

Resistance Profile to Antimicrobials of *Salmonella* spp. Isolated from Human Infections

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ABSTRACT

The purpose of this study was to analyse the profile of antimicrobial resistance among 21 strains of *Salmonella* isolated from patients with gastroenteritis symptom. It was observed that *S. enteritidis* was the serotype prevalent. These strains were sensitive to the majority of the antimicrobials tested, however, high resistance was observed in *S. typhimurium* and *S. enterica* subsp. *enterica* serotype 4,5,12:i:-. Surveillance and an efficient monitoring should be priority for the public health for the containment of antimicrobial resistance in foodborne infections.

Key words: *Salmonella*, humans, serotypes, resistance, antimicrobials

INTRODUCTION

Salmonella bacterium is one of the commonest causes of food poisoning worldwide. Food, such as egg, meat, milk and other dairy products, are the commonest source of salmonella for humans (Winokur et al., 2000). The *Salmonella* genus belongs to the *Enterobacteriaceae* family, according to current taxonomy, and consists of two species: *S. enterica* and *S. bongori*. *Salmonella enterica* is divided into six subspecies: *enterica*, *salamae*, *arizonae*, *indica*, *diarizonae* and *houtenae*. These species and subspecies can be classified according to the identification of antigenic factors (O-somatic, Vi-capsular and H-flagellar) in about 2400 serotypes (Popoff and Le Minor 1992). *S. enteritidis* is the most common serotype in Brazil and in the world. An increase in the number of cases of diseases in humans

caused by food contaminated by this serotype has been observed, but most of the isolates have been sensitive to the antimicrobials chosen for treatment of systemic salmonellosis (Bäumler et al., 2000; Tavechio et al., 1999; Fernandes et al., 1999; Reis et al., 1999). Although most *Salmonella* isolates belong to the Enteritidis serotype, *S. typhimurium* has increased resistance dissemination to antimicrobials, especially *S. typhimurium* phage type 104 (DT104) that has chromosome resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline (ACSSuT) (Gross et al., 1998; Glynn et al., 1998). The aim of this study was to verify the resistance profile to antimicrobials among *Salmonella* serotypes isolated from patients in two major hospitals in Londrina-PR.

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MATERIALS AND METHODS

Clinical specimens

Twenty-one strains of *Salmonella* spp. were isolated in two major hospitals in the city of Londrina-PR, between October 1999 and May 2000. They were isolated from feces, except one that was isolated from blood. Feces were collected in clean recipients and blood in hemoculture flask.

Isolation and biochemical identification

The fecal samples were inoculated in Hektoen-Enteric agar (Merck), MacConkey agar (Merck), and enriched Selenite-Cystine broth (Merck) followed by incubation for 18-24 hours at 37°C. The colonies that grew on both solid media were inoculated in culture medium for biochemical identification: EPM, MILi (Toledo et al., 1982 a,b) and Simmons Citrate (Merck), and also incubated for 18-24 hours at 37°C. Selenite-Cystine broth was inoculated in MacConkey agar and Salmonella-Shigella agar (Merck) and incubated for 18-24 hours at 37°C. Biochemical identification was then done, as previously mentioned. The blood sample was inoculated onto Blood agar and MacConkey agar, and the colonies isolated from MacConkey agar were biochemically identified as described above.

Serological identification

Strains with *Salmonella* biochemical characteristics were confirmed with polyvalent anti-*Salmonella* serum (Probac do Brasil, São Paulo, Brazil) and those positive were sent to the Instituto Adolfo Lutz, São Paulo, for serotyping.

Antimicrobial susceptibility

The antimicrobial susceptibility was determined by the disk diffusion method on Müller-Hinton agar (Biobrás), as described by Bauer et al. (1966). Initially, 16 antimicrobials were tested; those which corresponded to the most routinely used in clinical laboratory, such as, amikacin, ampicillin, aztreonam, carbenicillin, cephalotin, cefepime, cefotaxime, cefoxitin, ceftriaxone, ciprofloxacin, chloramphenicol, gentamicin, imipenen, pefloxacin, trimethoprim+sulfamethoxazole and tobramycin. The interpretation of the inhibition halos to the antimicrobials was according to the National Committee for Clinical Laboratory Standards (NCCLS, 2000a). When the results were analyzed it was suspected that four strains

might contain the phage type 104 (ACSSuT phenotype) because they presented resistance to two antimicrobials reported to this phenotype (ampicillin and chloramphenicol). Therefore, the resistance profiles of these strains were evaluated for streptomycin, sulfonamide and tetracycline in addition.

RESULTS

The results showed that *S. enteritidis* was the most frequently isolated serotype (66.7%). The other serotypes, *S. infantis*, *S. newport*, *S. typhimurium*, *S. javiana*, *S. brandenburg*, *S. glostrup* and *S. enterica* subsp. *enterica* serotype 4,5,12:i:- were isolated at frequency of 4.76%. All the serotypes were isolated from feces except *S. enterica* subsp. *enterica* serotype 4,5,12:i:- that was isolated from blood. Table 1 shows the resistance profiles to 16 antimicrobials initially tested. Seven *Salmonella* strains (50%) belonging to Enteritidis serotype were sensitive to all the antimicrobials tested and the other seven were resistant to one or more antimicrobials, and the highest percentage of resistance was found for ampicillin (85.7%). *S. infantis*, *S. newport*, *S. javiana* and *S. brandenburg* were sensitive to all the antimicrobials, and *S. glostrup* was resistant only to gentamicin. However, two strains belonging to Typhimurium serotype and *enterica* serotype 4,5,12:i:- were resistant to 11 antimicrobials. All strains tested were sensitive to amikacin, pefloxacin, imipenen and ciprofloxacin.

Four strains (two *S. enteritidis*, one *S. typhimurium*, and one *S. enterica* subsp. *enterica* serotype 4,5,12:i:-) that were suspected of containing the phage type 104 inserted in their chromosome, presented resistance to ampicillin, chloramphenicol, streptomycin and sulfonamide but were sensitive to tetracycline.

Table 1 - Antimicrobial resistance profiles of *Salmonella* spp. strains isolated from patients in two major hospitals in the city of Londrina-PR. AMI, amikacin; AMP, ampicillin; AZT, aztreonam; CAR, carbenicillin; CEF, cephalotin; CTX, cefotaxime; CFO, cefoxitin; CFP, cefepime; CRO, ceftriaxone; CIP, ciprofloxacin; CLO, chloramphenicol; GEN, gentamicin; IMP, imipenen; PEF, pefloxacin; SUT, sulfamethoxazol+trimethoprim; TOB, tobramycin.

Serotype	Resistance profile	Number of strains
<i>S. enteritidis</i>	AMP, CEF, CLO, SUT,	1
	AMP, CAR, CEF	1
	AMP, CLO	1
	AMP, SUT	1
	AMP	2
	GEN	1
<i>S. typhimurium</i>	AMP, AZT, CAR, CEF, CFO, CFP, CLO, CRO, CTX, GEN, TOB	1
<i>S. enterica</i> subsp. <i>enterica</i> 4,5,12:i:-	AMP, AZT, CAR, CEF, CFO, CFP, CLO, CRO, CTX, GEN, TOB	1
<i>S. glostrup</i>	GEN	1

DISCUSSION

The data obtained for the frequency of *Salmonella* strains isolated were according to the literature, and showed prevalence of *S. enteritidis* (Bäumler et al., 2000; Tavechio et al., 1999; Fernandes et al., 1999; Reis et al., 1999). Bäumler et al. (2000) attributed the increase of salmonellosis cases by this serotype because of the replacement of *S. pillorum* and *S. gallinarum* in an ecological niche that was previously occupied by the last two serotypes of avian origin. These three serotypes have a common antigen (O9) and the immune response produced by the avian *Salmonella* strains did not allow birds to be infected by the Enteritidis serotype. By eradication of these serotypes, at the beginning of the second half of the last century, *S. enteritidis* started to infect birds and consequently humans.

According to the resistance patterns to the antimicrobials tested, it was found that 50% of the *S. enteritidis* isolates were sensitive to all the antimicrobials and that the other 50% presented resistance to one or more antimicrobials. Attention was drawn to strains resistant to ampicillin, trimethoprim+sulfamethoxazole, chloramphenicol and third generation cephalosporine because the NCCLS (2000b) recommended that only ampicillin, a quinolone and trimethoprim+sulfamethoxazole be tested and reported for the *Salmonella* isolates of intestinal infection and a third generation cephalosporine and chloramphenicol should be tested and reported

only for extraintestinal infections. According to our results, three strains of *S. Enteritidis* were resistant to ampicillin, one to ampicillin and chloramphenicol, one to ampicillin and trimethoprim+sulfamethoxazole and other to ampicillin, trimethoprim+sulfamethoxazole and chloramphenicol. *S. typhimurium* and *S. enterica* subsp. *enterica* serotype 4,5,12:i:- were resistant to third generation cephalosporine, ampicillin and chloramphenicol. Therefore, monitoring programs are needed to detect these resistant strains before they become widely disseminated. The current concern has been the isolation of *S. enteritidis* strains that produce Extended Spectrum Beta Lactamase (ESBL) capable of hydrolyzing first, third and fourth generation cephalosporine and monobactamic, but do not act on cefamicines (second generation cephalosporine) (Nastasi et al., 2000; Rankin and Coyne, 1998; Ling et al., 1998). No *S. enteritidis* strain with this resistance mechanism was isolated in this study. A second resistance mechanism has recently been detected mainly in Typhimurium serotype, concerned to a Beta Lactamase type Amp-C mediated by plasmid, conferring additional resistance to cefamicines (Winokur et al. 2000).

In the present study, our strains of *S. typhimurium* and *S. enterica* subspecies *enterica* serotype 4,5,12:i:- presented a multiresistance pattern and among these resistance patterns were detected from first to fourth generation cephalosporines, such as cephalotin (1st generation); cefoxitin (2nd generation); cefotaxime and

ceftriaxone (3rd generation); cefepime (4th generation), and also to the monobactamic aztreonam. Therefore, it could be suggested that phenotypic and genotypic tests should be done to detect ESBL and Amp-C in these strains. It has been mentioned in the literature the concern about *Salmonella* strains that may contain phage type 104 (ACSSuT phenotype). In this study of the four strains that were suspected of containing this phage, all presented the ACSSu phenotype, being sensitive to tetracycline. A contributing factor for resistance dissemination observed in certain *Salmonella* serotypes has been the unnecessary use of antimicrobials for self limited gastroenteritis treatment. It has been suggested that these treatments be reserved only for extraintestinal salmonellosis and in cases of child gastroenteritis or elderly and immune suppressed patients where antibioticotherapy is fundamental to control these diseases (Winokur et al., 2000; Prats et al., 2000). Regarding antimicrobials resistance of enteric pathogens, the most worrying factor has been the increase of multiresistance dissemination of *S. typhimurium* and also, on a smaller scale, by other serotypes due to the use of these antimicrobials in animal feeding for prophylaxis and growth promotion. Therefore, it is expected that prophylactic measures will soon be introduced to decrease the use of antimicrobials in products of animal origin, which certainly will result in a decline in strains resistance to antimicrobials in the food chain (Threlfall et al., 2000).

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RESUMO

O objetivo deste estudo foi analisar o perfil de resistência à antimicrobianos, de *Salmonella* isoladas de 21 amostras provenientes de pacientes com sintomatologia de gastroenterite. Verificou-se que *S. enteritidis* foi o sorotipo com maior prevalência. Estas cepas mostraram-se sensíveis à maioria dos antimicrobianos testados, no entanto, as cepas *S. typhimurium* e *S. enterica* subsp. *enterica* sorotipo 4,5,12:i:-, mostraram-se resistentes à vários antimicrobianos. Vigilância e

um monitoramento eficiente, para diminuir a resistência antimicrobiana em microrganismos que causam infecções veiculadas por alimentos, devem ser prioridade para a saúde pública.

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