Full Length Research Paper

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

Chaiba Abdellah[1], Rhazi Filali Fouzja[1*], Chahlaoui Abdelkader[1], Soulaymani Bencheikh Rachida[2] and Zerhouni Mouloud[3]

[1]Department of Biology, College of Sciences, Moulay Ismail University. BP 11 201. Zitoune, Meknès. Morocco.

[2]Anti Poison Center, BP 6671, Rabat institutes, Madinat Al Irfane- Morocco.

[3]Laboratory of Epidemiology and hygiene, Delegation of Public health, Meknès. Morocco.

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 food-borne 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).

*Corresponding author. E-mail: abchaiba@yahoo.fr. Tel.: +2126 4488238.

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 g), gentamicin (15 g), neomycin (30 UI), tetra-cyclin (30 g), nalidixic acid (30 g), flumequin (30 g), sulpho-namides (200 g), trimethoprim-sulphamethoxazole (1.25 g/25.75 g), trimethoprim (5 g), chloramphenicol (30 g), colistin (50 g) and amoxicillin-clavulanic acid (20/10 g). The categories suscep-tible or resistant were assigned on the basis of the critical points recommended by the French committee on guidelines for suscep-tibility 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	n	n r	S	GM	N	TE	NA	UB	SSS	SXT	TMP	C	CL	AMC	Recapitulatory		
															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	SXTTMP	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 under-cooked 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 activities should be undertaken to alert the public to the risks of the unnecessary use of antibiotics.

ACKNOWLEDGEMENTS

REFERENCES

Bada-Alambedji R, Fofana A, Seydi M, Akakpo AJ (2006). Antimicrobial resistance of *salmonella* isolated from poultry carcasses in Dakar (Senegal). Brazilian J. Microbiol. 37: 510-515.

Breuil J, Brisabois A, Casin I, Armand-Lefevere L, Fremy S, Collatz E (2000). Antibiotic resistance in *Salmonella*e isolated from humans and animals in France: comparative data from 1994 and 1997. J. Antimicrob. Chemother. 46: 965–971.

Bryan FL, Doyle MP (1995). Health risks and consequences of *Salmonella* and *Campylobacter* jejuni in raw poultry. J. Food Prot. 58: 326–344.

Cardinale E, Perrier Gros -Claude JD, Tall F, Cissé M, Guèye EF, Salvat G (2003). Prevalence of *Salmonella* and *Campylobacter* in Retail Chicken Carcasses in Senegal, Élev. Méd. Vét. Pays trop., 56: 13-16.

Carraminana JJ, Yanguele J, Banco D, Rota C, Agustin AI, Arino A, Herrera A (1997). *Salmonella* incidence and distribution of serotypes throughout processing in a Spanish poultry slaughterhouse. J. Food Prot., 60: 1312-1317.

Carraminana JJ, Rota C, Agustin I, Herrera A. (2004). High preva-lence of multiple resistance to antibiotics in *Salmonella* serovars isolated from a poultry slaughterhouse in Spain. Vet. Microbiol. 104:133–139

Comité de l'Antibiogramme de la Société Française de Microbiologie (C.A.-S.F.M.). Communiqué 2006. Edition de Janvier 2006.

Cox NA, Bayley JS (1987). Pathogens associated with processed poul-try. In: Cunninghan, F.E., Cox, N.A. (Eds.), Microbiology of Poultry Meat Products. *Academic Press, London*, pp. 293– 310.

D'Aoust JY, Sewell AM, Daley E, Greco P (1992). Antibiotic resistance of agricultural and foodborne *Salmonella* isolates in Canada: 1986–1989. J. Food Prot. 55: 428–434.

Dominguez C, Gomenz I, Zumalacarregui J (2002). Prevalence of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. Int. J. Food Microbiol., 72: 165-168.

El-Leithy MA , Rashad FM (1989). Bacteriological studies on ground meat and its products. *Archiv für Lebensmittelhygiene*, 40: 58-61.

Lee LA, Threatt VL, Puhr ND, Levine P, Ferris K, Tauxe RV (1993). Antimicrobial-resistant *Salmonella* spp. Isolated from healthy broiler chickens after slaughter. J. Am. Vet. Med. Assoc. 202: 752–755.

Molla B, Kleer J, Sinell JH (1999). Occurrence, distribution and level of *Salmonella* in selected food items in Addis Ababa (Ethiopia). Fleischwirtsch. Int., 4: 37-39.

Mulder RW (1999). Hygiene during transport, slaughter and processing. In: Poultry Meat Science. Poultry Science Symposium Series. Richardson and Mead (ed.)*,* 25: 277-285.

Roberts D (1982). Bacteria of public health significance. In: Brown, M.H. (Ed.), Meat Microbiology. Applied Science Publishers, London, pp: 319– 386.

Sanders P, Gicquel M, Humbert F, Perrin-guyomard A, Salvat G (2002). Plan de surveillance de la résistance aux antibiotiques chez les bactéries indicatrices isolées de la flore intestinale des porcs et de la volaille 1999-2001. Bull. Acad. Vet. France. 155 : 267-276.

Scott E (1996). Foodborne diseases and other hygiene issues in the home. J. Appl. Bacteriol. 80: 5-9.

Seyfarth AM, Wegener HC, Frimodt-Moller N (1997). Antimicrobial resistance in *Salmonella* enterica subsp. Enterica serovar typhi-murium from humans and production animals. J. Antimicrob. Chemother. 40: 67–75.

Singh RD, Mandal LN, Pandey JN (1984). Isolation of aerobic microorganisms from poultry meat at central poultry farm and retail shop. Patna. Poult. Guide, 21: 71–74.

Tibaijuka B, Molla B, Hildebrandt G, Kleer J, Salah W (2002). Antimicrobial resistance to *Salmonella*e isolated from retail raw chicken meat and giblets in Ethiopia. Bull. Anim. Hlth. Prod. Afr. 50: 86-95.

Todd ECD (1994). Surveillance of foodborne disease. In Foodborne Disease Handbook. Vol.1. Marcel Dekker, Inc, New York, pp. 461-536.

Train TF, Khai LTL, Nguyen TT, Akita M, Ogasawara N, Shinoda D, Hayashidani H (2004). Prevalence of *salmonella* spp. in pigs, Chickens and Ducks in the Mekong Delta, Vietnam.J. Vet. , Med. Sci., 66: 1011- 1014.

Uyttendaele MR, Debevere JM, Lips RM, Neyts KD (1998). Prevalence of *Salmonella* in poultry carcass and their products in Belgium. Int. J. Food Microbiol. 40: 1-8.

Waldroup AL (1996). Contamination of raw poultry whit pathogens. World Poult. Sci. J. 52: 7 – 25.

Yashoda KP, Sachindra NM, Sakhare PZ, Rao DN (2001). Micro-biological quality of broiler chicken carcasses processed hygienically in a small scale poultry processing unit. J. Food Quality 24: 249–259.