Antimicrobial resistance in *Salmonella* Enteritidis from foods involved in human salmonellosis outbreaks in southern Brazil

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SUMMARY

The antimicrobial resistance of *Salmonella* Enteritidis (n=79) isolated from foods involved in human salmonellosis outbreaks in Southern Brazil during the period of 2001 to 2002 was analysed. The isolates were individually tested using the disc diffusion method against 10 antimicrobial agents. Most isolates were susceptible to all drugs tested. No *S.* Enteritidis isolates were resistant to sulfamethoxazole/trimethoprim or sulfazotrim and only one was resistant to chloramphenicol. The predominant resistance observed was to nalidixic acid (21.5%), gentamicin (12.7%), and streptomycin (11.4%), while intermediate resistance was observed most often for kanamycin (29.1%), neomycin (17.7%), and streptomycin (13.9%). Resistance was verified in 30 isolates (30.97%) grouped in 14 different patterns. Resistance to more than one agent was verified in 13 (16.46%) of the isolates. Two isolates were resistant to four drugs and only one strain presented resistance to three antibiotics.

KEY WORDS: antibiotic, S. Enteritidis, salmonellosis, RS/BRAZIL

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INTRODUCTION

Salmonella is one of the most important microorganisms involved in human foodborne diseases throughout the world. Non-typhoidal salmonellosis in humans is usually a self-limiting illness confined to the gastrointestinal tract, but when infection spreads beyond the intestine, or when immunocompromised persons are affected, effective antimicrobial treatment is essential (Cruchaga *et al.,* 2001). However, probably as a consequence of the extensive use of antibiotics, especially in livestock production, surveillance networks have indicated that the incidence of human salmonellosis caused by antimicrobial resistant *Salmonella* is rising in many countries (Breuil *et al.,* 2000). Increasing antimicrobial resistance in *Salmonella* can limit the therapeutic options available for clinical cases that require antimicrobial treatment. Furthermore, illness caused by resistant *Salmonella* can be more severe and difficult to control. As an example, infections caused by *S.* Typhimurium DT104 resistance to ampicillin, tetracycline, streptomycin, chloramphenicol, and sulphonamides presented higher rates of admission to hospital and increased mortality (Wall *et al.,* 1994). Currently, the serotypes *S.* Typhimurium and *S*. Enteritidis have been recognized as the two major etiologic agents of foodborne salmonellosis in humans (Darwin and Miller, 1999; Baay and Int Velt, 1993; Rodrigue *et al*., 1990). Even though antimicrobial resistance in *S*. Enteritidis has been considered low compared to the increasing of resistance presented by some *S*. Typhimurium isolates (Yang *et al.,* 2002), resistant *S*. Enteritidis have been isolat-

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ed very often from foods or human cases (Breuil *et al.,* 2000). In Brazil, epidemiological data demonstrated *S.* Enteritidis as the main cause of foodborne diseases in different States, and strains presenting antimicrobial resistance have been identified (Tavecchio *et al.,* 1996; Geimba *et al.,* 2005). Specifically in Rio Grande do Sul (RS), a State in southern Brazil, a significant increase in the isolation of *Salmonella* (Costalunga and Tondo, 2002) and, more recently, S. Enteritidis from foods involved in foodborne outbreaks has been reported (Geimba *et al.,* 2004). Based on this, the present work aimed to analyse the antimicrobial resistance of *S.* Enteritidis isolated from foods involved in human foodborne salmonellosis outbreaks in RS during the period from 2001 to 2002.

MATERIALS AND METHODS

Bacterial isolates

Salmonella Enteritidis (n=79) studied in this work were obtained from foods involved in foodborne salmonellosis outbreaks in different regions of the Rio Grande do Sul State, Brazil, during the period from 2001 to 2002. The salmonellosis outbreaks were investigated by the Division of Health Surveillance Service (DVS/RS) that carried out sampling collection and conducted suspected foods to the Central Laboratory of Rio Grande do Sul (LACEN/RS), or accredited laboratories, to perform bacterial isolation and biochemical identification according to FDA (1992). Strains were serotyped in Instituto Adolfo Lutz (São Paulo, Brazil) following methods described by Kauffman (1972).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted in Instituto de Ciência e Tecnologia de Alimentos (ICTA/UFRGS). The isolates were analyzed for susceptibility to 10 antimicrobial agents by the disc diffusion method according to the NCCLS (2001). All antimicrobial discs used were supplied by Oxoid (Hampshire, UK). The antimicrobial drugs and their respective concentrations (μg/disc) were: ampicillin (AMP), 10; tetracycline (T), 30; neomycin (NEO), 10; kanamycin (K), 30; sulfamethoxazole/trimethoprim (SXT), 25; gentamicin (GEN), 10; nalidixic acid, 30; chloramphenicol (C), 30; streptomycin (S), 10; sulfazotrim (SUL), 25. Resistance was determined according to reference zone diameter interpretative standards of NCCLS (2001). *Escherichia coli* ATCC 25922 was used as a reference strain.

RESULTS

The incidence of resistance of *S.* Enteritidis analysed in this study is presented in Table 1. The highest rates of resistance were observed for gentamicin (12.7%), streptomycin (11.4%), and nalidixic acid (21.5%) while most expressive intermediate resistance was observed for kanamycin (29.1%), neomycin (17.7%), and streptomycin (13.9%). In general, high percentages of susceptibility were found for all 10 drugs. The highest rates of sensitivity were demonstrated for ampicillin (94.9%), tetracycline (91.1%) and chloramphenicol (98.7%). No resistance was observed to sulfamethoxazole/trimetoprim and sulfazotrim.

TABLE 1 - *General percentages of antimicrobial resistance among s. Enteritidis isolated from foods involved in foodborne outbreaks occurred in the south of Brazil, 2001 to 2002*

	Antimicrobial resistance (%)									
	AMP	*T*	*NEO*	*K*	*SXT*	*GEN*	*C*	*S*	*NAL*	*SUL*
Sensitive	94.9	91.1	75.9	68.35	100	83.5	98.7	74.7	78.5	100
Intermediate	2.5	8.9	17.7	29.11	0	3.8	0	13.9	0	0
Resistant	2.5	2.53	6.3	2.5	0	12.7	1.3	11.4	21.5	0

ampicillin (AMP); tetracycline (T); neomycin (NEO); kanamycin (K); sulfamethoxazole/trimethoprim (SXT); gentamicin (GEN); chloramphenicol (C); streptomycin (S); nalidixic acid (NAL); sulfazotrim (SUL).

Comparing the antimicrobial resistance of the strains isolated in 2001 to those isolated in 2002, an increment in the percentage of resistant isolates was observed to tetracycline (0% to 5.4%), kanamycin (2.4% to 2.7%), nalidixic acid (19.0% to 24.3%), and chloramphenicol (0% to 2.7%). Results of intermediate resistance for neomycin, kanamycin, gentamycin, and streptomycin also increased from 2001 to 2002. Table 2 summarizes the resistance patterns of *S.* Enteritidis studied in this work. Overall, resistance was verified in 30 isolates (37.9%) grouped in 14 different patterns. Resistance to more than one drug was presented in 13 (16.5%) of the isolates. Resistance to one and two antimicrobial drugs was demonstrated by 13, and 10 isolates, respectively. Only one isolate (4056) demonstrated resistance for three drugs (tetracycline, streptomycin, and nalidixic acid) and two isolates (983 and 5100) presented resistance to four antimicrobial drugs (nalidixic acid, streptomycin, kanamycin, and gentamicin; streptomycin, tetracycline, gentamicin, and chloramphenicol, respectively).

In relation to resistance profile, pattern D (resistance to nalidixic acid) grouped the highest number of isolates (n=11). Patterns H (resistance to neomycin and gentamycin) and I (resistance to gentamicin and streptomycin) were composed of 3 isolates each, and were the next most frequent patterns observed.

Resistance to nalidixic acid was verified in association with streptomycin, gentamicin, and neomycin, this drug resistance being verified in 3 patterns (G, J, and K).

TABLE 2 - *Antimicrobial resistance pattern of S. Enteritidis isolated from food involved in Salmonellosis outbreaks in South of Brazil, 2001-2002*

Pattern	*Phenotype*	*Number of isolates for each pattern*
A	AMP	2
B	GEN	1
C	S	1
D	NAL	11
E	K	1
F	NEO	1
G	NAL, GEN	1
H	NEO, GEN	3
I	GEN, S	3
J	NAL, NEO	1
K	S, NAL	2
L	NAL, S, T	1
M	NAL, S, K, GEN	1
N	S, T, G, C	1

ampicillin (AMP); tetracycline (T); neomycin (NEO); kanamycin (K); sulfamethoxazole/trimethoprim (SXT); gentamicin (GEN); chloramphenicol (C); streptomycin (S); nalidixic acid (NAL); sulfazotrim (SUL)

DISCUSSION

The increase in antimicrobial resistance in *Salmonella* isolated from humans and animals in different parts of the world may indicate an epidemic spread of resistant or multi-resistant strains (Cruchaga *et al.,* 2001). *Salmonella* isolated from foods involved in foodborne outbreaks have also demonstrated significant levels of antimicrobial resistance (Kiessling *et al.,* 2002), confirming the possibility of spread of resistant *Salmonella* from the food chain to humans. This is particularly important concerning foods of animal origin, mainly meat and poultry products, which are frequently involved in salmonellosis outbreaks. Considering that Brazil is the leading world producer of poultry, and many poultry industries are located in the southern region, the evaluation of the resistance of *Salmonella* isolated from salmonellosis outbreaks acquires great importance.

In this study, the highest percentages of resistance were found for gentamicin (12.7%), streptomycin (11.4%) and nalidixic acid (21.5%). Similar results were demonstrated by Geimba *et al*. (2005) who reported that the highest percentages of resistance among 73 *S.* Enteritidis isolated from foods linked to salmonellosis outbreaks in RS in the period of 1999 to 2000 were detected for these three drugs (gentamicin, 13.7%; streptomycin, 37%; and nalidixic acid, 13.7%). Different results were demonstrated by

Oliveira et al. (2005), who did not find resistance for gentamicin or streptomycin among 31 *S.* Enteritidis isolated from foods involved in salmonellosis outbreaks in the same region in a previous period (1995 to 1997). These findings could suggest that the resistance for gentamicin and streptomycin is increasing among *S.* Enteritidis involved in foodborne outbreaks of RS.

Compared to our findings, higher percentages of resistance to nalidixic acid (31%) but lower resistance to gentamicin (1%) and streptomycin (1%) were found by Cruchaga *et al.,* (2001) in *S.* Enteritidis isolates from humans in Spain 1998. Lower percentages of resistance for gentamicin (0 to 1%) and nalidixic acid (2 to 4%) were also found by Breuil et al. (2000) in *S.* Enteritidis isolated from human and animal samples analysed in 1994 and 1997 in France. Molbak *et al.,* (2002) examined 2,546 *S.* Enteritidis isolates in Denmark, in the period 1995-2000 and demonstrated that 82 isolates (3.2%) were resistant to nalidixic acid. The same authors showed that quinolone resistance increased from 0.8% in 1995 to 8.5% in 2000. From 1994 to 1997, in England and Wales, quinolone resistance in *S.* Enteritidis increased from 0.4 % to 1.3% (Threlfall *et al.,* 1999). Several recent studies have reported that *Salmonella* mainly from poultry has emerged as particularly resistant to quinolones (Breuil *et al.,* 2000; Oliveira *et al.,* 2005). To avoid cross-resistance for quinolones, the use of fluoroquinolones in the poultry industry was considered inappropriate as this drug is one of the first-line antimicrobials in the therapy of gram-negative sepsis in humans (Garau *et al.,* 1999). However, the incidence of *Salmonella* strains resistant to quinolones increased substantially in the years following the licensing of flouroquinolones, such as enrofloxacin for veterinary use (Oliveira *et al.,* 2005). In Brazil, enrofloxacin is commonly used in veterinary practices and this could explain the resistance of *Salmonella* to nalidixic acid.

According to Tassios et al. (1997), resistance to ampicillin replaced resistance to doxycycline as the main resistance trait in isolates obtained in Greece after 1991, increasing from 10% in 1987 to 52% of the total isolates. High levels of resistance to ampicillin among *S.* Enteritidis isolated from humans, animals and foods were presented by different researchers (Breuil *et al.,* 2000; Cruchaga *et al.,* 2001). However, our results demonstrated that only 2.5% of the *S.* Enteritidis isolates were resistant to this drug. This is important as this drug can be used in the treatment of human salmonellosis.

In our study, 12.9% of the *S.* Enteritidis were resistant to streptomycin. Other studies reported similar results in poultry isolates (Lee *et al.,* 1993; Tessi *et al.,* 1997; Manie *et al.,* 1998; Gouws and Brözel, 2000; Usera *et al.,* 2002; Nayak *et al.,* 2004). Low percentages of resistance were found for tetracycline (2.35%), though a higher resistance is expected as this drug has been one of the antibiotics most widely used therapeutically for production animals.

Multi-resistant serotypes of *Salmonella* have been found in different parts of the world, and this may indicate the spread of multi-resistant clones, which might have a greater potential for infections and for the development of additional resistance to new antibiotics (Cruchaga *et al.,* 2001). The case of *S.* Typhimurium is a good example for this, as this serotype has been found in animals and food products, and is considered the serotype with the highest occurrence of multiple resistance among the human strains. The *S.* Typhimurium DT104 type ACSSuT (resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline) is particularly frequent among multi-resistant isolates of *S.* Typhimurium, and its dramatic spread has been detected in many countries. Cruchaga *et al.,* (2001) reported that the chromosomal genes coding for resistance to ACSSuT could have been transferred horizontally to other *S.* Typhimurium strains, contributing to the increasing frequency of resistance in this serotype. Based on the work of Yang *et al*. (2002), the detection and monitoring of multi-resistant *S.* Typhimurium and *S.* Enteritidis are important to substantiate the choice of antibiotics for the treatment of clinical salmonellosis and to assess the risk of expansion of multi-resistant strains. In this study, the *S.* Enteritidis isolates presented a relatively low incidence of resistance to two drugs or more. Low percentages of multiple resistance in *S.* Enteritidis strains have been pointed out by other authors (Simango and Mbewe, 2000; Threfall, 2002; Kiessling *et al.,* 2002; Wybo *et al.,* 2002; Mammina *et al.,* 2002). For example, in England and Wales, during the period of 1996 to 2000, the incidence of multi-resistant *S.* Enteritidis

from humans was very low. In 1996, only 0.4% of the almost 19,000 isolates studied by Threfall (2002) proved multi-resistant, and in 2000, the percentage of multiple resistant isolates remained very low (2.0%) among more than 8,400 isolates studied. To compare the differences between antimicrobial resistance in *Salmonella* serotypes, the same author reported that the multiple resistance presented by more than 10,000 *S.* Typhimurium isolates ranged from 59 to 81%, in the same period. Cruchaga *et al.,* (2001) reported that only one isolate among 125 food-related *S.* Enteritidis isolates was multi-resistant. Yang *et al.,* (2002) demonstrated that 100% of the *S.* Typhimurium, and 21% of the *S.* Enteritidis isolated from animals in Korea were resistant.

In the current work, 14 antimicrobial resistance patterns were identified, and most of the resistant isolates were resistant to only one or two drugs. Only 1 and 2 isolates were multi-resistant to 3 and 4 antimicrobial drugs, respectively (Table 2). Almost the same number of resistance patterns (n=15), but with different antibiotic composition, were verified by Geimba *et al.,* (2005) among *S*. Enteritidis isolated in 1999 to 2000 from salmonellosis-related foods in RS. Oliveira *et al.,* (2005) found 18 resistance patterns among *S*. Enteritidis isolated from humans, foods, broiler carcasses and poultry sampled in the same region. However, patterns were also different from the patterns verified by our results. If all these data are considered together, no predominant resistance pattern was verified among *S*. Enteritidis analysed in RS. Considering the number of resistance patterns, other authors have reported similar results studying *S.* Enteritidis (Tassios *et al.,* 1997; Hernandez *et al*., 2002; Yang *et al.,* 2002; Fernandes *et al.,* 2003).

Currently, *S.* Enteritidis is one of the most important *Salmonella* serotypes worldwide, being often associated with poultry and poultry products involved in foodborne outbreaks. Even though poultry feed frequently contains antimicrobials, *S.* Enteritidis seems to maintain high levels of sensitivity to many drugs unlike *S*. Typhimurium.

This research aimed to contribute to the monitoring of the antimicrobial resistance of *S.* Enteritidis involved with salmonellosis outbreaks in southern Brazil. The isolates studied in this work had relatively low levels of resistance, and no predominant resistance pattern could be identified compared with previous reports on *S.* Enteritidis isolated from the same State (Oliveira *et al.,* 2005; Geimba *et al.,* 2005). However, attention should be given to the isolation of multi-resistant *S.* Enteritidis in RS State and in other parts of Brazil (Tavechio *et al.,* 1996).

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