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Antibiotic Resistance Pattern of *Salmonella* Typhimurium obtained from animal sources in India

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Abstract

Antimicrobial resistance pattern of 104 isolates of *Salmonella* Typhimurium identified over last three decades revealed 83 antibiotic resistance patterns. Highest resistance was observed with doxycycline hydrochloride (36.53%), tetracycline (36.53%), neomycin (33.65%) and kanamycin (32.69%). On the other hand, *Salmonella* Typhimurium were found to be the most sensitive to gentamicin (92.31%), cefoperazone (86.53%), ceftazadime (83.65%), ciprofloxacin (83.65%) and streptomycin (85.57%). From 83 antibiotic resistance patterns one of the isolates showed resistance to as many as 12 drugs and similarly another isolate showed resistance to 10 drugs. There were 27 isolates which showed resistance to 5 to 9 antibiotics. Furthermore, resistance to 4 antibiotics was shown by 8 isolates. The results also indicated that there was marked increase in resistance to most of the antibiotics tested after the year 1980 with variations in between. However, some of the antibiotics such as cefoperazone, gentamicin, ciprofloxacin, streptomycin, and chloramphenicol did not show much change in resistance pattern over the years.

Key words: *Salmonella*, Typhimurium, Resistance

INTRODUCTION

Increased clinical use of antibiotics, easy accessibility to antibiotics in many countries of the world and international travel, as well as uncontrolled use of antibiotics in the environment and in meat-producing animals, has led to an increase of antibiotic resistance in many bacterial species. Emerging resistance in *Salmonella enterica* serotype Typhi has been described especially in Asia and Africa (Ling *et al*., 1996). *Salmonella* Typhimurium is a serovar of increasing economic significance worldwide (Sullivan *et al*., 1998). It is the second most common serovar isolated from England and Wales after serovar Enteritidis (Torre *et al*., 1993). Drug resistance is a major problem in controlling salmonellosis, and the *S*. Typhimurium shows high rate of resistance to multiple antibiotics (Weill *et al*., 2004; Isaac *et al*., 2007). *S.* Typhimurium is most important from food safety point of view and poultry is linked to be an important

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source of the organism in the food chain. No international standards exist for managing food safety problems related to antimicrobial resistance (Frank *et al*., 2005). In the Czech Republic, pentaresistant ACSSuT *S.* Typhimurium strains were for the first time isolated in humans in 1996 (Karpiskova *et al*., 1999). However, a retrospective study confirmed the occurrence of these multire-sistant epidemic strains *S.* Typhimurium in cattle in the Czech Republic as early as 1990 (Faldynova *et al.,* 2003).

Indiscriminate use of antibiotics to treat human, livestock and poultry has led to the emergence of multiple drug resistance to *Salmonella* and other enteric pathogens (Saikia *et al*., 2002). Therefore, it becomes pertinent to periodically study the drug resistance pattern, as this information will provide the incidence of resistant *Salmonella* Typhimurium strain against various drugs, which ultimately will help to devise control measures. The current study deals with antibiotic resistance pattern of *S.* Typhimurium identified over a 20 year period.

MATERIAL AND METHODS

From different regions of India at National *Salmonella* Centre (Vet), Indian Veterinary Research Institute, Izatnagar Bareilly, India, a total of 104 strains of *Salmonella enterica* serovar Typhimurium from various sources (poultry 39, pig 12, rabbit 18, goat 22, and monkey 13) over a 20 year period (1986-2005) were examined for drug resistance pattern using 16 antimicrobial agents by disc diffusion technique of Bauer *et al*. (1996). *Salmonella* strains were revived by inoculating in buffered peptone water and incubated at 37^0C for overnight in an incubator cum shaker. These cultures were streaked on hektoen enteric agar (HEA) and incubated at 37^0C for 24 h. Smooth and transparent black centered colonies with greenish surround were picked up and confirmed biochemically and by slide agglutination test. Confirmed single colony inoculated in BHI broth and incubated at 37^0C overnight. With the help of sterile cotton swab the broth culture was spread on the surface of nutrient agar plate and standard antimicrobial drugs were placed on the surface and incubated at 37^0C overnight. Antibiotic resistance pattern was classified as sensitive (S), intermediate (I) and resistance (R) on the basis of their zone of inhibition. Data of isolates were computerized and analyzed with Statistical Package for Social Science (SPSS) software (version 10.0) (SPSS Inc.) for determining relatedness among isolates.

Multiple antibiotic resistances (MAR) index for each resistance pattern was calculated by employing following formula:
Number of resistance antibiotics/ total Number of antibiotics tested

RESULT AND DISCUSSION

Use of antimicrobial drugs against salmonellosis is recommended only for cases with serious illness and multidrug resistant bacteria may have fatal consequences for the patients. As bacterial strains become increasingly resistant to standard antimicrobial therapy, measures to control and prevent this problem are essential (Matthew *et al*., 2007). We compared the antibiotic resistance pattern of all *Salmonella* Typhimurium strains isolated in last thirty years at National Salmonella Centre, IVRI,

Bareilly, in which highest resistance was observed with doxycycline hydrochloride (36.53%), tetracycline (36.53%), neomycin (33.65%) and kanamycin (32.69%). On the other hand, *Salmonella* Typhimurium were found to be the most sensitive to gentamicin (92.31%), cefoperazone (86.53%), ceftazadime (83.65%), ciprofloxacin (83.65%) and streptomycin (85.57%) (Table 1). Senthilkumar and Prabakaran, 2005 also reported that tetracycline was 83.33% resistance against *Salmonella* Typhimurium. Nogrady *et al*. (2005) reported most frequent resistance against tetracycline by different *Salmonella* Typhimurium strains.

Table-1.Resistance of *Salmonella* Typhimurium isolates to individual antimicrobial agents.

S.No.	Antibiotics drug	Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
1	Amoxycillin /clavulanic acid	31	29.80	-	-	73	70.20
2	Cefoperazone	4	3.84	10	9.61	90	**86.53**
3	Ceftazidime	17	16.34	-	-	87	83.65
4	Ceftriaxone	19	18.26	-	-	85	81.73
5	Doxycycline hydrochlorid	38	**36.53**	6	5.76	60	56.69
6	Tri-methoprime	23	22.11	-	-	81	77.88
7	Amikacin	20	19.23	7	6.73	77	74.03
8	Cephotaxime	17	16.34	5	4.80	82	78.84
9	Tetracycline	38	**36.53**	5	4.80	61	58.65
10	Gentamicin	8	7.69	-	-	96	**92.31**
11	Ciprofloxacin	17	16.34	-	-	87	83.65
12	Neomycin	35	33.65	5	4.80	64	61.55
13	Streptomycin	14	13.45	1	.96	89	85.57
14	Chloramphenicol	23	22.11	1	.96	80	76.92
15	Kanamycin	34	32.69	5	4.80	65	62.5
16	Furazolidone	27	25.96	-	-	77	74.03

Result was also analysed as per the year of isolation and for this purpose, the entire period of 30 years (from 1976-2005) was divided in 3 blocks *viz*., 1976-1985, 1986-1995 and 1996-2005. The results (Fig. 1) indicated that there was increase in resistance to 10 of the 16 antibiotics tested between the period 1976-1985, 1986-1995 and 1996-2005. Of these antibiotics chloramphenicol recorded highest decline in resistance *i.e.* from 30.00% during the period 1976-1985 to 17.39% during 2096-2005.

Table 2: Resistance pattern and multiple antibiotic resistance index of *Salmonella* Typhimurium isolated during the years 1976-1985.

S.No.	Resistance pattern	Source of Isolates					Total	Multiple antibiotic resistance index
		Poultry	Pig	Rabbit	Goat	Monkey		
1	T	-	-	1	-	-	1	0.0625
2	N,K	1	-	-	2	-	3	0.125
3	Ac,Tr	-	1	-	-	-	1	0.125
4	Do,Ak	-	1	1	-	-	2	0.125
5	Ca,C	-	-	-	-	1	1	0.125
6	Do,N	-	-	1	-	-	1	0.125
7	G,K	-	-	1	-	-	1	0.125
8	Ac,Do	6	-	-	2	-	9	0.125
9	Ci,Ce,N	-	-	1	-	1	2	0.1875
10	Ac,Ak,C	2	-	-	-	-	2	0.1875
11	Tr,Ca,C	1	-	-	-	-	1	0.1875
12	T,K,Fr	-	-	2	-	-	2	0.1875
13	Ca,Tr,Fr	-	-	-	-	1	1	0.1875
14	Ca,Tr,T	-	-	-	-	1	1	0.1875
15	Cr,Ak,N,C	-	-	1	-	-	1	0.250
16	Ca,Tr,N,C	-	-	-	-	1	1	0.250
17	Ca,Do,Ce,N,K,Fr,	-	2	-	-	-	2	0.375
18	Ac,Do,Ak,T,Cr,N,C,K,Fr	-	-	-	1	-	1	0.5625
19	Ac,Ca,Ci,Do,Tr,Ce,T,Cr,S,C,K,Fr	-	-	-	1	-	1	0.750
	Total	**10**	**4**	**8**	**6**	**5**	**33**	**0.2236**

Table 3: Resistance pattern and multiple antibiotic resistance index of *Salmonella* Typhimurium isolated during the years 1986-1995.

S.No.	Resistance pattern	Source of Isolates					Total	Multiple antibiotic resistance index
		Poultry	Pig	Rabbit	Goat	Monkey		
1	Cf	-	1	-	-	-	1	0.0625
2	S	-	-	1	-	-	1	0.0625
3	N,K	-	1	-	1	-	2	0.125
4	Ci,Ce	1	-	-	-	-	1	0.125
5	S,Fr	3	-	-	-	1	4	0.125
6	Cf,Fr	1	--	1	-	-	2	0.125
7	Ca,Ci,Ak	2	-	-	-	-	2	0.1875
8	T,Cf,N	-	-	-	-	1	1	0.1875
9	Do,Ce,N	1	-	-	-	-	1	0.1875
10	Do,S,Fr	1	-	-	-	-	1	0.1875
11	Ca,Ci,Cf, C	-	-	1	-	1	2	0.250
12	Cf,N,K,Fr	-	-	-	1	-	1	0.250
13	Ac,Ci,C,T	-	-	-	1	-	1	0.250
14	Cf,N,C,Fr	-	-	-	1	-	1	0.250
15	Ci,Do,Ak,N,K	1	-	-	-	-	1	0.3125
16	Ac,Do,N,S,K,Fr	-	-	1	-	1	2	0.375
17	Ac,DoTr,Ak,N,K	-	1	-	-	-	1	0.375
18	Ac,Do,Tr,T,S,K	1	-	-	-	-	1	0.375
19	Ac,Do,Ca,T,S,C	1	-	-	-	-	1	0.375
20	Ac,Do,Tr,Ak,T,N,C,Fr	-	-	--	1	-	1	0.500
21	Ac,Ca,Ci,Do,Tr,Ak,Ti.Cf,C,K	-	-		1	-	1	0.625
	Total	**12**	**3**	**4**	**6**	**4**	**29**	**0.2529**

Among the 104 strains of *S.* Typhimurium 83 resistance pattern were observed (Table 2, 3 and 4). Fourteen of the isolates were not resistant to any of the drugs tested. One each of the isolates showed resistant to as many as 12 and 10 drugs. There were 27 isolates which showed resistance to 5 to 9 antibiotics. Rest of the isolates were resistant to 1- 4 drugs. Considering the number of strain studied for their sensitivity/ resistance pattern, the proportion of resistance pattern appeared too high. Such observation were also recorded by Sojka *et al*. (1986), who noted that number of drug resistance patterns increased progressively from 35 to 62 between 1975 to

1978. Arvanitidou *et al*., (1998) found 18 different resistance profile in 62 *Salmonella* strains in which atleast one antibiotic was observed in 36 isolates. Kumar *et al*, 2008 also found 55 different resistance patterns in 109 *Salmonella* Virchow isolates. It was interesting to observe that during 1986 -1990 there were 11 isolates which showed resistance to 1 antibiotic but subsequently resistance to two or more antibiotics was observed.

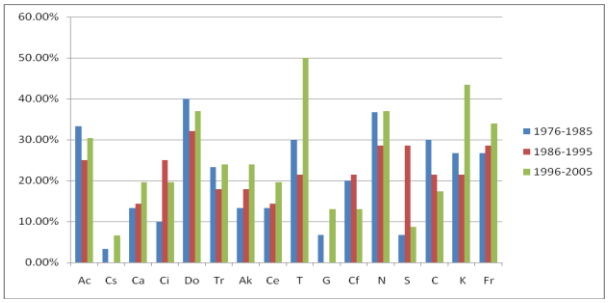
Table 4: Resistance pattern and multiple antibiotic resistance index of *Salmonella* Typhimurium isolated during the years 1996-2005.

S.No.	Resistance pattern	Source of Isolates					Total	Multiple antibiotic resistance index
		Poultry	Pig	Rabbit	Goat	Monkey		
1	T	1	-	-	-	-	1	0.0625
2	Cr	1	-	-	1	-	2	0.0625
3	T,Fr	1	-	-	-	-	1	0.125
4	Ce,K	-	1	-	-	-	1	0.125
5	N,K	-	-	-	2	-	2	0.125
6	Cr,Fr	1	-	-	-	-	1	0.125
7	Tr,K	-	-	-	1	-	1	0.125
8	Do,T,N	1	-	-	-	-	1	0.1875
9	CI,Tr,N	1	-	-	1	-	2	0.1875
10	Ac,N,K	-	-	-	1	-	1	0.1875
11	Ac,Do,T,C	1	-	-	-	-	1	0.250
12	Ca,Cf,C,Fr	2	-	-	-	-	2	0.250
13	Ac,T,Cf,K,	-	2	-	-	-	2	0.250
14	Ca,Ce,N,K	-	-	-	1	-	1	0.250
15	Ca,Do,N,K	-	-	-	1	-	1	0.250
16	Ca,T,N,C	1	-	-	-	-	1	0.250
17	Ca,Tr,T,C	1	-	-	-	-	1	0.250
18	Tr,T,S,K	-	-	-	1	-	1	0.250
19	Cs,Ci,Ak,Do	-	-	1	-	-	1	0.250
20	Ac,Do,Ak,K	-	-	-	-	1	1	0.250
21	Tr,Ak,T,K	-	-	-	-	1	1	0.250
22	Ac,Tr,T,C,Fr	-	-	-	-	1	1	0.3125
23	AC,Ci,Do,T,S	-	1	-	-	-	1	0.3125
24	CA,Do,Cf,N,K	-	-	1	-	-	1	0.3125
25	Do,T,Cf,N,Fr	-	-	1	-	-	1	0.3125
26	Do,Ak,Ce,T,Cf	1	-	-	-	-	1	0.3125
27	Cs,Ci,Do,Ce,T,N	1	-	-	-	-	1	0.375
28	Ac,Do,Ce,T,C,Fr	-	1	-	-	-	1	0.375
29	Ac,Do,Tr,Ak,T,K	-	-	-	-	1	1	0.375
30	Cs,Do,Ak,G,C,K	-	-	1	-	-	1	0.375
31	Ci,Tr,Ce,T,N,Fr	1	-	-	-	-	1	0.375
32	Ac,Tr,CeT,G,C,K	1	-	-	-	-	1	0.4375
33	Ac,Do,Tr,Ak,G,N,Fr	-	-	-	1	-	1	0.4375
34	Ac,Ca,Do,Ce,G,N,S	-	-	2	-	-	2	0.4375
35	AC,Do,Tr,T,G,C,K,Fr	1	-	-	-	-	1	0.500
36	Ci,Do,Ak,Ce,T,Cf,N,S,K	1	-	-	-	-	1	0.5625
	Total	**17**	**5**	**6**	**10**	**4**	**42**	**0.2743**

Multiple antibiotic resistance (MAR) index was also indicated rich in resistance over years. The average MAR index for the year 1976-1985 was 0.2236 (Table 2) which increased to 0.2529 during the period 1986-1995 and remained 0.2743 for the period 1996-2005 (Table 3 and 4). In 2008 Kumar *et al*., also found that MAR index increase during increasing year periods.

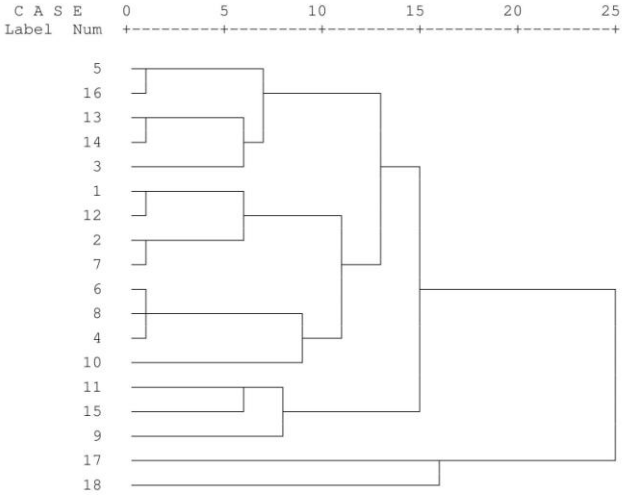
During the years 1976 -1985, AcDo was the dominant profile exhibited by 9 of the 33 isolates from different sources *viz*., poultry and goat. SFr was dominant profile during 1986 1995 period shown by 4 of the 29 isolates from poultry and monkey origin.

Fig 1: Year wise antimicrobial resistance among *Salmonella* Typhimurium isolates



However, during 1996 -2005 as many as 36 profiles were observed among 42 isolates. Another fact recorded in this study was the emergence of new resistance patterns during different years.

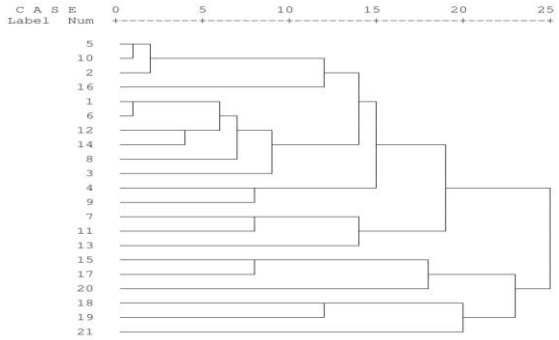
Fig 2: Cluster analysis for antibiotic resistance pattern for relatedness among *S*. Typhimurium isolates for the years 1976-1985.



Dendograms were generated for three different periods by cluster analysis using average linkage method (Fig. 2, 3 and 4). During 1976 – 1985 only 1 cluster was

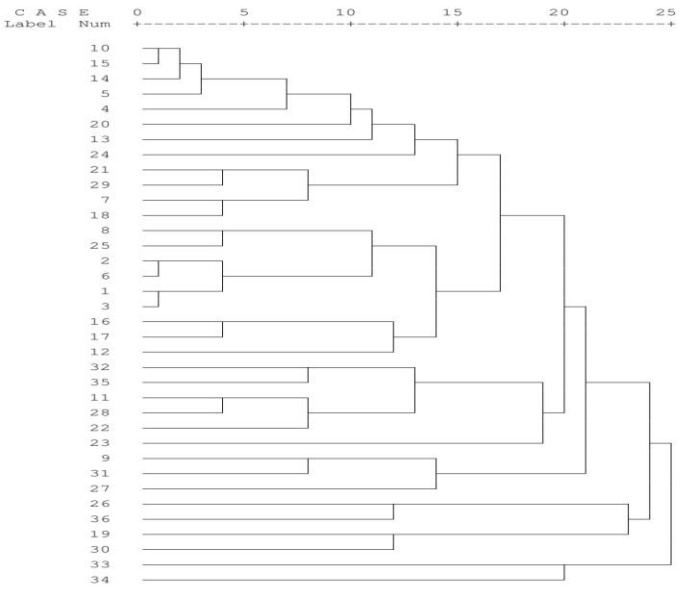
observed whereas, during 1986-1995 two and 1996-2005 three clusters were seen.

Fig 3: Cluster analysis for antibiotic resistance pattern for relatedness among *S.* Typhimurium isolates for the years 1986-1995.



Furthermore, these clustering did not reveal any linage among the isolates of different origin indicating that the antibiotic resistance was not confined to any specific animal species and isolates freely circulated among different animal species.

Fig 4: Cluster analysis for antibiotic resistance pattern for relatedness among *S.* Typhimurium isolates for the years 1996-2005.



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