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Antimicrobial resistance and R-plasmid in *Salmonella* spp from swine and abattoir environments[1]

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ABSTRACT.- Lázaro N.S., Tibana A., Rodrigues D.P., Reis E.M.F., Quintaes B.R. & Hofer E. 2004. **Antimicrobial resistance and R-plasmid in *Salmonella* spp from swine and abattoir environments**. *Pesquisa Veterinária Brasileira 24(2):57-60*. Depto Epidemiologia e Saúde Pública, Instituto de Veterinária, UFRRJ, Seropédica, RJ 23890-000, Brazil. E-mail: nslazaro@aol.com

Salmonella serovars isolated from swine are of particular interest not only because of the pathogenic potential for this animal species, but also due to its relevance with regard to public health. On basis of the profile of resistance to antimicrobials, 13 *Salmonella* strains were selected which belonged to the serovars Muenster (7), Derby (4), Typhimurium (1), and Braenderup (1). They were isolated from healthy swine as well as from the abattoir environment in the state of Rio de Janeiro. All strains of *Salmonella* were subjected to bacterial conjugation, and the *E. coli* K12 Nal^r Lac^+ F standard strain was used as receptor, with the purpose to verify the ability to transfer the resistance marks. Gene transfer phenomenon was detected in seven strains, and except *Salmonella* Typhimurium which transconjugated to Sm, Tc and Su, the remaining ones were characterized by transferring mark Su only. By plasmidial analysis of strains used and their respective transconjugants, 63 Kb plasmid was found, which was probably related to *S.* Typhimurium resistance.

INDEX TERMS: *Salmonella*, swine, antimicrobial resistance, R-plasmid.

RESUMO.- [**Resistência a antimicrobianos e plasmidios R em *Salmonella* spp isoladas de suínos e do ambiente de abatedouro**.] Sorovares de *Salmonella* isolados de suinos são de particular interesse não só pelo potencial patogênico para esta espécie animal, como também pela sua relevância em Saúde Pùblica. Com base no perfil de resistência aos antimicrobianos foram selecionadas 13 amostras de *Salmonella* pertencentes aos sorovares Muenster (7 amostras), Derby (4), Typhimurium (1) e Braenderup (1), isoladas de suinos sadios e do ambiente de abatedouro no Estado do Rio de Janeiro. As amostras foram submetidas a conjugação bacteriana, utilizando como receptora *E.coli* K12 55 Nal^r Lac^+ F^-, com a finalidade de verificar a capacidade da transferência de marcos de resistência. O fenômeno de transferência gênica foi detectado em 7 amostras e, com exceção de *Salmonella* Typhimurium que transconjugou para Sm, Tc e Su, as demais se caracterizaram por transferir somente o marco Su. Na análise plasmidial das amostras doadoras e suas respectivas transconjugantes foi revelado um plasmídio de 63 Kb, provavelmente relacionado com a multirresistência de *S.* Typhimurium.

TERMOS DE INDEXAÇÃO: *Salmonella,* suínos, resistência antimicrobiana, plasmidios R.

INTRODUCTION

Salmonella serovars other than those related to disease are being identified in clinically healthy swine by the time of slaughter (Costa et al. 1972, Zebral et al. 1974, Lázaro et al. 1997). This fact has implications on Public Health, in so far as a considerable number of such serovars are also isolated from outbreaks of human salmonellosis (Hofer & Reis 1994, Lirio et al. 1998).

Its significance does not only lie on the attributes of virulence, but also on the capability of resistance to antimicrobials shown by some strains, as well as of the transfer of this feature through plasmids (Ishiguro et al. 1980, Simmons et al. 1988, Heffernan 1991).

The quick and widespread drug-resistance mediated by plasmidial genes in *Salmonella* isolates has been reported

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worldwide. During the past few decades, various countries have witnessed a significant increase in human isolates of multiresistant salmonellae (Holmberg et al. 1984, Carvalho & Hofer 1989, Rivera et al. 1991, Asensi & Hofer 1994, Ling et al.1998), as well as animal isolates (Hampton et al. 1995, Millemann et al. 1995, Alaniz et al. 1997, Izumiya et al. 2001).

Of particular interest is the fact that most plasmids acquire their resistance genes through transposons, whether from an other plasmid in the strain, from the chromosome or plasmids carried by other bacterial strains which are present in the host (Threlfall & Frost 1990). In the light of this, research was carried out which concentrated essentially on R factors of *Salmonella*, by means of conjugation tests (Timoney 1978, Vinhas & Almeida 1984, Simmons et al. 1988, Sant'Ana et al. 1995).

In view of the complexity of the factors associated with the dissemination of resistant *Salmonella* strains, this paper has the purpose of assessing the capability to transfer marks of resistance to antimicrobials by means of bacterial conjugation as well as by analysis of the plasmidial profile of *Salmonella* isolated from clinically healthy swine, and also originating from the environment where these animals were slaughtered, in the state of Rio de Janeiro.

MATERIALS AND METHODS

For the conjugation experiments 7 samples were selected belonging to serovar Muenster; 4 samples of serovar Derby, one belonging to serovar Typhimurium , and one to serovar Braenderup. These were isolated from swine and the abattoir environment; they were resistant and/or multiresistant to sulphonamide (Su), streptomycin (Sm) and tetracycline (Tc) (Table 1).

Conjugation experiments. For the determination of R factors in the samples under study, the methodology used was the one described by Dias & Hofer (1985). Conjugation was performed using as recipient *Escherichia coli* K12 55, F^- Lac^+ Nal^r, susceptible to all drugs except nalidix acid. Transconjugants were seleted on MacConkey agar (Oxoid) containing the antimicrobials to which the standard strain was originally sensitive. The resistance patterns of transconjugant strains was confirmed through the disc diffusion method according to NCCLS (1998), and considering the original profile of the corresponding donor colony.

Plasmidial analysis. The analysis of the plasmidial contents regarding the original strains and their respective transconjugants was performed by the alkaline lysis method of Birnboim & Doly (1979), modified by Sambrok et al. (1989). The plasmidial DNAs of *E. coli* V 517 and 29R861 were included as molecular weight controls.

Table 1. Profile of resistance of strains utilized as donors on conjugation tests

Strains nº	Sourcea	Serovar	Resistance profileb
1	MES1	Braenderup	Sm, Tc, Su
2	MES4	Muenster	Su
6	MES27	Muenster	Sm
8	Ton61	Muenster	Su, Sm
22	ING65	Muenster	Sm
24b	ING66	Muenster	Sm
36	ING72	Muenster	Sm
71	Ton98	Derby	Sm, Tc, Su
72	Ton99	Derby	Sm, Tc, Su
73c	Ton99	Muenster	Sm
76	ET8	Typhimurium	Sm, Tc, Su
81b	ST9	Derby	Sm, Tc, Su
90	ST9	Derby	Tc, Su

[a]Specimen corresponding to animal nº Ex.: MES4 = swine 4 mesenteric lymph node; MES= mesenteric lymph node; ING= inguinal lymph node; Ton= tonsil; TE= scalding tank; ET= evisceration table. For the environmental samples the number follows the origin corresponding to the order of collection (visit) , e.g. ET8= 8th visit.

[b]Sm= streptomycin; Su= sulfonamide; Tc= tetracycline.

RESULTS

Table 2 shows the conjugation positivity in seven (53.84%) out of the 13 donor strains, which evidences the total transference of model Su, Tc, Sm on *S.* Typhimurium and partial transfer of mark Su in regard to serovars Derby and Muenster.

Table 2. Degree of resistance transfer marks to *E. coli* LR1 (K12 55) by *Salmonella* spp strains

Serovar nº	Strains	Resistance profile[a]	Transconjugants (seletive drugs)	Transfer degree	Transferred Marks
Muenster	2	Su	T2 Su x LR1	10^{-1}	Su^R
Muenster	8	Sm, Su	T8 Su x LR1	10^{-1}	Su^R
Derby	71	Sm, Tc, Su	T71 Su x LR1	10^{-1}	Su^R
Derby	72	Sm, Tc, Su	T72 Su x LR1	10^{-1}	Su^R
Typhimurium	76	Sm, Tc, Su	T76 Sm x LR1	10^{0}	Sm^R, Tc^R, Su^R
Typhimurium	76	Sm, Tc, Su	T76 Tc x LR1	10^{-2}	Sm^R, Tc^R, Su^R
Typhimurium	76	Sm, Tc, Su	T76 Su x LR1	10^{-2}	Sm^R, Tc^R, Su^R
Derby	81b	Sm, Tc, Su	T81b Su x LR1	10^{-1}	Su^R
Derby	90	Tc, Su	T90 Su x LR1	10^{-1}	Su^R

[a] Su= Sulfonamide, Tc= Tetracyclin, Sm= Streptomycin.

Regarding the transfer degree determined in salmonellae by view of the growth of transconjugant samples in dilutions 10^o, 10^{-1}, and 10^{-2}, it was found that from the seven transconjugants, the isolation of transconjugating up to dilution 10^{-2} was obtained only with *S.* Typhimurium; the others were characterized by reaching up to dilution 10^{-1}.

The antimicrobial susceptibility tests confirmed the transfer of resistance marks in all of the transconjugant strains.

In Table 3 are listed plasmids transferred by the conjugation process between *Salmonella* (donor) and (receptor) *E. coli* K12 55 (LR1). Despite the donor serovars Muenster and Derby, which showed resistance transfer to mark Su for *E. coli*, the analysis of plasmidial DNA in transconjugant samples did not reveal plasmids which were evidenced in the donor samples.

Regarding *S.* Typhimurium (strain no. 76), marks Tc, Sm, and Su were transferred, and the analysis of transconjugants (Fig. 1) revealed the presence of plasmids showing approximate sizes (Kb) of 63-3.75 and 3.45 Kb for transconjugant Sm (T76Sm x LR1), and only the 63 Kb plasmid on transconjugants Tc and Su (T76&c x LR1, and T76Su x LR1). It is noteworthy that the antimicrobial susceptibility test in order to confirm transfer of R factors revealed simultaneous resistance to marks Sm, Tc, and Su in the three transconjugants resulting from *S. Typhimurium*.

Table 3. Antibiotic-resistance and plasmids R transferred via conjugation between *Salmonella* (donor) and *E. coli*, K12 55 Nal^r Lac$^+$ F (receptor)

Donor				Receptor		
Strains	Serovar	Resistance marks	Plasmíds (Kb)	Transconjugants	Transferred marks[a]	Transferred plasmids (Kb)
2	Muenster	Su	7,95	T2Su x LR1	Su	-
8	Muenster	Sm, Su	7,95 - 5,55 - 3,15 2,85	T8Su x LR1	Su	-
71	Derby	Sm, Tc, Su	5,4 - 10,8	T71Su x LR1	Su	-
72	Derby	Sm, Tc, Su	5,4 -10,8	T72Sux LR1	Su	-
76	Typhimurium	Sm, Tc, Su	63 - 3,75- 3,45	T76Sm x LR1	Sm, Tc, Su	63 - 3,75 - 3,45
				T76Tc x LR1	Sm, Tc, Su	63
				T76Su x LR1	Sm, Tc, Su	63
81b	Derby	Sm, Tc ,Su	Não determinado	T81bSu x LR1	Su	-
90	Derby	Tc, Su	Não determinado	T90Su x LR1	Su	-

[a]Su= Sulfonamide, Tc= Tetracycline, Sm= Streptomycin.

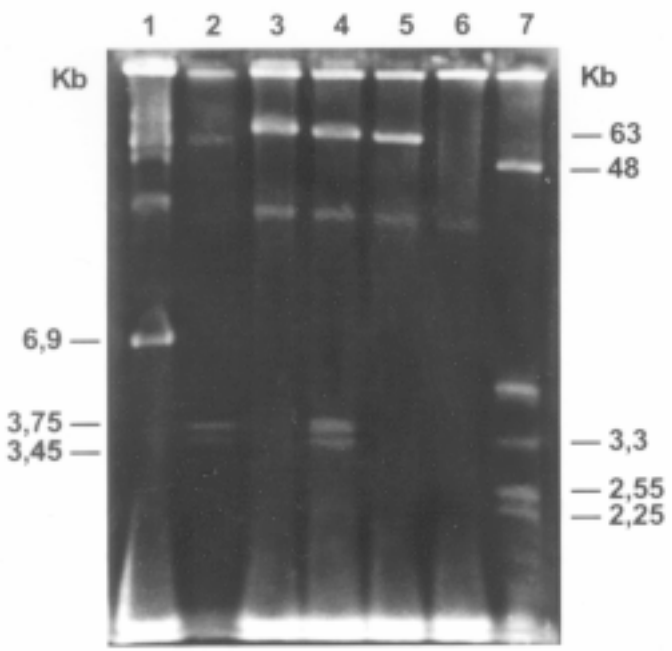

Fig. 1. Plasmid profile of resistant *Salmonella* Typhimurium and transconjugant lines: 1- *E. coli* 39R861; 2- *S*. Typhimurium; 3,4 and 5- transconjugants Su, Sm and Tc; 6- *E coli*. K 1255; 7- *E. coli* V517.

DISCUSSION

The information contained in the literature points out the transfer of genes among bacteria by means of conjugation, in varied environments as well as in the intestinal tracts of humans and animals (Smith 1969).

Although laboratorial experiments do not exactly reproduce – even under simulated conditions – the complexity existing in transfer processes occurring *in vivo*, they may constitute an important tool for making such inferences.

The negative result in the conjugation of the six *Salmonella* Muenster strains, as well as the absence of transconjugants for marks Sm in S. Muenster (no. 8), and Sm, Tc in *S*. Derby, may be related to one of the following features: the genic-chromosomic feature of these determinants; the non-existence of the transfer factor; the interference of nalidixic acid with selective plates for transconjugants; the inability of plasmid reception of the standard strain used (Barbour 1967); or even plasmid loss during handling of the samples.

The hypothesis is also admitted that these strains carry thermo-sensitive plasmids R encoding a resistance to Tc and Sm, which are effectively transferred at 25° C. Factors related to resistance to tetracycline seem to be effectively transferred outside the body, thereby decreasing transfer at 37°C (Timoney 1978). This may account for the difficulty of *in vivo* transfer on the part of plasmids bearing resistance of animal origin into the flora residing in man's intestine (Smith 1969). A wide distribution of thermo-sensitive plasmid in salmonellae isolated from swine was noted by Ishiguro et al. (1980) in Japan.

Analyzing Table 3 it was found that only *S.* Typhimurium was capable of transferring R- plasmid to *E. coli* . The close association between marks Su, Tc, Sm, along with the finding of simultaneous transfer to *S. typhimurium* transconjugants, suggests that such genic expression may be determined by the same plasmid (63 Kb), although there have been detected two additional plasmids (3.75 and 3.45 Kb) on transconjugant Sm of *S.* Typhimurium (T76Sm x LR1). This feature is found in the literature in so far a non-conjugating plasmid may be transferred to a receptor cell by cooperative action of a conjugative plasmid when they are present in the same cell. With regard to plasmids with relatively high molecular weights and encoding resistance to antimicrobials, in *Salmonella* Typhimurium these appears to exist a 40 Kb plasmid associated to resistance to amoxicilin, streptomycin, tetracycline, chloranphenicol, and sulfametoxazol-trimetroprin (Hansen et al. 1964), and another 80 Kb plasmid encoding resistance to marks Ap, Sm, Su, Tc (Hampton et al. 1995).

As to the absence of plasmids in transconjugants of *S.* Muenster and *S.* Derby, the hypothesis can scarcely be admitted that the determinant of resistance to mark Su is encoded by a large plasmid, which has not been demonstrated through the methodology employed. For this purpose, various methods and

procedures were set forth, the outstanding being the one developed by Kado & Liu (1981), which is the most convenient for extraction in view of plasmids with high molecular weight. Another explanation for this phenomenon could be the outcome of integration of the resistance-plasmid in the receptor's chromosome (Madigan et al. 1997); or, still, because of the poor stability during the storage period between tests.

The ease of *in vitro* transfer has led to the conclusion that similar pattern of resistance to antibiotics in different intestinal bacteria are mediated by the same resistance plasmid (R factor) and has the same origin (Cherubin 1981). In contrast, Avril et al. (1977) have shown that the same resistance pattern is expressed by different episomes, and in the same way in different *Salmonella* serovars. Whenever a *Salmonella* bearer is being treated with antibiotics and the microorganism develops a multi-resistance, similar resistance pattern may be found in the patient's intestinal flora (Aserkoff & Bennett 1969).

The genic transfer phenomenon observed in the samples which are the object of our study emphasizes the relevance of those factors in propagating resistant bacteria in different ecological niches, besides the progressive limitations in the therapeutics using antimicrobials mostly when the level of resistance to isolates is unknown.

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