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COLICINOGENY IN *SALMONELLA* SEROVARS ISOLATED IN BRAZIL

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A study of colicinogeny was made in 748 strains of Salmonella *(97 serovars) isolated from different sources: human (291), animal (119), environmental (141), food (102) and animal feed (95). Colicin production was detected in 64 strains (8.6%), particularly isolated from foods (30.4%). Col* E_1 *(53) and* I_a *(44) were the most frequently observed, especially in* S. agona *for environment and food sources. Col V production was identified in 5 strains of* S. typhimurium *within 8 producer cultures isolated from humans. Its relationship with the sources and serovars of* Salmonella *are discussed.*

Key words: *Salmonella* – colicins – serovars – human – animal – environment – food – animal feed

Salmonellosis continues to be a worldwide public health problem (Houston, 1984).

In the present epidemiological situation characterized by a constant diffusion of salmonellae in nature, it is of great importance not only to identify the circulating serovars, but also to compare these numerous strains.

A number of methods have been used to differentiate *Salmonella* isolates in epidemiological studies. Such techniques include biotyping (Duguid et al., 1975), phage typing (Tyc, 1980), antimicrobial susceptibility testing (Solari et al., 1984), plasmid profile analysis (Holmberg et al., 1984), and colicin typing (Barker & Old, 1979).

The purpose of the present investigation was to determine the incidence of colicinogeny among salmonellae isolated from different sources, and also to provide information on association of colicin patterns with the different sources and serovars of *Salmonella.*

MATERIALS AND METHODS

Media – Cultures were maintained at room temperature on Buffered Meat Extract Agar. This medium contained: Bacto Beef Extract (Difco), 0.3g%; Bacto Peptone (Difco), 10g%; Sodium Chloride (Merck), 0.3g%; Disodium Phosphate 12 H_2O (Baker), 0.2g%; Bacto Agar (Difco), 10g%; pH 7.3. Cultures were grown at 37°C for 18-24h on Nutrient Broth (Difco). Nutrient Agar (Difco) was used for colicinogeny assay.

Bacterial samples – The strains investigated included 748 *Salmonella* isolates (97 serovars) of different sources: human (291), animal (119), environmental (141), food (102) and animal feed (95). These cultures were isolated in the period of 1978-1983 from nine States in Brazil (Rio Grande do Norte, Pernambuco, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul and Goiás), and received for serotyping by the Department of Bacteriology, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro.

Colicin – Cultures were examined for colicin production by the overlay method (Ozeki et al., 1962) with indicator strain 22R80 of *Escherichia coli.* The type of colicin was determined by the use of a set of *E. coli* standard strains with resistances to specific colicins, listed in Table I. This collection of strains was kindly provided by B. Rowe, Central Public Health Laboratory, Colindale, London.

RESULTS

Out of the 748 *Salmonella* strains, 64 (8.6%) were colicinogenic (col^+), particularly those isolated from foods (30.4%), as shown in Table II.

Table III shows the 12 colicinogenic serovars identified; a higher number of colicin-producing cultures were noted among *S. agona* strains (24) than within other serovars. Also, all strains of *S. schleissheim* and *S. sachsenwald* tested were colicinogenic. The majority of col^+ serovars were of the group 04 (B).

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TABLE I

Standard colicin-sensitive and resistant strains of *Escherichia coli*

Indicator strain	Designation
22R80	*E. coli* K12 Row (colicin-sensitive strain)
20R675	*E. coli* K12 Col E_1
20R676	*E. coli* K12 Col E_2
22R81	*E. coli* K12 Col I, V^r
22R82	*E. coli* K12 Col I_a
22R83	*E. coli* K12 Col I_b
20R914	*E. coli* K12 Row K^r
20R915	*E. coli* K12 B^r, V^r
22R966	*E. coli* K12 Row B^r

TABLE II

Colicin production by the *Salmonella* isolates of different sources

Source	No. of strains tested	No. colicin positive (%)
Human	291	8 (2.7)
Animal	119	8 (6.7)
Environment	141	14 (9.9)
Food	102	31 (30.4)
Animal feed	95	3 (3.2)
Total	748	64 (8.6)

The results and their correlation of colicin typing with the sources and serovars of *Salmonella* are respectively illustrated in Tables IV and V. Thus, colicins E_1 (53) and I_a (44) were the most frequently observed, especially in samples from food and environment sources, and corresponded to the *S. agona* serovar. Col V production was identified in 5 strains of *S. typhimurium* within 8 cultures isolated from humans.

DISCUSSION

The percentage of colicinogenic strains (8.6%) in the 748 cultures examined (Table II) compared with those found in the literature, demonstrating that the frequency of colicin production in *Salmonella* range from 3.2% to 16.2% (Hamon & Péron, 1966; Barker, 1980; Câmara et al., 1982; Barker et al., 1982).

Of the 64 colicin-producing cultures, we identified a rather higher incidence of colicinogeny among *S. agona* strains (24), whereas only five strains of *S. typhimurium* were col^+ (Table III). These results are in marked contrast with a previous study, which reports colicinogenicity is relatively common among *S. typhimurium* strains and rarer among other serovars (Hamon & Péron, 1966).

It is important to point out that of the 12 colicinogenic serovars, 6 were of the group 04 (B), accounted for 52 of 64 colicin-producing strains (Table III). Considering that the col^+ character may play a complex role in the process of selection, survival and adaptation of bacterial cells to the environmental conditions (Israil, 1983), these findings could be helpful to explain the prevalence of some serovars in determinated niches or geographical regions (Hofer, 1974; Calzada et al., 1984).

TABLE III

Colicinogeny in *Salmonella* serovars

Serovar	Group	No. of strains tested	No. colicin positive (%)
S. agona	04 (B)	42	24 (57.1)
S. schleissheim	04 (B)	9	9 (100.0)
S. derby	04 (B)	155	7 (4.5)
S. saintpaul	04 (B)	39	6 (15.4)
S. infantis	07 (C_1)	23	6 (26.1)
S. typhimurium	04 (B)	114	5 (4.4)
S. anatum	03, 10 (E_1)	14	2 (14.3)
S. newport	08 (C_2, C_3)	8	1 (12.5)
S. oranienburg	07 (C_1)	17	1 (5.9)
S. dublin	09 (D_1)	101	1 (1.0)
S. wien	04 (B)	2	1 (50.0)
S. sachsenwald	040 (R)	1	1 (100.0)

TABLE IV

Colicins distribution among the *Salmonella* isolates of different sources

Source	No. of colicinogenic strains	Colicin type					
		I_a	I_b	E_1	E_2	K	V
Human	8	1	–	2	–	1	8
Animal	8	7	–	6	3	–	2
Environment	14	13	–	12	1	–	2
Food	31	22	1	31	1	–	–
Animal feed	3	1	–	2	–	–	1
Total	64	44	1	53	5	1	13

TABLE V

Colicin typing among the *Salmonella* serovars

Serovar	Colicin type					
	I_a	I_b	E_1	E_2	K	V
S. agona	24	1	24	4	–	–
S. schleissheim	–	–	9	–	–	–
S. derby	7	–	7	–	–	–
S. saintpaul	3	–	4	–	–	4
S. infantis	4	–	3	–	–	3
S. typhimurium	–	–	–	–	1	5
S. anatum	2	–	2	–	–	–
S. newport	1	–	1	1	–	–
S. oranienburg	1	–	1	–	–	–
S. dublin	–	–	–	–	–	1
S. wien	1	–	1	–	–	–
S. sachsenwald	1	–	1	–	–	–
Total	44	1	53	5	1	13

As to colicin typing, our data revealed a quite larger production of col E_1 (53) and I_a (44), especially in samples from food and environment sources (Table IV), and belonging to the *S. agona* serovar (Table V).

The incidence of colicin types among the *Salmonella* strains was different from other studies (Barker & Old, 1979; Barker, 1980; Barker et al., 1982). However, the national survey conducted by Solari et al. (1984) has also identified a predominant proportion of *S. agona* from food and environment sources producing col I_a.

Eight strains isolated from humans produced col V (Table IV), five of them belonging to the *S. typhimurium* serovar (Table V). This is a point of concern in view of the relationship between col V production and pathogenicity of bacteria (Smith, 1974; Israil, 1983; Tewari et al., 1986). Furthermore, *S. typhimurium* represents the most common serovar isolated in our country (Hofer, 1974; Calzada et al., 1984), frequently associated with nosocomial infections, in which the majority of the samples are multiple antibiotic-resistant (Pessôa et al., 1978; Esper et al., 1980; Riley et al., 1984).

Finally, the present study gives some information on the occurrence and distribution of colicins in common isolates of *Salmonella*. It could be interesting, therefore, to monitor in these serovars as in others the establishment of new col factors, as well as in combined use of different typing methods, in attempt to tracing the sources of infection and the routes of transmission of *Salmonella* strains.

RESUMO

Colicinogenia em sorovares de *Salmonella* isolados no Brasil – Investigou-se a produção de colicina em 748 amostras de *Salmonella* (97 sorovares) advindas de diferentes fontes: humana (291), animal (119), ambiental (141), de alimentos (102) e rações (95). Detectaram-se 64 amostras (8,6%) colicinogênicas, particularmente isoladas de alimentos (30,4%). Col E_1 (53) e I_a (44) foram as mais freqüentes, especialmente no sorovar *S. agona*, de origem ambiental e de alimentos. Identificou-se também a produção de col V em 5 amostras de *S. typhimurium* dentre 8 culturas produtoras de origem humana. Discute-se a relação entre a capacidade colicinogênica e as fontes e sorovares de *Salmonella*.

Palavras-chave: *Salmonella* – colicinas – sorovares – origem humana – animal – ambiente – alimento – ração

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