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LYSOTYPES AND PLASMIDIAL PROFILE OF *SALMONELLA* SEROVAR TYPHIMURIUM ISOLATED FROM CHILDREN WITH ENTERIC PROCESSES IN THE CITIES OF RIO DE JANEIRO, RJ, AND SALVADOR, BA - BRAZIL

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SUMMARY

The lysotypes, plasmidial profiles, and profiles of resistance to antimicrobial agents were determined in 111 *Salmonella* Typhimurium strains isolated from feces and blood of children treated in Rio de Janeiro and in Salvador. Six distinct lysotypes (19, 41, 97, 105, 120 and 193) were recognized, with a predominance of lysotype 193 (59.7%) in Rio de Janeiro and of phage type 105 (38.4) in Salvador. Approximately 86.7% of the lysotype 193 strains presented multiple resistance to more than six antimicrobial agents, whereas 93% of lysotype 105 strains were fully susceptible. More than 90% of the strains presented plasmids distributed into 36 different profiles in Rio de Janeiro and into 10 profiles in Salvador. A 40 MDa plasmid was the most frequent (47%) in the strains from Rio de Janeiro, whereas a 61 MDa plasmid predominated (14.5%) in Salvador. Combined analysis of plasmid profile and classification into lysotypes (especially those belonging to types 105 and 103, proved to be more discriminatory than the other methods applied).

KEYWORDS: *Salmonella*; Lysotypes; Resistance to antimicrobial agents; Plasmidial profile.

INTRODUCTION

Among the members of the genus *Salmonella*, serovar *Salmonella* Typhimurium is the most frequent in the etiology of acute diarrhea in different regions of the world (LING et al., 1987; CHALKER & BLASER, 1988; FARHOUDI-MOGHADDAM et al., 1990) and in Brazil (HOFER, 1974; PESSÔA et al., 1978; CALZADA et al., 1984; LEAL et al., 1988).

Several techniques have been used for typing *Salmonella* and epidemiologic studies especially for serovar Typhimurium. Among them include phenotypic characteristics such as biotyping, serotyping, antibiograms, colicin typing and phage typing (HOFER, 1979; BARKER & OLD, 1980; ANDERSON et al., 1977; THRELFALL et al., 1990) and molecular typing systems based on the DNA content of bacteria such as plasmid profile analysis (PPA) and restriction endonuclease analysis (RILEY et al., 1983; PLATT & BROWN, 1986; THRELFALL et al., 1986; RIVERA et al., 1991).

In the present investigation we used the tests of susceptibility to antimicrobial agents and lysotyping in combination with PPA in order to contribute to the epidemiologic analysis of *S.* Typhimurium strains isolated from children seen at the Fernandes Figueira Institute, Rio de Janeiro, RJ, and at the Martagão Gesteira Hospital and at the Center of Hydration and Recovery in Salvador, Bahia.

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MATERIALS AND METHODS

Bacterial strains: A total of 111 strains (39 from Salvador and 72 from Rio de Janeiro) were obtained from feces (89) and blood (22) of children submitted to medical treatment during the period from 1987 to 1992. They were identified as *Salmonella*, by determination of biochemical reactions described by EWING (1986), COSTA & HOFER (1972) and KAUFFMANN (1954). Serovar representation was done according to the criteria of LE MINOR (1988).

Susceptibility testing: The disk diffusion method [23] was used to test for antimicrobial susceptibility for ampicillin (AMP), 10 mcg; cephalothin (CET), 30 mcg; cefoxitin (COX), 30 mcg; ceftriaxone (CRO), 30 mcg; pefloxacin, 5mcg (PEF); gentamicin, 10mcg; (GEN); amikacin (AMK), 30 mcg; trimethoprim - sulfamethoxazole (SXT), 1.25/23.75 mcg; chloramphenicol (CMP), 30 mcg; tetracycline (TET), 30 mcg.

Phage typing: The *Salmonella* Typhimurium had been typing by Linda R. Ward at the Division of Enteric Pathogens, Central Public Health Laboratory, London and was carried out following a procedure of Routine Test Dilution of the phages, with a final determination until 204 phage types[1].

Plasmid profile analysis: Plasmidial DNA extraction was performed by the alkaline lysis method of BIRNBOIM & DOLY (1979), modified by SAMBROOK et al. (1989). Samples of 15 microliters of plasmid preparation were loaded into wells of a 0.8% agarose gel for a 7.5 cm length and run at 40V for 2h on a horizontal electrophoresis apparatus with a constant - voltage power source. Gels were stained with ethidium bromide and visualized on a UV transilluminator. Photographs were taken with Polaroid Type 667 film.The plasmidial DNAs of *Escherichia coli* R861 (THRELLFALL et al., 1986) and V517 (MACRINA et al., 1978) were included as molecular weight controls.

RESULTS AND DISCUSSION

The 72 strains from Rio de Janeiro (Table 1) consisted of two lysotypes, i.e., 193 (59.7%) and 97 (1.4%), plus 26 (36.1%) untypable with the phage preparations used, and two reacted with *S.* Typhimurium typing phages but did not conform to a recognizable pattern. They were therefore designated RDNC. The 39 strains from Salvador presented 5 distinct lysotypes with predominance of type 105 (38.4%), and lysotype 193 was detected in 25.6% of cases. Thus, it can be seen that lysotype 193 was the most frequent, being present in 53 (47.3%) of the strains isolated. However, most of these strains originated from Rio de Janeiro where this lysotype is in a situation that enables it to colonize a hospital environment. Indeed, lysotype 193 has also been described in other studies carried out in our midst (MAGALHÃES et al., 1975). Thus, HOFER et al. (1979), studying 356 strains isolated in different regions of the country from 1960 to 1970, noted that 164 of them (46.06%) belonged to lysotype 193.

Another aspect was the high frequency of nontypable strains, 34 (46.57%). PESSÔA et al. (1978) obtained similar results (73% untypable strains out of a total of 319) when they analyzed lactose-positive and lactose-negative *S.* Typhimurium strains by the international procedure (ANDERSON et al., 1977). These data indicate the need for continued research in order to obtain new phage preparations capable of revealing such lysotypes.

When evaluating the resistance profile of the most frequent lysotypes (Table 2), we noted that 50 (94.3%) of the 53 strains belonging to lysotype 193 presented more than one resistance marker, the most predominant one being AMP, CET, PEF, GEN, AMK, SXT, CMP, TET (47.1%). Conversely, 14 strains of the 105 lysotype (93.3%) were not resistant, and 59.4% of the 34 untypable strains presented more than six resistance markers.

The analysis of the resistance levels of strains from the two areas (Table 3) permitted us to determine that 58 (85.2%) of Rio de Janeiro strains presented more than six markers, as opposed to only 11 (52.3%) of Salvador strains. It is clear that strains Salvador were more susceptible, and this higher susceptibillity probably derives from the fact that the patients were infected with *S.* Typhimurium strains that have not yet colonized hospital wards. On the other hand, resistance to some drugs is thought to be an intrinsic property of some lysotypes adapted, for example, to hospital conditions.

TABLE 1

Frequency of *Salmonella* Typhimurium lysotypes isolated in Rio de Janeiro, RJ, and Salvador, BA.

City	LYSOTYPES								Total
	19	41	97	105	120	193	UT	RDNC	
Rio de Janeiro	-	-	1(1.4)*	-	-	43(59.7)	26(36.1)	2(2.77)	72
Salvador	1 (2.5)**	2(5.1)	-	15(38.4)	3(7.6)	10(25.6)	8(20.5)	-	39
Total	1	2	1	15	3	53	34	2	111

* Percentage from 72 strains; ** Percentage from 39 strains;
UT - untypable; RDNC - reacted with *S.* Typhimurium typing phages but did not conform to a recognizable pattern.

TABLE 2
Profile of resistance to antimicrobial agents occurring in the most frequent lysotypes of *Salmonella* Typhimurium

RESISTANCE PATTERN	LYSOTYPES							
	19	41	97	105	120	193	UT+RDNC	TOTAL
AMP, CET, CRO, PEF, GEN, AMK, SXT, CMP, TET	-	-	-	-	-	4	2	6
AMP, CET, CRO, PEF, GEN, AMK, CMP, TET	-	-	-	-	-	1	1	2
AMP, CET, PEF, GEN, AMK, SXT, CMP, TET	-	-	-	-	-	25	4	29
AMP, CET, COX, GEN, AMK, CMP, TET	-	-	-	-	-	1	-	1
AMP, CET, GEN, AMK, SXT, CMP, TET	-	-	-	-	-	10	3	13
AMP, CET, PEF, GEN, SXT, CMP, TET	-	-	-	-	-	2	1	3
AMP, CET, CRO, GEN, AMK, SXT, CMP	-	-	-	-	-	-	1	1
AMP, CET, CRO, GEN, AMK, CMP	-	-	-	-	-	-	1	1
AMP, CET, GEN, AMK, SXT, CMP	-	-	-	-	-	-	5	5
AMP, CET, GEN, AMK, CMP, TET	-	-	-	-	-	-	3	3
AMP, PEF, GEN, AMK, CMP, TET	-	-	-	-	-	1	-	1
AMP, CET, PEF, GEN, SXT, TET	-	-	-	-	-	1	-	1
AMP, CET, GEN, SXT, CMP, TET	-	-	-	-	-	1	-	1
AMP, CET, PEF, AMK, SXT, TET	-	1	-	-	-	-	1	2
AMP, CET, GEN, AMK, CMP	-	-	-	-	-	-	4	4
AMP, CET, PEF, GEN, AMK	-	-	-	-	-	1	-	1
AMP, PEF, GEN, CMP, TET	-	-	-	-	-	1	-	1
AMP, CET, GEN, AMK	-	-	-	-	-	-	1	1
AMP, CET, CFX, CRO	-	-	-	-	1	-	-	1
AMP, CET, SXT, TET	1	-	-	-	-	1	-	2
AMP, CET, SXT, CMP	-	-	-	-	-	-	3	3
AMP, GEN, SXT, CMP	-	-	-	-	-	-	1	1
GEN, SXT, CMP, TET	-	-	-	-	-	-	1	1
AMP, CMP	-	-	1	-	-	-	-	1
SXT, TET	-	-	-	-	-	1	-	1
CMP, TET	-	-	-	-	-	-	1	1
TET	-	-	-	1	-	1	-	2
Without Resistance	-	1	-	14	2	2	3	22
TOTAL	1	2	1	15	3	53	36	111

TABLE 3
Multiple - antibiotic resistance of *S.* Typhimurium isolates.

Nº of Markers	City		
	Rio*	Salvador	Total
1	-	2	2
2	3	-	3
4	1	8	9
5	6	-	6
6	11	3	14
7	11	7	18
8	31	-	31
9	6	-	6
Total	69	20	89

*Rio de Janeiro

In the present investigation, we also intended to use the determination of plasmidial profile as an epidemiologic screening tool in combination with other methods such as phage typing and antibiogram. Thus, analysis of the palsmidial profile of Salvador strains (Table 4) permitted us to recognize 10 different profiles with predominance of the 61 MDa plasmid, detected in 10 of the 14 strains of lysotype 105. The relation between this result and those obtained by lysotyping demonstrates that phage types 105 and 193 have heterogeneous plasmidial profiles, suggesting a possible different origin of involved individuals.

A similar phenomenon was observed in Rio de Janeiro strains, in which the most frequent lysotype, 193, showed 24 different profiles (Table 5). The analysis of

TABLE 4

Plasmidial profile according to the different lysotypes of *S.* Typhimurium isolated in Salvador, BA.

Plasmid Pattern	Lysotypes						
	19	41	105	120	193	UT	Total
105, 1.5	-	-	-	-	1	-	1
98	1	-	-	-	-	-	1
82	-	-	3	1	-	-	4
61	-	-	9	-	1	1	11
61, 2.1, 1.6	-	-	-	-	1	-	1
61, 3.7, 3.2, 2.2, 0.9, 0.7	-	-	1	-	-	5	6
40, 1.5	-	-	-	-	5	-	5
2.4, 2.0	-	1	-	-	-	-	1
1.5, 1.3	-	-	-	-	-	1	1
1.4	-	-	-	-	1	-	1
Total	1	1	13	1	9	7	32

UT - Untypable

TABLE 5

Plasmidial profile of *S.* Typhimurium strains lysotype 193 isolated in Rio de Janeiro, RJ

Plasmid Pattern MDa	No. of Strains
105*	1
105, 3.1	1
105, 1.4, 1.3	1
105, 40, 3.7, 2.0, 1.4	4
105, 4.1, 3.8, 3.1, 1.8, 1.4*	1
94, 2.0, 1.8, 1.0*	2
61, 3.1, 2.8	1
61, 40, 1.6, 1.3*	2
61, 2.3, 1.7, 1.5	1
61, 40, 3.1, 2.6, 2.5, 1.7, 1.5*	5
61, 40, 3.6, 3.3, 1.8, 1.6, 1.4*	1
61, 40, 2.4, 1.4*	2
53, 6.0, 1.6, 1.4	1
40*	1
40, 1.4*	1
40, 1.4, 1.0, 0.7*	2
40, 3.7, 2.0, 1.4	1
40, 1.8*	1
40, 2.0, 1.4*	5
40, 3.8, 2.0, 1.4*	4
40, 2.2, 2.0, 1.4, 1.2*	1
40, 3.8, 1.8, 1.4, 1.2	1
40, 3.1, 2.1, 1.8, 1.4, 1.1, 1.0*	1
Total	41

*Resistance to AMP, CET, PEF, GEN, AMK, SXT, CMP, TET

the table also shows that the 40 MDa plasmid was observed in 16 (66.6%) of the strains analyzed.

Figure 1 demonstrates the variability of the plasmidial profiles of lysotype 193 isolated in Rio de Janeiro, the strains are different, although some of them have similar plasmids. It is observed the presence of high molecular weight plasmids, frequently cited as coding for virulence mechanisms (JONES et al., 1982; VINHAS & ALMEIDA, 1984; BAIRD et al., 1985; JONES & OSBORNE, 1991).

CHART 1

Data of plasmidial profile analysis from the two cities

Data	Rio*	Salvador
Strains with plasmids	94.44%	91.42%
Mean number of different plasmids	33	16
Mean number of different profiles	39	10
Mean number plasmids/strain	4.2	2.8
Mean number of strains/profile	1.8	3.5
Most frequent plasmid (MDa)	40(47%)	61(14.4%)
Total number of strains analyzed	72	35

*Rio de Janeiro, RJ

The higher frequency of 40 MDa and 61 MDa plasmids in each city (Chart 1), suggests that local epidemiologic conditions may imply the existence and predominance of distinct strains. However, it is interesting to note that the 5 strains of lysotype 193 from Salvador also have the 40 MDa plasmid, which was detected in 66.6% of the strains of the same lysotype from Rio de Janeiro. On the other hand, more than 90% of the strains presented plasmids: those from Rio de Janeiro presented a mean number of 4.2 plasmids and those from Salvador, 2.8, although the latter revealed a more homogeneous profile with 3.5 strains/profile. We should emphasize the diversity of plasmidial profiles - 39 in the 72 strains from Rio de Janeiro, probably explained by the peculiar characteristics of the hospital which treats children from different nearby municipalities.

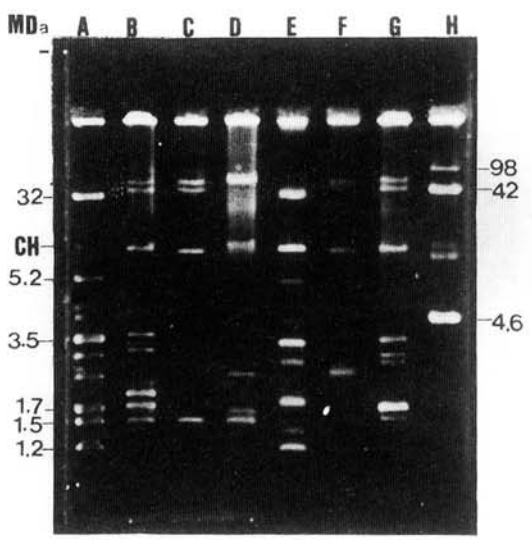

Fig. 1 - Plasmidial profiles from different strains of *Salmonella* Typhimurium lysotype 193. Lane A: V517; Lanes B - G: (strains lysotypes 193); Lane H: R861.

These results indicate the value of determining the plasmidial profile in combination with lysotype characterization in the Typhimurium serovar, in full agreement with data reported (BRUNNER et al., 1983; HOLMBERG et al., 1984; PLATT & BROWN, 1986; FANTASIA et al., 1990; THRELLFALL et al., 1990; RIVERA et al., 1991; SORENSEN et al., 1991). This aspect acquires even greater importance, especially in regions where there is a marked predominance of a single lysotype with the same resistance profile, as is the case for Rio de Janeiro for lysotype 193 and Salvador for lysotype 105.

RESUMO

Lisotipos e perfil plasmidial de *Salmonella* Serovar Typhimurium isoladas de processos entéricos infantis nas Cidades do Rio de Janeiro, RJ e Salvador, BA - Brasil.

Os lisotipos e os perfis de resistência a antimicrobianos e plasmidial foram caracterizados em 111 amostras de *Salmonella* Typhimurium isoladas de fezes e sangue de crianças atendidas nas cidades do Rio de Janeiro e Salvador. A lisotipia evidenciou seis tipos fágicos distintos (19, 41, 97, 105, 120 e 193) com uma predominância do lisotipo 193 (59,7%) na primeira cidade e do fagotipo 105 (38,4) na segunda. Cerca de 86,7% das amostras do lisotipo 193 apresentaram resistência múltipla para mais de seis antimicrobianos, enquanto que 93,3% de 105 foram totalmente sensíveis.

Em mais de 90% das amostras, verificou-se a presença de plasmídios, distribuídos em 39 perfis diferentes nas do Rio de Janeiro e 10 nas de Salvador. Predominou o plasmídio de 40 MDa nas amostras do Rio de Janeiro (47%), enquanto o de 61 MDa (14,4%) prevaleceu em Salvador.

A associação da análise do perfil plasmidial com a fagotipagem, principalmente naqueles pertencentes aos lisotipos 105 e 193, comprovou ser mais discriminatória que os outros métodos aplicados.

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