibiotics (tetracycline + nalidixic acid + trimethoprim + streptomycin + trimethprim-sulphamethoxazole).

## DISCUSSION

The incidence of *Salmonella* in chicken products obtained by other authors varied between 0 and 100% (Cox and Bayley, 1987; Bryan and Doyle, 1995; Waldroup, 1996). The level of *Salmonella* contamination of chicken samples similar to ours (7.9%) was found by Train et al. (2004) in chicken carcasses in Vietnam. However, the contamination level higher than ours (32%) was found by Cardinale et al. (2003) in chicken carcasses from retail shops in Dakar.

Previously other studies have reported some of serotypes that were identified in our study (Carraminana et al., 1979; Molla et al., 1999; Uyttendaele et al., 1998). It

should be noted that the presence and distribution of *Salmonella* serotypes could vary from region to region (Dominguez et al., 2002; Uyttendaele et al., 1998). It should also be mentioned that isolation rates depend upon the country where the study was carried out, the sampling plan and the detection limit of the methodology (Roberts, 1982; Uyttendaele et al., 1998).

The isolation of invasive *Salmonella* serotypes such as *S. Typhimurium* and other pathogenic *salmonella*s in our study indicate the public health significance of these serovars as contaminated chicken meat and meat products may pose health hazards. This risk may further be higher if chicken meat or giblets are consumed undercooked or cross contamination in the kitchen with *Salmonella* during meal preparation (Scott, 1996; Uyttendaele et al., 1998).

It is estimated that nearly 90% of all antibiotic agents use is in food animals, are given at subtherapeutic concentrations prophylactically or to promote growth (Lee et al., 1993). The antimicrobial susceptibility patterns of the *Salmonella* strains isolated indicated that a large proportion of the isolates were resistant to a variety of the drugs tested particularly tetracycline, sulfamides, trimethoprim and streptomycin.

The percentages of resistance obtained with these antibiotics are comparable with those reported in other studies in France (Sanders et al., 2002) and in Senegal (Bada-Alambedji et al., 2006). Our results were lower than those reported by Carraminana et al. (2004). However, they were higher than the results obtained by Tibaijuka et al. (2002) in Ethiopia.

The high level of contamination of chicken meat and giblets with *Salmonella* observed in this paper indicates the need for an improvement in the microbiological quality of retail chicken. There is also a need for a comprehensive epidemiological study and control of *Salmonella* contamination at various levels of chicken production and retail outlets in Morocco.

The high rates of resistance found in the present study can be explained by the spread of use of antibiotics agents given to poultry in Morocco as prophylaxis, growth promoters or treatment. The multiple resistance observed was to those antimicrobials frequently employed in veterinary practices. We recommend more restrictions on the irrational use of antibiotics and public awareness activeties should be undertaken to alert the public to the risks of the unnecessary use of antibiotics.

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