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PHAGE TYPING AND MULTIDRUG RESISTANCE PROFILE IN *S.* TYPHIMURIUM ISOLATED FROM DIFFERENT SOURCES IN BRAZIL FROM 1999 TO 2004

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ABSTRACT

Salmonella Typhimurium has become a widespread cause of salmonellosis among humans and animals worldwide. In Brazil, *Salmonella* Typhimurium (STM) is one of the most prevalent serovars isolated from food for human consumption. The uncontrolled sale and use of antimicrobials in agriculture and for treating human patients contributes to increase multidrug resistance of this serovar. In the present study, a total of 278 STM isolates from different sources and regions of Brazil over the period 1999 to 2004 were phage typed and analyzed for their antimicrobial resistance profile at Laboratory of Enterobacteria, Oswaldo Cruz Institute, FIOCRUZ. The main STM phage types isolated were DT 193 (64.3%), DT 19 (17.4%) and DT 18 (4%). Others phage types as DT 10 (2%), DT 27 (3.24%), DT 13 (0.36%), DT 22 (0.36%), DT 28 (0.36%), DT 29 (0.36%) and DT 149 (0.36%) were obtained in low percentages. A total of 54% STM strains were resistant to three or more antimicrobial classes, while no resistance to third generation cephalosporin or ciprofloxacin was identified in these strains. Those results show the STM phage types circulating among animals, food for human consumption and humans in Brazil as well as the increasing of multidrug resistance. The surveillance of STM strains based on phage typing and antimicrobial resistance profile are useful for detecting outbreaks, identifying sources of infection and implementing prevention and control measures.

Key-words: *Salmonella* Typhimurium, Phage Types, Multidrug resistance

INTRODUCTION

Gastroenteritis associated with *Salmonella* enterica is an important foodborne disease throughout the world. More than 2500 *Salmonella* serovars are recognized and most of them are capable of infecting a variety of animal species, including humans (6).

In Brazil, *Salmonella* Typhimurium (STM) is the most prevalent serotype isolated from animals (especially swine) and food for human consumption. An important characteristic of this serovar is the emergence of multidrug resistant phenotypes. The rapid spread of multidrug resistance is a result of many factors such as the uncontrolled sale and extensive use of antimicrobials in agriculture and for treating patients. This phenomenon may be complex especially when bad hygiene is applied at rendering animal to human consumption and also when there is mishandling of food at home (7).

Phage typing has enabled differentiation of *Salmonella* Typhimurium into more than 200 definitive phage types (DTs). Some of these phage types appear with high prevalence in certain geographical areas. Phage type DT 104, one of the most common multidrug resistant, is pandemically distributed while phage type 193 is becoming an important phage type for Public Health and often associated with swine products (5).

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The purpose of this retrospective study is to present the distribution of STM phage types in different sources in Brazil and the correspondent multidrug resistance profiles.

MATERIAL AND METHODS

A total of 278 *Salmonella* Typhimurium (STM) strains isolated between 1999 and 2004 at the Laboratory of Enterobacteria, Oswaldo Cruz Institute, FIOCRUZ were phage typed using standard scheme (1) with 31 STM phage types, plus the phages 1, 2, 3, 10 and 18. Phage suspensions and the interpretative guide were kindly provided by the International Reference Laboratory for Enteric Phage Typing, Health Protection Agency (HPA), Colindale, London, UK.

The STM strains have been isolated from animals (145), food for human consumption (77), food stuff (22), humans (10), environment (03), quality control (02) and other sources (19). They were analyzed for antimicrobial resistance to ampicillin 10 µg (AMP), chloramphenicol 30 µg (CHL), tetracycline 30 µg (TCY), cefoxitin 30 µg (FOX), ceftriaxone 30 µg (CRO), cyprofloxacin 5 µg (CIP), gentamicin 10 µg (GEN), imipenem 10 µg (IMP), nalidixic acid 30 µg (NAL), trimethoprim/sulfamethoxazole 1.25 µg/23.75 µg (SXT), nitrofurantoin 300µg (NIT) and cephalotin 30 µg (CEP) using the disk diffusion method (CLSI, 2005). The interpretation of results were performed as described in the Clinical and Laboratory Standards Institute guidelines. Multidrug-resistance was defined as resistance to three or more classes of antimicrobials.

RESULTS

The STM strains were differentiated into 20 definitive phage types. DT 193 (64.3%) was the predominant phage type, followed by DT 19 (17.4%) and DT 18 (4%). Other phage types were obtained in lower percentages: DT 27 (3.24%), DT 10 (2%), DT 42 (1.08%), DT 89 (1.08%), DT 44 (0.72%), DT 168 (0.72%), DT 13 (0.36%), DT 22 (0.36%), DT 26 (0.36%), DT 28 (0.36%), DT 29 (0.36%), DT 45 (0.36%), DT 53 (0.36%), DT 55 (0.36%), DT 168A (0.36%), DT 149 (0.36%) and DT 184 (0.36%). Also, three phage types not considered definitive phages were isolated: U298 (0.72%), U291 (0.36%) and U294 (0.36%) (Table 1).

A total of 10 *Salmonella* Typhimurium isolates were isolated from humans during the period of the study. In 2002 the phage type DT193 was often isolated. In 2003, DT 19 (10%), DT 13 (10%) and DT 27 (10%) were also identified. Among them only DT 193 isolated in 2002 showed multidrug resistance for more than three antimicrobial classes (Table 2).

The distribution of STM strains according to different sources from 1999 to 2004 showed that *Salmonella* Typhimurium strains were most common in materials from animal origin (43.4%) and food for human consumption (32.7%)(Table 3). Among the 278 STM analyzed for antimicrobial resistance, DT 193 (58%), DT 19 (16%) and DT18 (4%) STM isolates showed characteristic to multiple antibiotic resistance in more than three antimicrobial classes but no resistance to third generation cephalosporin or cyprofloxacin (Tables 4 and 5). It was also observed that 14 different phage types non-DT 193 and 01 strain DT 19 were sensitive for the drugs tested (Table 6).

Table 1. *Salmonella* Typhimurium phage types isolated from different sources over the period of 1999 to 2004 at LABENT/IOC/FIOCRUZ, Brazil.

Phage Type	N (%)	Phage Type	N (%)
DT 193	178 (64.3)	DT 26	01 (0.36)
DT19	48 (17.4)	DT 28	01 (0.36)
DT 18	11 (4)	DT 29	01 (0.36)
DT27	09 (3.24)	DT 45	01 (0.36)
DT10	06 (2)	DT 53	01 (0.36)
DT 42	03 (1.08)	DT 55	01 (0.36)
DT 89	03 (1.08)	DT149	01 (0.36)
DT 44	02 (0.72)	DT 168A	01 (0.36)
DT168	02 (0.72)	DT 184	01 (0.36)
U 298	02 (0.72)	U 291	01 (0.36)
DT 13	01 (0.36)	U 294	01 (0.36)
DT 22	01 (0.36)		

N= Number of *Salmonella* Typhimurium strains, %=Percentage from the total *Salmonella* Typhimurium strains (278).

Table 2. *Salmonella* Typhimurium phage types isolated from humans over the period of 1999 to 2004 at LABENT/IOC/FIOCRUZ, Brazil, and their antimicrobial profile.

Year	Phage Type	N	Antimicrobial Profile
1999	DT 193	2	TCY
			TCY, SXT
2002	DT 193	3	CHL, TCY, SXT, NIT
			CHL, TCY, NAL, SXT, NIT
			AMP, CHL, TCY, CEP, NAL, SXT, NIT
2003	DT193	2	TCY
			AMP, TCY
	DT 13	1	TCY
	DT 19	1	-
	DT 27	1	-

AMP=Ampicilin, CEP=Cephalotin, CHL=Chloramphenicol, NAL=Nalidixic Acid, NIT=Nitrofurantoin; SXT=Trimethoprim/Sulfamethoxazole, TCY=Tetracycline.

Table 3. Distribution of *Salmonella* Typhimurium (STM) isolated according to their sources during the period of 1999 to 2004 at LABENT/IOC/FIOCRUZ, Brazil.

Year	Sources							Total
	AN	EN	FH	HU	FS	QC	US	
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
1999	14 (5)	03 (1)	21 (7.6)	02 (0.7)	12 (4.3)	-	01 (0.4)	**53 (19)**
2000	12 (4.3)	-	03 (1)	-	04 (1.4)	-	02 (0.7)	**21 (7.6)**
2001	09 (3.2)	01 (0,4)	01 (0.4)	-	02 (0.7)	-	-	**13 (4.7)**
2002	05 (1.7)	-	28 (10)	03 (1)	02 (0.7)	01 (0.4)	-	**39 (14)**
2003	110 (39.5)	-	16 (5.8)	05 (1.7)	01 (0.4)	-	-	**132 (47.5)**
2004	-	-	08 (2.8)	-	01 (0.4)	-	11 (3.9)	**20 (7.2)**
Total	**150 (54)**	**04 (1.4)**	**77 (27.6)**	**10 (3.6)**	**22 (8)**	**01 (0.4)**	**14 (5)**	**278 (100)**

N= Number of STM strains, %= Percentage of STM strains over the total (278), AN= Animal source, EN= Environmental source, FH= Food for human consumption source, HU= Human source, FS= Food stuff, QC= Quality Control source, US= unrelated source.

Table 4. Distribution of antimicrobial resistance profiles of *S.*Typhimurium DT 193 isolated from 1999 to 2004 at LABENT/IOC/FIOCRUZ.

Year	Source	N	Antimicrobial Resistance Profile
1999	FH	2	CHL, TCY, NIT CHL, TCY, SXT
	AN	4	AMP, CHL, TCY CHL, TCY, SXT TCY, NIT, GEN TCY, SXT, NIT
	FS	3	TCY, NIT, GEN CHL, TCY, NIT, GEN TCY, SXT, NIT, GEN
2000	FH	1	AMP, CHL, TCY, GEN
	AL	7	AMP, TCY, GEN AMP, CHL, TCY CHL, TCY, NAL AMP, CHL, TCY, SXT AMP, TCY, NAL, NIT CHL, TCY, SXT, NIT CHL, TCY, NAL, SXT, NIT
	US	2	TCY, NAL, NIT CHL, TCY, SXT
2001	EN	2	AMP, TCY, GEN CHL, TCY, SXT
	AN	6	TCY, NAL, NIT AMP, TCY, NAL, NIT CHL, TCY, SXT, NIT AMP, CHL, TCY, GEN, NIT AMP, TCY, NAL, SXT, NIT AMP, CHL, TCY, NAL, SXT, NIT
	FS	2	TCY, NAL, NIT AMP, CHL,TCY, SXT
2002	FH	8	TCY, NAL, NIT AMP, TCY, NIT CHL, TCY, NIT TCY, GEN, NAL TCY, NAL, SXT CHL, TCY, NAL, NIT CHL, TCY, SXT, NIT AMP, CHL, TCY, GEN, NIT
	AN	4	TCY, NAL, NIT AMP, TCY, NIT AMP, CHL, TCY, NIT AMP, CHL, TCY, CEP, NIT
	QC	1	CHL, TCY, NAL CHL, TCY, SXT, NIT
	HU	3	CHL, TCY, NAL, SXT, NIT AMP, CHL, TCY, CEP, NAL, SXT, NIT CHL, TCY, NAL, SXT, NIT
	FS	2	AMP, CHL, TCY, CEP, GEN, NAL, SXT, NIT
2003			CHL, TCY, NAL TCY, NAL, NIT CHL, TCY, GEN CHL, TCY, NIT TCY, GEN, NAL AMP, TCY, NIT CHL, TCY, SXT, NIT

2003	AL	13	AMP, CHL, TCY, GEN CHL, TCY, NAL, SXT AMP, CHL, TCY, NAL, NIT CHL, TCY, CEP, NAL, SXT CHL TCY, GEN , NAL, SXT CHL, TCY, GEN, NAL SXT, NIT
	AN	10	CHL, TCY, GEN CHL, TCY, NAL CHL, SXT, NIT AMP, TCY, NIT CHL, TCY, GEN, NAL AMP, CHL, TCY, NIT AMP, CHL, TCY, GEN TCY, GEN, NAL, SXT, NIT
			AMP, CHL, TCY, GEN, NIT AMP, CHL, TCY, NAL, SXT, NIT
	FS	1	AMP, CHL, TCY, NAL, SXT
	US	1	TCY, NAL, NIT
2004	US	5	TCY, SXT, NIT TCY, NAL, SXT TCY, NAL, SXT, NIT AMP, TCY, CEP, CRO, SXT AMP, CHL, TCY, CEP, CRO, NIT
	FH	1	CHL, TCY, NIT

N = antimicrobial profile number AMP = Ampicilin, CEP = Cephalotin, CHL = Chloramphenicol, CRO= Ceftriaxone,GEN = Gentamicin, NAL = Nalidixic Acid, NIT = Nitrofurantoin, SXT = Trimethoprim/ Sulfamethoxazole, TCY = Tetracycline.

Table 5. *Salmonella* Typhimurium non-DT 193 phage types isolated from 1999 to 2004 from different sources and classified according to their antimicrobial resistance profile.

Phage Types	Year/ Source	N	Antimicrobial Resistance Profile	**Total strains**
PT 10	2004/US	03	NAL, SXT AMP, CHL, TCY AMP, TCY, SXT, NIT	**03**
PT 13	2003/HU	01	TCY	**01**
PT 18	2000/AN 2001/AN 2002/FH 2004/US	01 01 05 02	AMP, TCY, NAL, NIT TCY, NAL, NIT CHL, TCY, NIT TCY, NAL, NIT TCY, NAL, SXT, NIT CHL, TCY, NAL, NIT TCY, NAL, SXT AMP, CHL, TCY, NIT	**09**
PT 19	2003/AN	38	TCY CHL, SXT TCY, NIT AMP, TCY TCY, NAL, NIT CHL, TCY, SXT AMP, TCY, NIT AMP, TCY, CEP, NIT AMP, CHL, TCY, GEN AMP, CHL, TCY, GEN, NIT CHL, TCY, NAL, SXT, NIT AMP, CHL, TCY, GEN, SXT AMP, CHL, TCY, NAL, SXT, NIT	**38**
PT 22	2003/US	01	AMP, CHL, TCY, NAL, SXT	**01**
PT 28	2002/FH	01	TCY, NAL, NIT	**01**
PT 29	1999/FH	01	TCY	**01**
PT 149	2003/US	01	AMP, TCY, SXT	**01**

N= Number of *Salmonella* Typhimurium isolated according to year and source, AN= Animal source, FH= Food for human consumption source, HU= Human source, US= Unrelated source; AMP=Ampicilin, CEP=Cephalotin, CHL=Chloramphenicol, GEN= Gentamicin, NAL= Nalidixic Acid, NIT=Nitrofurantoin; SXT=Trimethoprim/Sulfamethoxazole, TCY=Tetracycline.

Table 6. *Salmonella* Typhimurium non-DT193 phage types isolated from 1999 to 2004 and sensitive to all drugs tested.

Phage Types	N (%)
DT 19	1 (4.3)
U291	1 (4.3)
DT 26	1 (4.3)
DT 27	1 (4.3)
DT45	1 (4.3)
U294	1 (4.3)
DT 168A	1 (4.3)
DT 53	1 (4.3)
DT 184	1 (4.3)
DT 168	2 (8.7)
U 298	2 (8.7)
DT 44	2 (8.7)
DT 55	2 (8.7)
DT 42	3 (13)
DT 89	3 (13)
Total	**23 (100)**

N= Number of *Salmonella* Typhimurium strains, %=Percentage of *Salmonella* Typhimurium strains over the total (23).

DISCUSSION

In the present study the prevalence of *Salmonella* Typhimurium isolated from animals and food for human consumption associated with multidrug resistance in Brazil has determined the need to monitor the spread of this microorganism (8).

Phage typing distinguished serotype Typhimurium variants in 23 different phage types. Among them a high prevalence of resistant strains was especially detected in phage type DT 193, which can be corroborated with studies that demonstrate the emergence of this phage type associated with swine, food for human consumption and antimicrobial resistance (7).

In Brazil, *Salmonella* Typhimurium phage types isolated from humans appear to have a geographical distribution pattern. The main phage type isolated in areas such as Rio de Janeiro was DT 193, whereas DT 19, DT 41, DT 97, DT 105, DT 120 and DT 193 were isolated in Salvador city. In both cases, all strains were isolated from children hospitalized with enteric processes (2).

In the present investigation it was observed that DT 193 was the most prevalent phage type isolated from humans associated with multidrug resistance to more than three antimicrobials. Other phage types were also isolated, such as DT 13 resistant only to tetracycline, and DT 19 and DT 27 sensitive for all drugs tested.

Although our findings did not indicate the presence of DT 104, considered an emergent phage type, this retrospective study allows isolating *Salmonella* phage types DT 193 and non 193 associated with a high level of multidrug resistance. Moreover, the wide variety of phage types isolated from different sources, including human source, shows the rapid spread of *Salmonella* Typhimurium circulating in Brazil from 1999 to 2004 (4).

Taking into account the data presented in this paper, it is important that a prudent use of antimicrobials in veterinary field, agriculture and treatment patients should be encouraged, in order to contribute in minimizing the emergence of multidrug resistance. Furthermore, the surveillance of *Salmonella* Typhimurium strains based on phage typing is useful for detecting outbreaks, identifying sources of infection and implementing prevention and control measures.

RESUMO

Fagotipagem e Perfil de multirresistência antimicrobiana em *S.* Typhimurium isoladas de diferentes fontes no Brasil de 1999 a 2004

Salmonella Typhimurium é considerada uma das principais bactérias causadoras de salmonelose nos animais e no homem em todo o mundo. No Brasil, *Salmonella* Typhimurium é um dos mais prevalentes sorovares isolados de alimentos para consumo humano. O uso indiscriminado de antibióticos em produtos agrícolas e no tratamento de pacientes humanos tem contribuído para aumentar a multirresistência desse sorovar a diversos antimicrobianos. No presente estudo, 278 cepas de STM foram selecionadas de diferentes fontes e regiões do Brasil, no período de 1999 a 2004 e realizadas a fagotipagem e análise do perfil de resistência antimicrobiana no Laboratório de Enterobactérias, Instituto Oswaldo Cruz, FIOCRUZ. Os principais fagotipos isolados foram DT 193 (64,3%), DT 19 (17,4%) e DT 18 (4%). Os fagotipos DT 10 (2%), DT 27 (3,24%), DT 13 (0,36%), DT 22 (0,36%), DT 28 (0,36%), DT 29 (0,36%) e DT 149 (0,36%) foram isolados em menores percentuais. Um total de 54% das cepas de STM foi resistente a três ou mais classes de antimicrobianos e não foi observada resistência a cefalosporinas de terceira geração ou ciprofloxacina. Esses resultados indicam os principais lisotipos de *Salmonella* Typhimurium circulantes entre os animais, alimentos de consumo humano e seres humanos no Brasil, bem como o aumento da multirresistência antimicrobiana. O monitoramento de cepas de *Salmonella* Typhimurium baseado na fagotipagem e no padrão de resistência antimicrobiana são ferramentas úteis para detectar surtos, identificar a fonte de infecção e implementar programas de prevenção e controle de salmonelose.

Palavras-chave: *Salmonella* Typhimurium, Fagotipagem, Multirressistência

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