ANTIMICROBIAL SUSCEPTIBILITY OF *Salmonella* Enteritidis STRAINS FROM DIFFERENT SOURCES

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ABSTRACT

The antimicrobial susceptibility occurrence in *Salmonella* Enteritidisin the period from 1995 to 1997 is described in strains isolated from healthy and ill chickens, human sporadic gastrenteritis outbreaks related to egg products consumption, poultry meats, broiler chicken pipped embryos, meat meal/aviculture environment, swine feces and foods (cheese, mayonnaise, cake mass, cake cover and bacon). It was observed that 72.2% were sensitive, whereas 9.2% and 17.8% were resistant and intermediate, respectively. Resistance was verified in 6.2% of the human isolates, in 5.0% of poultry meats, in 36.4% of foods, in 4.2% of chickens, in 23.7% of pipped embryos, in 5.7% of meat meal/aviculture environment and in 13.3% of swine. The obtained results revealed high proportion of strains with intermediate sensibility (18.1% of total). We observed 100% of sensibility to six antibiotics. Considering the resistant strains, 15 (57.7%) presented resistance to only nine antibiotics. Four (15.4%) (chickens and pecked eggs) presented double resistance; triple resistance was detected in one strain (3.8%), whereas six (23.1%) presented multiresistant profiles, to 4-9 drugs, identified in the human strains, foods, chickens and pecked eggs. Although the resistance index described here may be considered low, there was high frequency of multiresistant profiles and of intermediate sensibility to tetracycline, particularly in samples associated to chickens.

KEY WORDS: Antibiotic multirresistance, food, chickens, human gastrenteritis.

RESUMO

SUSCEPTIBILIDADE ANTIMICROBIANA DE AMOSTRAS DE *Salmonella* Enteritidis ISOLADAS DE DIFERENTES FONTES

A susceptibilidade antimicrobiana de *Salmonella* Enteritidis é descrita em amostras isoladas de galinhas saudáveis e doentes, casos esporádicos de gastrenterite humana relacionadas ao consumo de produtos contendo ovos, carne de frango, ovos bicados, farinha de carne/ambiente de avicultura, fezes de suínos e alimentos. No período de 1995 a 1997 foi observado que 72,2% foram sensíveis, enquanto que 9,2% e 17,8% foram resistentes e intermediárias, respectivamente. Constatou-se resistência em 6,2% das isoladas do homem, 5,0% de carnes de frangos, 36,4% de alimentos, 4,2% de galinhas, 23,7% de ovos bicados, 5,7% de farinha de carne/ambiente de avicultura e 13,3% de suínos. Os resultados obtidos revelaram elevada proporção de amostras com sensibilidade intermediária (18,1% do total) e 100% de sensibilidade a seis princípios. Considerando as amostras resistentes, quinze (57,7%)

apresentaram resistência única a nove antimicrobianos. Quatro (15,4%) (galinhas e ovos bicados) apresentaram resistência dupla; resistência tripla foi detectada em uma amostra (3,8%), enquanto que seis (23,1%) apresentaram seis perfis de multirresistência, frente a 4-9 drogas, identificados em amostras do homem, alimentos, galinhas e ovos bicados. Embora o índice de resistência aqui descrito possa ser considerado baixo, deve ser destacada a alta frequência de sensibilidade intermediária à tetraciclina, particularmente em amostras associadas a aves.

PALAVRAS-CHAVES: Alimentos, galinhas, gastrenterite humana, multirresistência a antibióticos.

INTRODUCTION

The genus *Salmonella* can be classified in agreement with the identification of antigenic factors in approximately 2.400 serotypes, amongst which Salmonella Enteritidisis included (POPPFF et al., 1997).

In several countries of Europe and of the Americas, *S.* Enteritidis emerged as the principal serotype responsible for the etiology of human *salmonellosis*, surpassing *S.* Typhimurium, that until then occupied the first place (CDC, 1990, RODRIGUE et al., 1992). In Brazil, 1in the last 10 years, an increased incidence of *S.* Enteritidis was observed and isolated of human, alimentary and animal sources.

Among the characteristic points of salmonellosis epidemiology, stands out the increase in resistance to antibiotics, its intersection with AIDS, prevalence of infections caused by *S.* Enteritidis associated to the consumption of eggs, and the occurrence of dispersed outbreaks (TIETJEN & FUNG, 1995).

The transfer of bacterial resistance to antibiotics has been studied widely. The plasmids R are the most common genetic instrument for that transfer (HARADA & MITSUHASHI, 1977).

This study intends to show the susceptibility profile of *S.* Enteritidis isolated from different sources in Brazil.

MATERIAL AND METHODS

A total of 277 *S.* Enteritidis strains, isolated during the period from 1995 to 1997. Ninety-four (94) strains were from chickens (healthy and ill), 48 in sporadic gastroenteritis outbreaks related to egg products consumption from human, 38 from poultry meat (chicken thighs, drumsticks, breast, skin, wings and carcass), 38 from pipped embryos (broiler), 34 from meat meal/aviculture environment (rat stool, paper pads, broiler litter and drag swabs), 14 from swine feces and 11 from foods (cheese, mayonnaise, cake mass, cake cover and bacon).

The Vitek automated system was adopted (Biomerieux Vitek System Inc., USES), and the cards used were the GNS-F1 and GNS-F2. The first includes amikacin, cefazolin, cefotetan, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, cephalothin, gentamicin and tobramycin; the second includes ampicilin, aztrionam, carbenicillin, ciprofloxacin, imipenem, mezlocillin, piperazine, tetracycline, ticarcillin, ticarcillin-Clavulonic Acid (CA) and Trimethoprim/sulfamethoxazole. Chloramphenicol (30 mg/mL), kanamycin (30 mg/mL), nalidixic acid (30 mg/mL), neomycin (30 mg/mL), nitrofurantoin (300 mg/mL) and sulfonamides (300 mg/mL) were tested by the standard method of diffusion of disks in agar (NCCLS, 1993).

RESULTS AND DISCUSSION

Table 1 presents antimicrobial susceptibility of *S.* Enteritidis from different origins against 28 drugs. The results showed that 72.2% were sensitive, whereas 9.2% and 17.8% were resistant and intermediate, respectively, to the antibiotics used in this study.

RODRIGUE et al. (1992) reported similar results in strains of animal origin and of sporadic cases of human salmonellosis (24% and 23%, respectively) isolated in the decade of 80. Their results also showed that the resistance profile remained unchanged from one decade to the other – the same as our results show – and, likewise, those in the study developed by QUINTAES et al. (1997), in Brazil.

Considering the isolation origin, higher sensibility was observed in isolates of chicken

meats (86.8%), followed by swine (85.7%) and chickens (80.0%, each), human (77.1%), meat meal/aviculture environment (70.6%), foods (45.4%) and pipped embryos (broiler) (42.1%) (Table 1).

TABLE 1. Susceptibility to different antibiotics in *S.* Enteritidis strains, and isolation origin, in the period from 1995 to 1997

Origen	Resistant		Sensítive		Intermediate		Total
	no. [1]	(%)[2]	no.	(%)	no.	(%)	
Chicken	04	4.2	76	80.0	15	15.8	94
Human	03	5.3	37	77.1	08	16.7	48
Poultry meats	02	5.0	33	86.8	03	7.5	38
Pipped embryos	09	23.7	16	42.1	13	34.2	38
meat meal/aviculture environment	02	5.9	24	70.6	08	23.5	34
Swine	02	14.3	11	80.0	01	6.7	14
Foods	04	36.4	05	45.4	02	18.2	11
Total[3]	26	9.4	202	72.2	50	18.1	277

1. Number of resistant, sensitive or intermediate strains in each isolation origin; 2. percentage calculated in relation to total strains examined in each isolation origin; 3. percentage calculated in relation to total of strains examined.

The highest sensibility level verified in the strains of meats of chickens obtained in the retail trade contrasts with the indexes described by SON et al. (1995), in Malaysia, in *S.* Enteritidis strains isolated of carcasses, between 1992 and 1993. They observed resistance of all strains to at least one of the examined antibiotics, as well as multiresistance, in 63% of the strains. PERESI et al. (1997) described 100% of sensibility to the 15 antibiotics examined in strains of *S.* Enteritidis isolated of carcasses of chickens marketed in São José do Rio Preto, Brazil, where likeness with our results is observed.

The human isolated strains were less sensitive than the 275 strains examined by LING et al. (1998), isolated in Hong Kong from1986 to 1995, that displayed 99% of sensibility to 17 of the 19 examined antibiotics.

FANTASIA et al. (1991) describe 100% of sensibility to the examined antibiotics, in 81 *S.* Enteritidis strains of human origin (outbreaks and sporadic cases) and foods consumed by the patients in those outbreaks, in Italy. However, intermediate resistance to tetracycline was verified in five strains.

In our study, resistance was verified in 6.2% of the isolates from sporadic cases of human salmonellosis, 5.0% of meats of chickens, 36.4% of foods, 4.2% of chickens, 23.7% of pipped embryos (broiler), 5.7% of meat meal/aviculture environment and 13.3% of swine (Table 1). The results from the isolated strains of meat meal/aviculture environment were different from those observed by BERCHIERI-JÚNIOR (1985), for certain serotypes of *Salmonella*. Our results showed resistance to sulfazotrin, bacitracin and penicillin in 100% of the analyzed strains, and the resistance to nalidixic acid, nitrofurantoin, amikacin and cefoxitin was verified in 18%, 2%, 16% and 0.7%, respectively.

It is important to point out that the human strains' resistance to trimethoprim-sulfamethoxazole, to tetracycline, gentamicin and to penicillins, tended to maintain a certain difference in relation to the resistance presented by strains of other isolation origins, particularly the isolated of chickens and pipped embryos (broiler), although the strains of chickens and those associated to birds have exhibited resistance mainly to the antibiotics with indication and approval of BRASIL (1996), for therapeutic purposes and as growth promoters.

Results in Table 1 still illustrate the high proportion of strains with intermediate sensibility,

with the largest percentile detected in the isolates of pipped embryos (broiler) (34.2%), meat meal/ aviculture environment (23.5%), foods (18.2%), human (16.7%) and chickens (15.8%), followed by strains isolated of chicken meats (7.5%) and swine (6.7%).

Table 2 displays the results referring to the susceptibility of the total of the stumps, according to antibiotics used. We observed that all were sensitive to Amikacin, aztreonam, cefotetan, cefoxitin, ceftazidime and ciprofloxacin, whereas the smallest percentile was verified for tetracycline (84.4%). Sensibility to nitrofurantoin, chloramphenicol, ceftriaxone, cefuroxime sodium and imipenem was observed in 99.7%, to neomycin, cefazolin and sulfonamides in 99.3%, to kanamycin in 98.9%, to Trimethoprim/ sulfamethoxazole in 98.6%, to cefuroxime-axetil, cephalothin, ticarcillin/CA and tobramycin in 98.2%, to nalidixic acid, ampicilin, carbenicillin and gentamicin in 97.9%, and to mezlocillin, piperacillin and ticarcillin in 97.5%.

Our results are close to those of FERREIRA et al. (1997), who verified 100% of sensibility to cephalothin, cefoxitin, imipenem, amikacin, chloramphenicol, ofloxacin and gentamicin. And our study also observed 2,1% of resistance to gentamicin.

Still in Table 2, resistance was observed to at least one of these 19 antibiotics: 2.1% to ampicilin, carbenicillin, gentamicin, tetracycline and ticarcillin; 1.8% to piperacillin and tobramycin, 1.4% to nalidixic acid, cephalothin, mezlocillin and Trimethoprim-sulfa, 1.1% to kanamycin, 0.7% to neomycin, sulfonamides and ticarcillin/CA, 0.3% to nitrofurantoin, chloramphenicol, cefazolin and imipenem. Although in indexes considered very low (0.4% to 2.1%), this resistance was characterized against a high diversity of drugs, and multiresistance was found up to nine antibiotics.

THRELFALL (1992) already pointed a decrease in the global frequency of resistance presented by human strains of *S.* Enteritidis and of animals for consumption, in 1990, when compared with those isolated in 1981, and the percentiles fell from 15% to 11%, whereas multiple resistance increased. The author points out the small increase observed in the resistance to ampicilin, starting from 1981 (1% for 4%), and decrease in the resistance to furazolidone; some strains isolated in 1990 demonstrated resistance to nalidixic acid, although the general frequency of resistance to the other antibiotics has remained practically unaffected.

Intermediate sensibility was verified against at least one of these 11 drugs: tetracycline (13.5%), cefuroxime-axetil (1.8%), mezlocillin and ticarcillin/CA (1.1%), nalidixic acid and piperacillin (0.7%, each) and Cefazolin, Ceftriaxone, sodium cefuroxime, cephalothin and ticarcillin (0.3%, each) (Table 2).

Increased resistance indexes to tetracyclines are being reported for decades, and the resistance to this antibiotic is completely disseminated among the strains of *Salmonella* spp. (TANAKA et al., 1976; HADAD & JEMEL, 1990; MORINIGO et al, 1990; THRELFALL, 1992; SON et al., 1995). On the other hand, starting from 1981, resistance to ampicilin reaches growing importance in salmonellas of poultry origin, besides *S.* Enteritidis (THRELFALL, 1992; RODRIGUE et al., 1992). These results are in agreement with our study, independently of the isolation sources; the largest percentiles of resistance were obtained against penicillins (ampicilin, carbenicillin, and ticarcillin), aminoglycoside gentamicin and tetracycline. More recent Brazilian data indicate fall in the percentile of resistance, and show levels here closer to the obtained data, for the antibiotics in general (PERESI et al., 1997; QUINTAES et al., 1997).

As suggested by OLSEN et al. (1993), this circumstance makes indispensable the continuous monitoring of the isolated strains, with the objective of maintaining and updating data regarding modifications in the patterns of resistance of bacteria. Like this, the importance epidemic pass to occupy secondary position to the implications for therapy and the agent control, in spite of the generated data could be used quickly for epidemic purposes.

TABLE 2. Susceptibility to different antibiotics in *S.* Enteritidis of different origins, in the period from 1995 to 1997.

Antibiotics	Resistant		Sensitive		Intermediate	
	No. =277[1,2]	(%)	No. =277	(%)	No. =277	(%)
Nalidixic acid	04	1.4	271	97.9	02	0.7
Neomycin	02	0.7	275	99.3	00	0.0
Nitrofurantoin	01	0.4	276	99.6	00	0.0
Chloramphenicol	01	0.4	276	99.6	00	0.0
Kanamycin	03	1.1	274	98.9	00	0.0
Sulfonamides	02	0.7	275	99.3	00	0.0
Amikacin	00	0.0	277	100.0	00	0.0
Ampicilin	06	2.1	271	97.8	00	0.0
Aztreonam	00	0.0	277	100.0	00	0.0
Carbenicillin	06	2.1	271	97.8	00	0.0
Cefazolin	01	0.4	276	99.6	01	0.4
Cefotetan	00	0.0	277	100.0	00	0.0
Cefoxitin	00	0.0	277	100.0	00	0.0
Ceftazidime	00	0.0	277	100.0	00	0.0
Ceftriaxone	00	0.0	276	99.6	01	0.4
Cefuroxime Sodium	00	0.0	276	99.6	01	0.4
Cefuroxime-axetil	00	0.0	272	98.2	05	1.8
Cephalothin	04	1.4	272	98.2	01	0.4
Ciprofloxacin	00	0.0	277	100.0	00	0.0
Gentamicin	06	2.1	271	97.8	00	0.0
Imipenem	01	0.4	276	99.6	00	0.0
Mezlocillin	04	1.4	270	97.5	03	1.1
Piperacillin	05	1.8	270	97.5	02	0.7
Tetracycline	06	2.1	233	84.1	38	13.7
Ticarcillin	06	2.1	270	97.5	01	0.4
Ticarcillin-CA	02	0.7	272	98.2	03	1.1
Tobramycin	05	1.8	272	98.2	00	0.0
Trimethoprim-sulfa	04	1.4	273	98.6	00	0.0

1. Total of strains examined 2. Presence of multiresistant strains.

Considering the 26 resistant strains, 15 (57.7%) presented resistance only to Trimethoprim-sulfamethoxazole, imipenem, tobramycin, nalidixic acid, nitrofurantoin, cephalothin, gentamicin, tetracycline and sulfonamides. Four (15.4%) (chickens and pipped embryos) presented double resistance, with the following profiles: neomycin/kanamycin, gentamicin/tobramycin and gentamicin/sulfonamide; triple resistance was detected in one strain (3.8%) (tetracycline/neomycin/kanamycin), whereas six isolates (23.1%) showed six different multiresistance patterns, against 4-9 drugs, identified in the human strains (2 profiles, with 4-8 drugs), in foods (2 profiles, with 7-8 drugs), chickens (1 profile, with 9 drugs) and pipped embryos (broiler) (1 profile, 6 drugs) (Table 3).

FERREIRA et al. (1997) identified double resistance to ampicilin-trimethoprim/sulfamethoxazole in one, among the twelve strains of *S.* Enteritidis isolated from patients between 1995 and 1996, in Rio de Janeiro (RJ), whereas in our study multiresistant strains prevailed among the identified three, with this origin.

Multiresistance results described in this work are in agreement with other studies, that register increase multiresistance gradate among strains of *S.* Enteritidis, although the frequencies of resistance in this serotype are low (THRELFALL & FROST, 1990; THRELFALL, 1992; OLSEN et

al., 1993). On the other hand, they thwart WARD et al. (1990), who showed the simultaneous resistance of this serotype, to more than four drugs, as a little common factor, attributing increase in multiresistance rates to the triple resistance.

THRELFALL (1992) suggests that, starting from 1970, the therapeutic and prophylactic uses of antibiotics are responsible for the multiresistance. The author marks that the evident increase in the use of furazolidone and its associated compositions, in avian production, has been selecting resistant strains of *S.* Enteritidis and *S.* Virchow. RAMPLING et al. (1990) suggest that the appearance of resistant strains of the previous serotypes results from the use of nitrofurans in aviculture.

TABLE 3. Resistance profile in *S.* Enteritidis strains from different sources

Resistance number	Nº. [1]	Resistance profile	Isolation origins
Only Resistance (15)	4	Nalidixic Acid	Foods (2), broiler chicks pipped embryos (2)
	2	Cephalothin	Broiler chicks pipped embryos
	1	Gentamicin	broiler chicks pipped embryos
	1	Imipenem	Poultry meats
	1	Nitrofurantoin	Chicken
	1	Sulfonamides	Swine
	3	Tetracycline	Swine(1), meat meal/aviculture environment (2)
	1	Tobramycin	Poultry meats
	1	Trimethoprim-sulfa	Human
Double Resistance (4)	1	Neomycin / Kanamycin	Chicken
	2	Gentamicin / Tobramycin	Chicken and broiler chicks pipped embryos
	1	Gentamicin / Sulfonamides	Broiler chicks Pipped embryos
Triple Resistance (1)	1	Tetracycline / Neomycin / Kanamycin	Broiler chicks Pipped embryos
Multiresistance (6)[2]	1	Ampicilin – Carbenicillin – Mezlocillin – Piperacillin – Ticarcillin – Gentamicin – Tetracycline – Trimethoprim-sulfa	Human
	1	Ampicilin - Carbenicillin – Ticarcillin – Trimethoprim-sulfa, with intermediate sensibility to Mezlocillin e Piperacillin	
	1	Ampicilin - Carbenicillin – Mezlocillin - Piperacillin - Ticarcillin - Ticarcillin/CA- Cephalothin, with intermediate sensibility to Tetracycline /Cefazolin	Foods
	1	Carbenicillin – Mezlocillin – Piperacillin – Ticarcillin – Ticarcillin/CA- Cephalothin –Cefazolin, with intermediate sensibility to Tetracycline /cefuroxime-axetil	
	1	Ampicilin – Carbenicillin – Piperacillin – Ticarcillin – Gentamicin – Kanamycin – Tobramycin – Tetracycline – Chloramphenicol, with intermediate sensibility to Mezlocillin – Ticarcillin /CA	Chicken
	1	Ampicilin - Carbenicillin – Mezlocillin - Piperacillin - Ticarcillin - Trimethoprim-sulfa, with sensibility intermediate to Ticarcillin/CA	Broiler chicks Pipped embryos

[1] nº. – Number of strains; [2] In the profile of strains with multiresistance showed intermediate sensibility.

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