

ANTIMICROBIAL RESISTANCE OF *SALMONELLA* ISOLATED FROM POULTRY CARCASSES IN DAKAR (SENEGAL)

Rianatou Bada-Alambédji^{1*}; Aïssatou Fofana¹; Malang Seydi²; Ayayi Justin Akakpo¹

¹Service de Microbiologie-Immunologie-Pathologie Infectieuse, Ecole Inter-Etats des Sciences et Médecine Vétérinaires, Dakar, Sénégal; ²Service d'Hygiène et Industries des Denrées Alimentaires d'Origine Animale, Ecole Inter-Etats des Sciences et Médecine Vétérinaires, Dakar, Sénégal

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ABSTRACT

This study was undertaken to estimate the antimicrobial resistance of *Salmonella* isolated from raw chicken. From November 2003 to April 2004 a total of 120 chicken carcasses were collected from 36 randomly selected sale points (supermarkets, traditional market, poultry slaughter house, flocks) in the urban and periurban zones of Dakar, Sénégal, and examined for the presence of *Salmonella*. *Salmonella* was isolated from 75 (62.5%) of the examined samples. Out of the 90 *Salmonella* isolates obtained, twenty one serotypes were identified, from which the most prevalent were *S. Kentucky* 30%, *S. Muenster* (13.3%), *S. Brancaster* (8.8%), *S. Enteritidis* and *S. Hadar* (6.6%). All *Salmonella* isolates were tested for their susceptibility to 16 selected antimicrobial agents by the agar diffusion method. Seventy one (78.9%) isolates were resistant to one or more antimicrobials. Out of 71 resistant *Salmonella* isolates, 33 (46.5%) showed multiple resistance to five or more different antimicrobials. Resistance to ampicillin, trimethoprim, trimethoprim-sulphamethoxazole, tetracycline and sulphonamides was the most frequent. We found 36 different patterns of multiresistant strains. The high level of antibiotic resistance of foodborne *Salmonella* isolates in the present study is an indication of indiscriminate and continuous use of subtherapeutic doses of antibiotics in animals. Furthermore, the results showed the possible significance of chicken meat as a source of multiple antimicrobial-resistant *Salmonella* for human infections and suggest the need for detailed epidemiological study.

Key words: *Salmonella* sp, poultry, antimicrobial resistance

INTRODUCTION

The extensive use of antimicrobials in human and animals has led to an increase in bacterial multidrug resistant among several bacterial strains. This phenomenon of multiple resistance represents a worldwide problem both for veterinary and public health sectors. Bacterial resistance is observed especially when the antibiotics are abundantly used and that the bacteria can be transmitted easily between the individuals. Various antimicrobials in intensively managed food animals including chickens are often administered through feed or drinking water either for therapy, prophylaxis or growth promotion. *Salmonella* sp is one of the most frequently isolated bacteria in avian

production units. The increasing single and multiple antimicrobial-resistant *Salmonella* strains isolated from human cases of salmonellosis has been associated with widespread use of antimicrobial agents in food animal production. This may represent a public health risk by transfer of resistant *Salmonella* strains to humans through the consumption of contaminated food and food products. Studies on antimicrobial resistance in *Salmonella* have usually been undertaken in countries (1,10,15,20), that worked out excellent system to monitor resistance to antibiotics (3,9,11,17). In developing countries like Senegal, the situation of antimicrobial resistance is more complex and difficult. This is because *Salmonella* and other zoonotic bacterial pathogens are not routinely cultured

*Corresponding Author. Mailing address: Service de Microbiologie-Immunologie-Pathologie Infectieuse, Ecole Inter-Etats des Sciences et Médecine Vétérinaires, BP 5077, Dakar, SÉNÉGAL. E-mail: riana@refer.sn

and their resistance to commonly employed antimicrobials both in public health and veterinary practices is rarely determined. In Senegal, a new multiple resistant *Salmonella* serotype which emerged quasi simultaneously in food and human was identified (6). The purpose of the present study was to determine through the microbiological quality of chicken carcasses, the frequency and pattern of antimicrobial resistant *Salmonella* isolated from poultry in Dakar (Sénégal).

MATERIALS AND METHODS

Samples

A total of 120 whole chicken carcasses were purchased in 13 sale points and 23 flocks from November 2003 to April 2004 in the urban and periurban zones of Dakar. Sampling was done using a random sampling method. The number of carcasses purchased in poultry flocks was determined by the flock size. The samples were packed in iceboxes and transported immediately to the Hygiene and Animal Foodstuffs Inspection laboratory of Ecole Inter-Etats des Sciences et Médecine Vétérinaires of Dakar. The carcasses were frozen until their analysis.

Isolation and identification of *Salmonella*

The samples were examined for the presence of *Salmonella* according to the technique NF V 08-052 recommended by l'Association Française de Normalisation (AFNOR). Briefly, frozen samples were thawed at room temperature for about 3 hours. Twenty five grams of each sample were excised using a sterile scalpel. Each sample was put into a sterile stomacher bag containing 225 mL of buffered peptone water. The samples were then mixed for 2 minutes using a STOMACHER ND and incubated at 37°C for 16 to 20 hours. After incubation, 0.1 mL of the pre-enriched sample was transferred into 10 mL of Rappaport Vassiliadis (Bio-Rad) broth and incubated at 42°C for 18 to 24 h. Another 1 mL aliquot was transferred into 10 mL of Selenite Cystine (Bio-Rad) broth and incubated at 37°C for 24 hours. Following incubation, a loopful of each culture was streaked onto brilliant green and Hecktoen agars (Bio-Rad) which were incubated at 37°C for 18 to 24 hours. Presumptive *Salmonella* colonies chosen from each plate were inoculated onto nutrient agar (Bio-Rad) and grown overnight at 37°C. The cultures are then conveyed to the Microbiology-Immunology-Infectious Pathology Unit of Ecole Inter-Etats des Sciences et Médecine Vétérinaires of Dakar for identification. *Salmonella* isolates were screened biochemically using triple sugar iron, citrate, lysine decarboxylase, urease test, and indole test. Colonies that exhibited typical reactions were further biochemically characterised using API 20 E (Biomérieux) as recommended by the manufacturer. The isolates, which tested positive for *Salmonella*, were subcultured and sent for serotyping to the Medical Biology Unit, Pasteur Institute of Dakar.

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 16 antimicrobials tested were those commonly used in poultry herds or in human. There are: ampicillin (10 µg), nalidixic acid (30 µg), cephalotin (30 µg), cefoxitin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), colistin (50 µg), flumequin (30 µg), furan (300 µg) gentamicin (15 µg), neomycin (30 UI), streptomycin (10 µg), sulphonamides (200 µg), tetracyclin (30 µg), trimethoprim (5µg) and trimethoprim-sulphamethoxazole (1.25 µg/25.75 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.) (5).

RESULTS

Salmonella were isolated from 75 (62.5%) of the 120 collected samples. Out of the 90 *Salmonella* isolates, twenty one different serotypes were identified of which *S. Kentucky* (30.0%), *S. Muenster* (13.3%), *S. Brancaster* (8.8%), *S. Enteritidis* and *S. Hadar* (6.6%) were the most prevalent ones. The distribution of serotypes is indicated in Table 1. From the total of 90 *Salmonella* isolates, 71 (78.9 %) were resistant to one or more antimicrobials. Among the 71 resistant *Salmonella* isolates, 33 (45.6%) showed a multiple resistance pattern to 5 antimicrobials (ampicillin, trimethoprim, trimethoprim-sulfamethoxazole, sulphonamides and tetracyclin). The most significant percentages of resistance were obtained by decreasing order with tetracyclin (46.6%), trimethoprim (42.2%), sulphonamides (41.1%), Trimethoprim-Sulfamethoxazole (40%) and ampicillin (34.4%). Table 1 shows the frequency of resistance to one or more antimicrobials for the 21 identified serotypes.

Among the 21 serotypes identified, resistance was found in 16 of them. Fifteen multiresistant serotypes (to 2 or more antimicrobials) were identified, including 7 resistant serotypes where resistance to more than 5 antimicrobials was noted. Thirty-six different antimicrobial multiresistance patterns were displayed by Table 2.

Five serotypes did not show any resistance with respect to antimicrobials tested: *S. Altona* (2 isolates), *S. Duisburg* (2 isolates), *S. Banana* (1 isolate), *S. Give* (1 isolate) and *S. Tado* (1 isolate).

DISCUSSION AND CONCLUSION

The level of contamination of chicken with *Salmonella*, observed in this study was higher than that obtained by Tall (unpublished data, 2003) in Senegal but comparable with those reported in some other African countries (13,19). Birds coming

Table1. Resistance of *Salmonella* serotypes isolated from poultry to antimicrobial agents

Serotypes	n	Antibiotics													Recapitulatory						
		AM	CF	FOX	S	M	N	TE	NA	CIP	UB	SSS	SXT	TMP	C	CS	FT	0	1	2-5	5+
Kentucky	27	12	11	10	5	0	0	6	3	1	0	7	9	8	0	0	10	6	3	16	2
Muenster	12	6	5	5	4	0	0	4	0	0	0	9	9	9	0	1	6	3	0	2	7
Brancaster	8	0	0	0	8	0	3	8	0	0	0	8	7	7	0	0	0	0	0	3	5
Enteritidis	6	2	2	2	2	0	0	4	4	0	1	0	2	1	0	0	4	0	1	3	2
Hadar	6	1	0	0	6	0	1	6	0	0	0	3	3	3	0	0	0	0	0	4	2
Bredeney	4	2	3	3	0	1	0	1	0	0	0	0	0	3	0	0	3	0	1	1	2
Johannesburg	4	0	0	0	0	0	0	4	0	0	0	1	0	0	0	0	0	0	3	1	0
Vitkin	3	3	0	0	0	0	0	3	0	0	0	3	3	3	0	0	0	0	0	1	2
Chester	3	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	2	0	1	0
Mbandaka	3	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0	1	1	1	0
Schwarzengrund	2	2	0	0	0	0	0	2	0	0	0	2	2	2	0	0	0	0	0	2	0
Altona	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Duisburg	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Banana	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Corvallis	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Duval	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Give	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Hull	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0
Maastricht	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Tado	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0
Tshiongwe	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0
Total	90	31	25	22	25	1	4	42	8	1	1	37	36	38	0	2	25	19	10	39	22

n = number of isolates;

0 = susceptible;

1 = resistance to one antibiotic;

2-5 = resistant to 2-5 antibiotics;

5+ = resistance up to 5 antibiotics.

AM = ampicillin, CF = cephalotin, FOX = ceftiofuran, S = streptomycin, GM = gentamicin, N = neomycin, TE = tetracyclin, NA = nalidixic acid, CIP = ciprofloxacin, UB = flumequin, SSS = sulphonamides, SXT = trimethoprim-sulphamethoxazole, TMP = trimethoprim, C = chloramphenicol, CS = colistin, FT = furans

out of rearing units can be the principal source of contamination of the poultry meat by *Salmonella*. The results of the investigation showed that some stockbreeders do not respect prophylactic sanitary measures, which makes it possible for the bacteria to be maintained in the herd. The presence of 3 to 6 different serotypes in the same breeding reflects this lack of hygiene. However, plucking, evisceration and carcass washing, are all stages of the preparation which contribute to the whole process of contamination (Tall, unpublished data). The number of serotypes identified is significant: 21 from the 90 isolates. They are potentially pathogenic and different from those isolated by Tibaijuka *et al.* (19) in Ethiopia. They reported that from 244 poultry meat samples, 9 different serotypes were isolated from which the most prevalent were *S. Braenderup*, *S. Anatum*, *S. Uganda* and *S. Saintpaul*. In our study, serotypes Enteritidis and Hadar which play a significant role in collective food-borne diseases, were isolated. 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 tetracyclin, trimethoprim, sulphonamides, trimethoprim-sulfamethoxazole, ampicillin, cephalotin and streptomycin. The percentages of resistance obtained with these antibiotics are comparable with those reported in other studies in France (17) and in Ethiopia (19). In Ethiopia, the level of resistance of 31 *Salmonella* isolates obtained from chicken meat samples was 60%.

In the present study, all *Salmonella* isolates were susceptible to chloramphenicol and only one was resistant to gentamicin. This may be explained by the limited availability and high cost of the above groups of antimicrobials that would reduce their frequent utilization in veterinary practice or public health practices in Senegal. Our results showed that *Salmonella* isolates were still largely susceptible to quinolones (9.7% of resistance to NA, 1.1% to flumequine). Although quinolones resistant *Salmonella* strains are seldom isolated (4,8), the reduction in the susceptibility must be regarded as an alarm

Table 2. Multiple antimicrobial resistance patterns of 15 *Salmonella* serotypes isolated from poultry.

Serotype	Number of isolate(s) tested	Resistant isolates	resistance pattern (Resistance to two or more)							Number of multiresistant isolates			
<i>S. Kentucky</i>	27	21	AM	CF	FOX							3	
			AM		FT								1
			AM	FT	CF	TE							1
			AM	FT	CF	FOX							2
			AM	FT	CF	FOX	S						1
			AM	FT	CF	FOX	SXT						1
			AM	FT	CF	FOX	TMP						1
			AM	FT	CF	FOX	TE	TMP					1
			AM	FT	CF	FOX	TE	SSS	SXT	TMP			1
			NA	CIP	FT								1
			SSS	SXT	TMP								1
			SSS	SXT	TMP	TE							1
			SSS	SXT	TMP	S	TE						1
			SSS	SXT	TMP	FT	TE						1
			SSS	SXT	S	TE							1
<i>S. Muenster</i>	12	9	AM	CF	FOX	SSS	SXT	TMP				1	
			AM	CF	FOX	FT	SSS	SXT	TMP			1	
			AM	CF	FOX	FT	SSS	SXT	TMP	TE		2	
			AM	CF	FOX	FT	SSS	SXT	TMP	S	TE	1	
			AM	FT	SSS	SXT	TMP	S	TE			1	
			SSS	SXT	TMP							1	
			SSS	SXT	TMP	S	TE					1	
<i>S. Brancaster</i>	8	8	SSS	SXT	TMP	CS	FT	S				1	
			SSS	N	S	TE						1	
			SSS	SXT	TMP	S	TE					5	
			SSS	SXT	TMP	S	TE	N				2	
<i>S. Enteritidis</i>	6	6	AM	CF	FOX	FT	NA	S	TE			1	
			AM	CF	FOX	FT	NA	S	TE	UB		1	
			NA	FT								1	
			SXT	TE								1	
<i>S. Hadar</i>	6	6	SXT	TMP	FT	TE						1	
			AM	SSS	SXT	TMP	S	TE				1	
			N	SSS	SXT	TMP	S	TE				1	
			S	TE								3	
<i>S. Bredeney</i>	4	4	SSS	SXT	TMP	S	TE				1		
			AM	FT	CF	FOX						1	
			AM	FT	CF	FOX	TMP					1	
<i>S. Vitkin</i>	3	3	CF	FOX	FT	GM	TMP	TE			1		
			AM	SSS	SXT	TMP	TE					3	
			AM	SSS	SXT	TMP	TE					2	
<i>S. Schwarzengrund</i>	2	2	AM	SSS	SXT	TMP	TE				2		
<i>S. Corvalis</i>	1	1	AM	CF	FOX						1		
<i>S. Hull</i>	1	1	AM	CF	FOX	FT	TE				1		
<i>S. Tado</i>	1	1	AM	CF	FT	TE					1		
<i>S. Tshiongwe</i>	1	1	AM	CF	NA	TE					1		
<i>S. Chester</i>	3	1	SSS	SXT	TMP						1		
<i>S. Mbandaka</i>	3	2	SSS	TMP							1		
<i>S. Johannesburg</i>	4	4	SSS	TE							1		

signal, since quinolones are considered last resort antibiotics against multiple-drug resistant *Salmonella* strains. The evolutionary aspect of the resistance mechanisms calls for caution. Acquired resistances to furans are rare and develop slowly. The percentage of resistance to furans observed in our study was 29.0%. It is higher than the ones obtained by Poppe *et al.* (16) from *Salmonella* strains isolated from animals of which the percentages of resistance to furans were respectively 7.1%, 10.4%, 5.8%, and 5.1% over the years 1994 to 1997. This may be explained by the frequent use of furans (23.5%) for therapy in the poultry breeding and often by stockbreeders own decisions to medicate animals as reported by Bada-Alamedji *et al.* (2). Besides the increasing resistance to commonly used antibiotics in animals and humans, there is a concurrent increase in multiple resistant *Salmonella* isolates worldwide (7,14). The multiple resistance observed in the present study was to those antimicrobials commonly employed in veterinary practices and particularly to ampicillin, trimethoprim, trimethoprim-sulfamethoxazole, tetracyclin, sulphonamides and streptomycin, which are also used for treatment of different human bacterial diseases. In a study of 54 *Salmonella* strains isolated from a total of 301 chicken meat and giblets in Ethiopia, 31 were resistant to one or more antimicrobials. Out of 31 resistant *Salmonella* isolates, 17 (54.8%) exhibited multiple resistance to up to 6 different antimicrobials (ampicillin, streptomycin, trimethoprim, sulfamethoxazole, trimethoprim-sulfamethoxazole and spectinomycin (19). In our study, all *S. Brancaster* and *S. Hadar* isolated showed multiple-drug resistance, followed by *S. Enteritidis* (83.3%), *S. Muenster* (75%), and *S. Kentucky* (66.6%). *Salmonella* Hadar isolates were resistant to streptomycin and tetracyclin. These frequencies of resistance are comparable with those reported by Poppe *et al.* (16). A study carried out in Ontario (Canada), showed that among strains of *S. Hadar*, isolated from poultry, 85.7% were resistant to these same antibiotics (12). On the other hand, *S. Hadar* isolates were susceptible to quinolones, contrary to an evolution to nalidixic acid resistance of *S. Hadar* isolated from animals in Belgium and reported by Chaslus-Dancla and Martel (8). Resistance to Nalidixic acid and tetracyclin was more frequent among *S. Enteritidis* isolates. Other studies covering several years that were done in the United Kingdom showed an increase in the frequency of nalidixic acid resistance among *S. Enteritidis* strains isolated from animals (18).

The results of the present study indicate single or multiple resistance of *Salmonella* strains isolated from poultry, that constitutes a potential source of transmission of these resistant strains to man and poses a problem in public health. This suggests the need for more prudent use of antibiotics by farmers, veterinarians and physicians. Further detailed epidemiological and molecular studies are essential on the frequency, sources of acquisition of resistant genes and distribution of antimicrobial

resistant *Salmonella* among food animals, food products and humans in Sénégal.

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RESUMO

Resistência antimicrobiana de *Salmonella* isolada de carcaças de frango em Dakar, Sénégal

O presente estudo foi realizado com o propósito de avaliar a resistência aos antibióticos dos sorovares da *Salmonella* isolados da carne de frango crua. De novembro de 2003 até abril de 2004, um total de 120 carcaças de frango foram compradas em 13 pontos de venda e 23 centros de criação de frangos. As *Salmonella* foram isoladas a partir de 75 (62,2%) carcaças analisadas. Vinte e um (21) sorotipos diferentes foram identificados, sendo os mais frequentes *S. Kentucky* (30%), *S. Muenster* (13,3%), *S. Brancaster* (8,8%), *S. Enteritidis* e *S. Hadar* (6,6%). Todos os sorovares de salmonela foram examinados a fim de determinar a resistência à 16 antibióticos. Setenta e quatro (79,6%) foram resistentes à um antibiotico ou mais, das quais 33 (45,6%) mostraram resistência múltipla a cinco antibióticos (ampicilina, trimetoprim, trimetoprim-sulfametoxazol, tetraciclina e sulfonamidas). Foram encontrados 36 perfis diferentes de resistência múltipla. O nível elevado de resistência dos isolados de *Salmonella* encontrados na carne do presente estudo, é um indicador do uso indevido e contínuo de doses subterapêuticas de antibióticos nos animais. Por outro lado, os resultados do estudo demonstram a importância da carne de frango como fonte potencial de sorovares de *Salmonella* multiresistentes transmissíveis ao homem e sugerem um estudo epidemiológico detalhado.

Palavras-chave: *Salmonella* sp, frangos, resistência à antimicrobianos

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