Short Communication

Reduced Minimum Inhibitory Concentration of Antibiotics Associated with DT 104 Phage Type of *Salmonella Enterica Serovar Typhimurium*

Mudasir Ahmad[1], Rajesh Kumar Agarwal[2], Jinu Mary John[3], Joy Lalmuanpuia Kataria[1], Blessa Sailo[4]

[1]Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., India; [2]National Salmonella Centre (Vet), Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., India; [3]College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut; [4]ICAR Research Complex for NEH region, Manipur Center, Lamphelpat, Imphal, India.

Abstract | There has been worldwide emergence of a multi-drug resistant S. typhimurium phage type DT104. It is reported to be resistant to five antibiotics *viz*., ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracycline (ACSSuT) and a major health concern for human and animals alike. A study was undertaken to illuminate the MIC levels of five antibiotics among Indian isolates of *S. typhimurium*. Examination of 100 strains of *S. typhimurium* revealed that all the strains were sensitive to chloremphenicol and streptomycin and 95% of tested strains were sensitive to ampicilline. However, sulfisoxazole and tetracycline expressed resistance of 93% and 57%, respectively. The MIC values of all the antibiotics determined in this study were much below the break point except for one isolate, which showed MICvalue of >240 µg/ml for sulfisoxazole. The study provides valuable information on the MIC value of *S. typhimurium* and it appears that this organism circulating in India have not yet acquired resistance against most of these antibiotics. Judicious use of these antibiotics in human and veterinary practice and regular monitoring is required to keep a check on the situation.

Keywords | S. typhimurium, DT 104 phage type, Indian, Antibiogram, Multi drug resistance

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.
Received | November 01, 2014; **Revised** | November 13, 2014; **Accepted** | November 14, 2014; **Published** | November 21, 2014
***Correspondence** | Rajesh Kumar Agarwal, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., India; **Email:** grace_bly@yahoo.com
Citation | Ahmad M, Agarwal RK, John JM, Kataria JL, Sailo B (2014). Reduced minimum inhibitory concentration of antibiotics associated with DT 104 phage type of *Salmonella enterica Serovar typhimurium*. Adv. Anim. Vet. Sci. 2 (10): 592-598.
DOI | http://dx.doi.org/10.14737/journal.aavs/2014/2.10.592.598
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

Salmonellosis is one of the important diseases affecting human and animal health worldwide. Many serovars of *Salmonella* have been identified to cause infection both in humans and animals (Agarwal et al., 1999; Crump and Mintz, 2010; Singh et al., 2013). Among different serovars, *S. typhimurium* is most commonly associated with enteric infection in man and animals (de Jong et al., 2012). *S. typhimurium* has a diverse host range, which include humans, cattle, pig, sheep, horse, rodent and birds (Townsend et al., 2001; Chaudhuri et al., 2013). *S. typhimurium* and other serovars can cause a more invasive infection in immunocompromised patients such as those with AIDS, certain cancers and under chemotherapy, which can be life threatening (Hachfi et al., 2009). It is also recognized as the most common saviour of *Salmonella* causing cardiovascular, bone and

joint infections (Kalpana et al., 1998).

The increasing concern is the emergence of multidrug resistant phenotypes among *Salmonella* serotypes, in particular S. *typhimurium* (Mirza and Wamola, 1989; Singh et al., 2011). Antibiotic resistance in many bacteria including zoonotic salmonellas has been attributed to the injudicious use of antibiotics in food-producing animals with onward transmission to humans through the food chain (Threlfall, 2002; Tiwari and Dhama, 2014).

S. typhimurium definitive phage type DT104 resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (ACSSuT) was first isolated in the 1960s and later emerged in the 1990s as major pathogen (de Jong et al., 2012; Sahu et al., 2013). About 15% of isolates have been found to exhibit decreased susceptibility to ciprofloxacin (Threlfall, 2002). MDR DT104 has caused numerous outbreaks throughout the world (Threlfall et al., 2005).

This strain of *S. typhimurium* is recognized to be particularly virulent in animal and human hosts and has been isolated increasingly from humans and animals in the United Kingdom and several other European countries and more recently, in the United States and Canada (Poppe et al., 1998). Humans may acquire the infection from foods of animal origin contaminated with the infective organism.

There is no report/very little information available on the DT 104 phage type and MIC levels of antibiotics to which it is resistant. Therefore, the present study was undertaken to detrmine the MIC levels of five antibiotis to which *S. Typhimurium* D 104 are resistant.

A total of 100 strains of *Salmonella typhimurium* were taken from the repository of the National Salmonella Center (Veterinary), Indian Veterinary Research Institute, Izatnagar, India. All the strains were tested for their purity, morphological and biochemical characteristics (Barrow, 1993; Edwards and Ewing, 1972) and serotypically by slide agglutination test using polyvalent antisera and tube agglutination test using somatic and flagellar group specific and factor antisera available at National Salmonella Centre (Veterinary), I.V.R.I., Izatnagar.

All the *S. typhimurium* strains were initially examined for their antimicrobial drugs susceptibility/ resistance pattern in triplicate by disc diffusion method (CLSI, 2005) on Mueller Hinton agar (Himedia, Mumbai, India) using 5 different antimicrobial agents *viz*., streptomycin 300 µg,, ampicillin 10 µg, tetracycline 30 µg, sulfisoxazole 300 µg and chloramphenicol 30 µg.

All the strains were subjected to testing for minimum inhibitory concentration (Table 1) by HiComb MIC test (Himedia, Mumbai). Briefly, young broth culture of each isolate was swabed on Mueller Hinton agar plates three times, turning the plate at 60° angle between each streaking to get the homogenous growth of culture. The inoculum was allowed to dry for 5-15 min HiComb MIC strip was applied to the agar surface with the MIC scale facing upwards. Plates were incubated at 37°C and examined after 24hrs. The zone of inhibition was in the form of ellipse. MIC value was determined as the value at which the zones convene the comb like projections of the strips and not the handle. If the MIC value interpreted from part A of the strip was not same for part B, in that case MIC was interpreted as lower of the two.

Table 1: Antibiotics used for MIC determination

S. No.	Name of antibiotic	Range (µg)
1	Ampicillin (A)	A: 256 - 0.1 B: 4 - 0.001
2	Chloramphenicol ©	A: 240 - 0.01 B: 8 - 0.001
3	Streptomycin (S)	A: 240 - 0.01 B: 30 - 0.001
4	Sulfisoxazole (Sf)	A: 240 - 0.001 B: 30 - 0.001
5	Tetracycline (T)	A: 240 - 0.01 B: 5 - 0.01

One hundred strains of S. *typhimurium* were initially subjected to testing for 5 individual antibiotics. Results revealed that all the strains were sensitive to chlorompheniсol and streptomycin and 95% of strains were sensitive to ampcillin. However, resistance was observed with sulfisoxazole by 93% strains and with tetracyclin by 43% strains. The high degree of chlorompheniсol and streptomycin susceptibility to *S. enterica* isolates has also been reported from many other parts of India (Mandal et al., 2004; Singh et al., 2010; Choudhary et al., 2013). This may be due to less use of these drugs in human and animal practice. However, sensitivity to ampiciln appears to be new phenomenon. Recently, Choudhary et al. (2013) observed 90% *Salmonella* Typhi and Paratyphi strains to be sensitive to ampicillin, which may be due to its reduced use in clinical practice.

Table 2: Result of minimum inhibitory concentration of individual *S. typhimurium* strain (R=Resistance)

S. No.	*S. typhimurium* isolates	MIC (µg/ml)				
		Ampicillin	Chloramphen-icol	Streptomycin	Sulfisoxaz-ole	Tetracyclin
1	E-4767	0.512	0.1	1.0	R	R
2	E-2924	0.512	0.1	1.0	R	R
3	E-421	0.256	0.1	0.1	R	R
4	E-2919	0.512	0.1	1.0	R	R
5	E-4638	0.256	0.1	1.0	>240	0.1
6	E-828	0.512	0.1	1.0	R	R
7	E-2563	0.512	0.1	0.1	R	R
8	E-4231	0.512	0.1	1.0	R	0.1
9	E-2928	0.128	0.1	1.0	R	R
10	E-773	0.256	0.1	0.01	R	0.1
11	E-2416	0.512	0.1	1.0	R	0.1
12	E-5215	R	0.1	0.1	R	0.1
13	E-2700	0.512	0.1	0.01	R	R
14	E-2393	0.512	0.1	0.1	R	R
15	E-557	0.016	0.5	1.0	R	R
16	E-5257	0.256	0.1	0.01	R	R
17	E-4256	0.256	0.1	0.01	3	0.01
18	E-4806	0.512	0.1	1.0	R	0.01
19	E-1159	0.256	0.1	1.0	R	R
20	E-4885	0.512	0.1	1.0	R	0.01
21	E-427	1.024	0.1	1.0	R	R
22	E-2443	0.256	0.1	0.01	R	R
23	E-2382	R	0.1	0.01	R	R
24	E-4483	0.512	0.1	1.0	R	R
25	E-4808	0.512	0.1	1.0	R	R
26	E-5268	0.512	0.1	1.0	R	R
27	E-4807	0.256	0.1	1.0	R	R
28	E-4836	0.512	0.1	1.0	R	R
29	E-2381	R	1.0	3.0	R	R
30	E-4810	0.256	0.1	1.0	R	0.01
31	E-4851	0.064	0.1	1.0	R	0.01
32	E-420	0.256	0.01	0.01	R	R
33	E-872	0.256	0.1	1.0	R	R
34	E-5220	0.256	0.1	1.0	R	0.01
35	E-5256	0.256	0.1	1.0	R	R
36	E-5266	0.128	0.01	0.001	R	R
37	E-2398	0.256	0.1	0.001	R	R
38	E-1114	R	0.1	0.001	R	R
39	E-5202	0.256	0.1	0.001	R	R
40	E-425	0.032	0.1	0.001	R	R
41	E-2391	0.512	0.1	0.1	R	R

42	E-4769	0.512	0.1	0.1	R	0.1
43	E-4891	0.512	0.1	0.1	R	0.01
44	E-2614	0.256	0.1	0.001	R	R
45	E-5262	0.256	0.1	0.01	R	0.01
46	E-2392	0.512	0.1	1.0	R	0.1
47	E-2959	0.512	0.01	0.01	R	R
48	E-5227	0.512	0.1	1.0	R	R
49	E-4857	0.512	0.1	1.0	R	0.1
50	E-2377	0.128	0.1	1.0	3.0	0.01
51	E-3139	0.512	0.01	0.01	3.0	0.1
52	E-2919	0.064	0.01	0.01	R	R
53	E-5255	0.512	0.01	0.01	R	0.1
54	E-4862	0.256	0.01	1.0	R	0.1
55	E-5264	0.256	0.01	1.0	R	R
56	E-608	0.512	0.1	1.0	R	R
57	E-4858	0.512	0.01	1.0	R	0.1
58	E-4242	0.512	0.1	1.0	R	R
59	E-4630	0.512	0.1	1.0	R	R
60	E-2689	1.024	0.1	0.1	R	R
61	E-4658	0.512	0.1	1.0	R	R
62	E-5270	0.256	0.1	0.001	R	R
63	E-4863	0.064	0.1	1.0	R	0.01
64	E-660	0.256	0.5	0.01	R	R
65	E-5226	0.064	0.1	1.0	R	R
66	E-4856	0.256	0.1	0.001	R	0.1
67	E-4803	0.512	0.1	1.0	R	0.1
68	E-5265	0.256	0.1	1.0	R	R
69	E-4775	0.256	0.1	1.0	R	0.01
70	E-2622	0.512	0.1	1.0	R	R
71	E-4809	1.024	0.1	1.0	R	0.1
72	E-4811	0.256	0.1	0.001	R	0.1
73	E-2443	0.128	0.1	0.001	R	R
74	E-2950	0.256	0.1	0.001	R	0.1
75	E-4854	0.128	0.1	0.01	R	0.01
76	E-2920	0.128	0.1	0.01	R	0.01
77	E-2597	0.256	0.1	0.01	R	R
78	E-2387	0.256	0.1	0.01	R	0.1
79	E-5235	0.256	0.1	1.0	R	R
80	E-2693	0.256	0.1	0.01	R	R
81	E-5158	0.128	0.1	1.0	R	0.1
82	E-5254	0.256	0.1	0.01	R	R
83	E-4227	0.256	0.1	0.01	R	R
84	E-5269	0.256	0.1	0.01	R	0.01
85	E-958	0.064	0.1	0.01	3.0	0.01

86	E-3135	0.256	0.1	0.01	3.0	0.1
87	E-4757	0.512	0.1	0.01	R	0.1
88	E-462	0.128	0.1	0.01	R	R
89	E-1115	0.256	0.1	0.01	R	R
90	E-556	0.256	0.1	1.0	R	R
91	E-381	0.064	2.0	1.0	R	R
92	E-711	0.256	0.1	1.0	R	R
93	E-3126	0.064	0.1	1.0	R	0.1
94	157B	0.256	0.1	1.0	R	0.01
95	E-2595	0.128	0.1	1.0	5.0	0.01
96	E-759	0.256	0.1	1.0	R	0.1
97	E-970	0.512	0.1	1.0	3.0	0.1
98	E-4629	0.256	0.1	1.0	R	0.1
99	E-774	0.512	0.1	1.0	R	1.0
100	E-3140	R	0.5	1.0	R	R
Total resistant		**5**	**0**	**0**	**93**	**57**

One of the tests prescribed by CLSI for determining minimum inhibitory concentration is E test. However, its cost and limited availability in India may restrict its use. HiComb MIC test (Himedia) is considered to be an alternative to the E test (Harish et al., 2008). In this study, we used HiComb MIC test for determining the minimum inhibitory concentration of selected antibiotics (ampicillin, chloramphenicol, streptomycin, sulfisoxazole and tetracyclin) against which S. typhimurium isolates were found to be sensitive.

The results are presented in table 2, MICs of ampicillin sensitive strains ranged between 0.016 µg/ml to 1.024 µg/ml. MIC level of 0.256 µg/ml was recorded in 40 isolates, 0.512 µg/ml in 34 isolates, 0.128 µg/ml in 9 isolates, 0.064 µg/ml in 7 isolates, 1.024 µg/ml in 3 isolates, 0.032 and 0.016 µg/ml in 1 isolate each. MICs of chloramphenicol sensitive strains ranged between 0.01 µg/ml to 2 µg/ml. MIC level of 0.1 µg/ml was recorded in 86 isolates, 0.01 µg/ml in 9, 0.5 µg/ml in 3, 1 and 2 µg/ml in 1 isolate each.

MICs of streptomycin sensitive strains ranged between 0.001 µg/ml to 3 µg/ml. MIC level of 1 µg/ml was recorded in 54 isolates, 0.01 µg/ml in 26, 0.001 µg/ml in 11, 0.1 µg/ml in 8 and 3 µg/ml in one isolate. MICs of sulfisoxazole sensitive strains ranged between 3.0 µg/ml to >240 µg/ml. MIC level of 3 µg/ml was recorded in 6 isolates, 5 µg/ml in 1, greater than 240 µg/ml in 1 isolate. MICs of tetracyclin sensitive strains was between 0.01µg/ml to 1.0 µg/ml. MIC level of 0.1 µg/ml was recorded in 24 isolates, 0.01 µg/ml in 18, 1 µg/ml in 1 isolate. The MIC results revealed that all the isolates were within the prescribed concentrations for sensitivity for the antibiotics tested viz., ampicillin (=8 µg/ml), chloramphenicol (=8 µg/ml), streptomycin (=8 µg/ml), sulfisoxazole (=256 µg/ml) and tetracyclin (=4 µg/ml). The observations were in agreement to the results of antibiotic sensitivity test performed in our experiment.

The determined minimum inhibitory concentration of the tested antibiotics (ampicillin, chloramphenicol, streptomycin, sulfisoxazole, tetracyclin) in our study, were much below to the findings of De Jong et al. (2009) who observed MIC value of >128 µg/ml for ampicillin, chloramphenicol, streptomycin, tetracyclin and 1024 µg/ml for sulfisoxazole. It appears that Indian isolates have not yet acquired resistance to these antibiotics. Highest MIC observed in our study for ampicillin, chloramphenicol, streptomycin, sulfisoxazole and tetracyclin were 1.024 µg/ml, 2 µg/ml, 3 µg/ml, >240 µg/ml and 1.0 µg/ml, respectively. The MIC results revealed that all the isolates were within the prescribed concentrations for sensitivity for the antibiotics tested *viz.*, ampicillin (=8 µg/ml), chloramphenicol (=8 µg/ml), streptomycin (=8 µg/ml), sulfisoxazole (=256 µg/ml) and tetracyclin (=4 µg/ml). The observations were in agreement to the results of antibiotic sensitivity test. The MIC values of all the antibiotics determined in this study were much below the break point except for one isolate which showed-

MIC value of >240 µg/ml for sulfisoxazole. The reason for higher breakpoint for this isolate is not clear. Overall, it appears that S. typhimurium isolates circulating in India have not yet acquired resistance against these antibiotics, which may be due to less use of these antibiotics in human and veterinary practice.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, Indian Veterinary Research Institute, Izatnagar (U.P) for providing all the necessary facilities to carry out the above research work.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

- Agarwal RK, Kapoor KN, Verma JC and Bachhil VN, Singh BR, Kumar A, Sachan N, Singh DK, Malik SVS (1999). First report and some properties of *Salmonella* Stockholm in India. Indian J. Comp. Microbiol. Immunol. Infect. Dis. 20(1): 50-52.
- Barrow PA (1993). *Salmonella* control-past, present, and future. Avian Pathol. 22(4): 651-669.
- Clinical and Laboratory Standards Institute (2005). Performance standards for antimicrobial susceptibility testing approved standard. M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.
- Choudhary A, Gopalakrishnan R, Senthur NP, Ramasubramanian V, Ghafur AK, Thirunarayan MA (2013). Antimicrobial susceptibility of *Salmonella enterica* serovars in a tertiary care hospital in southern India. Indian J. Med. Res. 137(4): 800-802.
- Chaudhuri RR, Morgan E, Peters SE, Pleasance SJ, Hudson DL, et al. (2013) Comprehensive assignment of roles for *Salmonella typhimurium* genes in intestinal colonization of food-producing animals. PLoS Genet. 9: e1003456. doi:10.1371/journal.pgen.1003456
- Crump JA, Mintz ED (2010). Global trends in typhoid and paratyphoid fever. Emerging Infect. 50(2): 241-246.
- De jong A, Bywater A, Butty P, Deroover E, Godinho K, Klein V, Marion H, Simjee S, Smets K, Thomas V, valle M, Wheadon A (2009). A pan European survey of antimicrobial susceptibility towards antimicrobial drugs among zoonotic and commensal enteric bacteria isolated from healthy food producing animals. J. Antimicrob. Chem. 63(4): 733-744.
- de Jong HK, Parry CM, van der Poll T, Wiersinga WJ (2012). Host–Pathogen Interaction in Invasive salmonellosis. PLoS Pathog. 8: e1002933. doi:10.1371/journal.ppat.1002933
- Edwards PR, Ewing WH (1972). In: Identification of Enterobacteriaceae, Third edition. Burgess Publishing Company, Georgia. Pp. 362.
- Hachfi W, Bellazreg F, Ladib M, Kaabia N, Khalifa M, Krifa H, Letaief A (2009). *Salmonella typhimurium* epidural empyema in an HIV infected patient. Infectious Dis. Reports. 1:e5 doi:10.4081/idr.2009.e5
- Harish BN, Menezes GA, Parija SK (2008). A case report and review of literature: ciprofloxacin resistant *S. enterica* serovar Typhi in India. J. Infect. Dev. Countr. 2(2): 324-327.
- Kalpana DS, Marica BG, Robert HR (1998). *Salmonella* infections. In: Infectious Diseases. Gorbach, S.L., Bartlett, j.G. and Blacklow, N.R. (eds), Pennsylvania. Pp. 699-712.
- Mandal S, Mandal MD, Pal NK (2004). Reduced minimum inhibitory concentration of chloramphenicol for *Salmonella enterica* serovar Typhi. Indian J. Med. Sci. 58(1): 16-23.
- Mirza NB, Wamola, IA (1989). *S. typhimurium* outbreak at Kenyatta National Hospital. East African Med. J. 66(7): 453-457.
- Poppe C, Smart N, Khakhria R, Johnson W, Spika J, Prescott J (1998). *Salmonella typhimurium* DT104: A virulent and drug-resistant pathogen. Can. Vet. J. 39(9): 561-565.
- Sahu SN, Anriany Y, Grim CJ, Kim S, Chang Z, Joseph, SW, Cinar HN (2013) Identification of virulence properties in *Salmonella typhimurium* DT104 using *Caenorhabditis elegans*. PLoS ONE 8(10): e76673.
- Singh BR, Agarwal M, Chandra M, Verma M, Sharma G, Verma JC, Singh VP (2010). Plasmid profile and drug resistance pattern of zoonotic *Salmonella* isolates from Indian buffaloes. J. Infect. Dev. Ctries. 4(8): 477-483.
- Singh S, Agarwal RK, Tiwari SC, Singh H (2011). Antibiotic resistance pattern among the *Salmonella* isolated from human, animal and meat in India. Trop. Anim. Health Prod. 44(3): 9953-9957.
- Singh S, Singh H, Tiwari SC, Prejit N, Agarwal RK (2013). Characterization of virulence factors among diverse *Salmonella* serotypes and sources. Adv. Anim. Vet. Sci. 1(2): 69-74.
- Threlfall EJ (2002). Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. FEMS Microbiol. Rev.

26(2): 141-148.
- Threlfall J, Hopkins KL, Ward LR (2005) Diversification in *Salmonella typhimurium* DT104. Emerg. Infect. Dis. 11(6): 980-981.
- Tiwari R, Dhama K (2014). Antibiotic resistance: a frightening health dilemma. Am. J. Pharmacol. Toxicol. 9(3): 174-176.
- Townsend SM, Kramer NE, Edwards R, Baker S, Hamlin N, Simmonds M, Stevens K, Malony S, Parkhill J (2001). *Salmonella enterica Serovar typhi* possesses a unique repertoire of fimbrial gene sequences. Infect. Immun. 69(5): 2894-2901.