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BRIEF COMMUNICATION

"MULTIPLEX PCR" IDENTIFICATION OF THE ATYPICAL AND MONOPHASIC *Salmonella enterica* subsp. *enterica* serotype 1,4,[5],12:i:- IN SÃO PAULO STATE, BRAZIL: FREQUENCY AND ANTIBIOTIC RESISTANCE PATTERNS

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SUMMARY

Salmonella spp. are the etiologic agents of salmonellosis, a worldwide spread zoonoses causing foodborne outbreaks and clinical diseases. By serological identification, *Salmonella enterica* subsp. *enterica* serotype 1,4,[5],12:i:- accounted for 8.8% of human and 1.6% of nonhuman *Salmonella* strains isolated in São Paulo State, during 1991-2000. A total of 28.6% of them amplified a fragment corresponding to H:1,2 (flagellar phase two) through PCR analysis and were further assigned as *S*. Typhimurium. Antimicrobial resistance was detected in 36.3% of the 369 PCR-negative strains tested, including the multiresistance to ampicillin, chloramphenicol, sulfonamides, tetracycline, and streptomycin.

KEYWORDS: *Salmonella*; Serotyping; Antimicrobial resistance; PCR analysis

Salmonella spp. strains are the etiologic agents of salmonellosis, a zoonotic disease worldwide spread, and/or enteric fevers depending on the serotypes involved. *Salmonella* serotypes have been traditionally defined through serological identification of somatic (O) and flagellar (H) factor antigens present in the bacterial cell. Most *Salmonella* strains are motile by peritrichous flagella and an important characteristic of the genus is the presence of two different flagellin genes on the bacterial chromosome, *fliC* and *fljB* (formerly *H1* and *H2*), which expression is done by a system called "phase variation"[8,14]. The great majority of the 2,501 *Salmonella* serotypes listed in the Kauffmann-White scheme[10] expresses alternatively both genes (phase one and phase two), being then called biphasic. The remaining serotypes displaying only one flagellar phase are designated monophasic.

Salmonella enterica subsp. *enterica* serotype 1,4,[5],12:i:- (*S*. I 1,4,[5],12:i:-) is an atypical and emergent monophasic serotype, which shares almost all antigenic factors with *S. enterica* subsp. *enterica* serotype Typhimurium (*S*. Typhimurium) (1,4,[5],12:i:1,2), a worldwide spread *Salmonella* serotype. Some studies have been carried out in order to determine the genetic relationship between these serotypes and to confirm the hypotheses that *S*. I 1,4,[5],12:i:- is a monophasic variant of *S*. Typhimurium[5,7]. As far as we know, this unusual serotype has been described since 1993 in Thailand[2], in Spain[4], and in the U.S.A.[1].

In São Paulo, Brazil, *S*. I 1,4,[5],12:i:- has been detected in samples of human and nonhuman origins through the last decades, with an increasing of up to five times in the 90´s, being particularly associated with human foodborne outbreaks and septicemia cases[11,12,13]. Besides that, *S*. I 1,4,[5],12:i:- has been found among the top five *Salmonella* serotypes isolated from human infections in our area, together with *S*. Enteritidis and *S*. Typhimurium. This study reports the "multiplex PCR" identification of *S*. I 1,4,[5],12:i:- and its frequency and antimicrobial resistance patterns, providing baseline for further characterization.

From January 1991 through December 2000, 389 (8.8%) out of 4,426 *Salmonella* human strains (including 61.0% from feces and 25.0% from blood) and 128 (1.6%) out of 7,827 nonhuman ones (mostly from foodstuffs and animals) were identified as *S*. I 1,4,[5],12:i:- at the Enteropathogens Laboratory, Instituto Adolfo Lutz, São Paulo. Those strains were isolated in different cities of São Paulo State. Isolation and identification of strains at the genus level were performed as described by EWING[6]. *Salmonella* subspecies determination and serotyping were carried out according to POPOFF[10]. Every somatic and flagellar antiserum used for *Salmonella* serotyping has been produced and maintained by this Enteropathogens Laboratory.

Those 517 strains were firstly assigned as *S*. I 1,4,[5],12:i:- by slide agglutination method with polyclonal antisera and stored in agar slant at room temperature. Afterwards, they were submitted to the "multiplex PCR" method described by ECHEITA & USERA[3] in order to detect the gene encoding for the flagellar phase two in the H1 antigenic complex (H:1,2, H:1,5, H:1,6, H:1,7). One hundred and forty-eight (28.6%) strains

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amplified the 394 bp-fragment specific for H:1,2 being, therefore, assigned as *S*. Typhimurium. These results indicate that the undetected flagellar phase two during the serologic identification is likely to be due to the low level of bacterial cells expressing this antigen. Thus, the "Multiplex PCR" proved to be a useful and complementary tool for the classical serological identification of *S*. I 1,4,[5],12:i:- strains.

The 369 (289 human and 80 nonhuman) PCR-negative strains were analyzed for antimicrobial resistance, determined by the disk diffusion method according to National Committee for Clinical Laboratory Standards criteria[9], against to the following antimicrobial agents (CECON): amikacin (30 µg), amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), aztreonam (30 µg), cefepime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), netilmicin (30 µg), nitrofurantoin (300 µg), sulfonamides (300 µg), streptomycin (10 µg), sulfamethoxazole-trimethoprim (25 µg), and tetracycline (30 µg).

The results showed that all strains were susceptible to aztreonam, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, and imipenem. Table 1 demonstrates that 8% of human and 5% of nonhuman strains were susceptible to all antimicrobials tested. A total of 208 (56.3%) strains (55% human and 62.5% nonhuman) presented intermediate resistance, mostly to tetracycline (91%) and streptomycin (64%). One hundred and thirty-four (36.3%) strains (37% human and 31% nonhuman) exhibited resistance to one up to 13 antimicrobial agents, distributed to 30 resistance patterns (Table 1). Such resistance was mostly to tetracycline (68%),

Table 1
Antimicrobial susceptibility patterns of the 369 *S*. I 4,5,12:i:- PCR-negative strains, isolated from human and non human sources during 1991-2000, in São Paulo State, Brazil

Susceptibility pattern	Nº (%) of strains	
	Human (n = 289)	Nonhuman (n = 80)
Susceptible	23 (8.0)	4 (5.0)
Intermediate resistance	158 (55.0)	50 (62.5)
Resistance profile*		
TET	53 (18.5)	11 (13.6)
NIT	13 (4.5)	5 (6.0)
SSS	-	1 (1.3)
STR	6 (2.1)	-
NAL	1 (0.3)	-
CEP, NIT	1 (0.3)	-
TET, NIT	3 (1.1)	2 (2.5)
TET, CEP	1 (0.3)	-
TET, KAN	3 (1.1)	-
SSS, NIT	3 (1.1)	1 (1.3)
NIT, STR	1 (0.3)	-
TET, NAL	2 (0.7)	-
TET, SSS, NIT	2 (0.7)	1 (1.3)
TET, CHL, CEP	1 (0.3)	-
TET, SSS, STR	1 (0.3)	-
SSS, SXT, STR	1 (0.3)	1 (1.3)
AMP, SSS, STR	1 (0.3)	-
AMP, SSS, SXT, STR	3 (1.1)	1 (1.3)
TET, SSS, SXT, STR	1 (0.3)	1 (1.3)
CHL, SSS, SXT, STR	1 (0.3)	-
TET, KAN, SSS, SXT, STR	1 (0.3)	-
TET, KAN, AMP, SSS, STR	1 (0.3)	-
TET, CHL, AMP, CEP, NIT	1 (0.3)	-
AMP, AMC, CEP, SSS, SXT, STR	1 (0.3)	-
TET, KAN, AMP, SSS, SXT, STR	1 (0.3)	-
TET, CHL, AMP, SSS, SXT, STR	1 (0.3)	1 (1.3)
TET, CHL, AMP, SSS, SXT, STR, NIT, NAL	-	1 (1.3)
NET, CHL, GEN, KAN, AMP, CEP, SSS, SXT, AMC, STR	1 (0.3)	-
NET, CHL, GEN, KAN, AMP, CEP, SSS, SXT, STR, AMK	1 (0.3)	-
NET, TET, CHL, GEN, KAN, AMP, CEP, SSS, SXT, AMC, STR, AMK, NAL	2 (0.7)	-

* AMK, amikacin; AMC, amoxicillin/clavulanic acid; AMP, ampicillin; CEP, cephalothin; CHL, chloramphenicol; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; NET, netilmicin; NIT, nitrofurantoin; SSS, sulfonamides; STR, streptomycin; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline.

nitrofurantoin (25%), sulfonamides (22.4%), and streptomycin (22%). Eighty percent of these strains were resistant to one or two agents and 20% displayed multiresistance to three or more antimicrobials. ECHEITA *et al.*[4] in Spain and AGASAN *et al.*[1] in the U.S.A. found a higher level of multiresistant (including the ampicillin, chloramphenicol, sulfonamides, streptomycin, tetracycline pattern) *S.* I 1,4,[5],12:i:- strains comparing to our results.

Although no strain was resistant to ciprofloxacin, six strains presented resistance to nalidixic acid. Approximately 90% of the human strains isolated from blood displayed some resistance or decreased susceptibility to the antimicrobials suggesting a special concern with such strains. Multiresistance was found in the same level (5%) for both human and nonhuman strains. No increase in antibiotic resistance was observed throughout the period under study.

Further studies on phenotypic and molecular characterization of *S.* I 1,4,[5],12:i:- and *S.* Typhimurium strains are in progress in this laboratory by some of us. Data obtained from those studies will be compared in order to confirm the genetic relationship between these two serotypes.

RESUMO

Identificação por "Multiplex PCR" do sorotipo monofásico e atípico *Salmonella enterica* subsp. *enterica* sorotipo 1,4,[5],12:i:-, no Estado de São Paulo, Brasil: freqüência e resistência antimicrobiana

Salmonella spp. é o agente etiológico da salmonelose, zoonose mundialmente distribuída e responsável por surtos de doenças transmitidas por alimentos e doenças clínicas. Sorologicamente, *Salmonella enterica* subsp. *enterica* sorotipo 1,4,[5],12:i:- correspondeu a 8,8% e 1,6% das cepas de *Salmonella* de origem humana e não-humana, respectivamente, isoladas no Estado de São Paulo, no decênio 1991-2000. Aproximadamente 28,6% destas cepas amplificaram o fragmento correspondente a H:1,2 (fase flagelar dois) em testes de PCR e foram, então, identificadas como *S.* Typhimurium. Das 369 cepas negativas em PCR, 36,3% apresentou resistência antimicrobiana, incluindo multirresistência a ampicilina, cloranfenicol, sulfonamidas, tetraciclina e estreptomicina.

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