Brief Original Article

Drug resistance patterns of *Salmonella* isolates of equine origin from India

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Abstract

Introduction: Salmonellosis is a zoonosis, and one of the most serious public health and animal health problems.
Methodology: We studied 111 isolates of *Salmonella* belonging to 14 *S. enterica* subspecies *enterica* serovars namely *S.* Abortusequi (45), *S.* Weltevreden (1), *S.* Dumfries (2), *S.* Tshiongwe (1), *S.I.* 4,5,12:r,i:1,5 (12), *S.* Bovismorbificans (3), *S.* Drogana (8), *S.* Lagos (4), *S.* Kottbus (3), *S.* Richmond (1), *S.* Typhimurium (6), *S.* Newport (7), *S.* Paratyphi B var Java (17) and *S.* Saintpaul (5) isolated from equids in India.
Results: All strains studied were resistant to one or more antimicrobials. Strains were resistant to ampicillin (18, 16%), ampicillin+cloxacillin (6, 5%), cefotaxime (6, 5%), chloramphenicol (2, 2%), ciprofloxacin (9, 8%), gentamicin (27, 24%), kanamycin (37, 33%), nalidixic acid (10, 9%), furazolidone (97, 87%), streptomycin (33, 30%), sulphamethoxazole (91, 82%), tetracycline (48, 43%) and trimethoprim (5, 4.5%). Multiple-drug-resistance was detected in 84 (75.7%) isolates and was seen in isolates of all serovars except of *S.* Kottbus, a rare serovar in India. *Salmonella* isolates could be classified into 51 resistotypes but 47 (42.3%) isolates belonged to six major resistotypes. Resistotype 13 (resistant to furazolidone, sulphamethoxazole and tetracycline) was most common, followed by resistotype 19 (resistant to nalidixic acid, sulphamethoxazole and tetracycline), resistotype 28 (resistant to furazolidone, streptomycine, sulphamethoxazole and tetracycline) and resistotype 40 (resistant to furazolidone, gentamycin, kanamycin, streptomycine, sulphamethoxazole and tetracycline) including 11, 8, 8 and 7 strains of different serovars, respectively.
Conclusions: This study revealed that antimicrobial drug resistance was common in *Salmonella* isolates from equids even towards those drugs not used in equids.

Keywords: *Salmonella*, MDR, equids, Antibiotic resistance

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Introduction

Salmonellosis is a zoonosis, and one of the most serious public health and animal health problems. Almost all warm-blooded and many cold-blooded animals are its natural hosts. It may vary in severity from undetectable infections to acute disease, which may be fatal to the very young, the old, or the debilitated individual. Salmonellosis in equids is a global problem with varying severity and prevalence varying from less than 2% to about 27% depending upon the topography and climatic conditions [1,2,3,4].

The earliest report of salmonellosis in equids was from cases of abortion in mares [5,6]; causative organism was identified as *Salmonella abortusequi*, now known as *S. enterica* subsp. *enterica* serovar Abortusequi (*S.* Abortusequi). Earlier *S.* Abortusequi was found globally; however, today the infections are mostly limited to Asian and African countries. *Salmonella* Typhimurium is another serovar commonly isolated from horses [7,8,9]. More recently, a number of other *Salmonella* serovars have also been recovered from equids [2,3,10-12]. In recent studies, *Salmonella* bacteria could be isolated from 2% to 15% of apparently healthy equids while *Salmonella* DNA could be detected by PCR from 27% to 40% equids [2,3,4].

The majority of *Salmonella* infections in equids are iatrogenic [13]. Important risk factors, which lead to precipitation of *Salmonella* infection in equids, include administration of antimicrobials, colic, surgery, and transportation stress. Animals with severe neutropenia (due to either any immunodeficiency problem or to prolonged anticancer chemotherapy) are also at increased risk for bacterial and fungal infections. [14-16].

Antimicrobial resistance in disease-causing

Table 1. Antimicrobial drug resistance in *Salmonella* isolates of equine origin in India.

***Salmonella* Serovars**	**Total**	**A**	**Ac**	**Ce**	**C**	**Cf**	**G**	**K**	**Na**	**F**	**S**	**Sm**	**T**	**Tm**
S. Abortusequi	45	3	0	2	0	5	9	28	9	41	9	31	1	3
S. Bovismorbificans	3	1	1	1	0	0	0	0	0	2	1	1	2	0
S. Drogana	8	1	0	1	0	0	0	0	0	7	2	8	8	0
S. Dumfries	2	0	0	0	0	0	1	1	0	2	2	2	1	0
S. Kottbus	3	0	0	0	0	0	0	0	0	0	0	3	0	0
S. Lagos	4	0	0	0	0	0	0	0	0	4	3	2	3	0
S. Newport	7	0	0	0	0	0	7	6	0	7	6	7	1	0
S. Paratyphi B Java	17	7	1	1	1	2	6	0	0	12	0	12	11	1
S. Richmond	1	1	1	0	0	0	0	0	0	0	0	1	1	0
S. Saintpaul	5	1	1	1	1	0	2	0	0	4	0	4	4	0
***S.I.* 4,5,12:ri:i,5**	12	3	1	0	0	0	0	1	0	11	8	12	12	1
S. Tshiongwe	1	0	0	0	0	0	1	0	0	1	1	1	0	0
S. Typhimurium	6	0	0	0	0	1	1	0	1	5	0	6	4	0
S. Weltevreden	1	1	1	0	0	1	0	1	0	1	1	1	0	0
Total Isolates	111	18	6	6	2	9	27	37	10	97	33	91	48	5
Percent resistant	100	16.2	5.4	5.4	1.8	8.1	24.3	33.3	9.0	87.4	29.7	82.0	43.2	4.5

A, ampicillin; Ac, ampicillin+cloxacillin; Ce, cefotaxime; C, chloramphenicol; Cf, ciprofloxacin; G, gentamicin; K, kanamycin; Na, nalidixic acid; F, furazolidone; S, streptomycin; Sm, sulphamethoxazole; T, tetracycline; Tm, trimethoprim

bacteria results in increased illness, deaths, and increased health-care costs [17-18]. Emergence of multi-drug-resistance (MDR) in empirical antimicrobial agents is a global problem, particularly in pathogens causing nosocomial infections. The increasing use of indwelling devices, as well as widespread and indiscriminate use of antibacterial agents in hospital settings, contributes to antimicrobial resistance among pathogens causing nosocomial infections [17, 19]. Although, much work has been conducted on the emergence of antimicrobial resistance to *Salmonella* in human health, [20-22], little is known about antimicrobial resistance in *Salmonella* strains prevalent in equids in India. We conducted antimicrobial drug sensitivity tests on 111 *Salmonella* isolates from equids isolated from different parts of India [12] and detected MDR in more than75% of the isolates.

Material and Methods

Salmonella *isolates*

Salmonella isolates (111) belonging to 14 serovars (Abortusequi, Weltevreden, Dumfries, Tshiongwe, 4,5,12:r,i:i,5, Bovismorbificans, Drogana, Lagos, Kottbus, Richmond, Typhimurium, Newport, Paratyphi B var Java, and Saintpaul) of *S. enterica* subspecies *enterica* (Table 1) were obtained from stocks at the National *Salmonella* Centre (Vet), Indian Veterinary Research Institute, Izatnagar, repository. All were confirmed through biochemical and serological methods [23] and maintained on nutrient agar slopes until tested. All *S*. Abortusequi isolates included in the study were isolated between 1982 and 1996 at Izatnagar from aborted fetal tissues and vaginal swabs submitted for diagnosis [10,12], while strains of other *Salmonella* serovars were isolated after 1991 either from healthy or apparently healthy equids [2,12,27] except for one *S*. Typhimurium isolate from a diarrhoeic foal [11].

Antimicrobial sensitivity assay

Antimicrobial sensitivity of *Salmonella* isolates was determined in triplicate by disc diffusion method on Mueller Hinton agar No. 4 using ampicillin 10 μg (Am), ampicillin+cloxacillin 20 μg (Ac), chloramphenicol 30 μg (C), cefotaxime 30 μg (Ce), ciprofloxacin 5 μg (Cf), furazolidone 300 μg (F), gentamicin 10 μg (G), kanamycin 30 μg (K), nalidixic acid 30 μg (Na), streptomycin 10 μg (S), sulphamethoxazole 300 μg (Sm), tetracycline 30 μg (T) and trimethoprim 5 μg (Tm) discs (Hi-media, Mumbai). Based on zone of inhibition, isolates were classified as sensitive or resistant according to CLSI (2006) standards. The strains resistant to three or more drugs were classed as multi-drug-resistant (MDR) strains. A reference *E. coli* K12 strain (E-382), sensitive to all antimicrobials was used as control.

Results

All 111 isolates were resistant to one or more antimicrobials. Resistance rate varied from 2% to 87% as follows: ampicillin (18, 16%), ampicillin+cloxacillin (6, 5%), cefotaxime (6, 5%), chloramphenicol (2, 2%), ciprofloxacin (9, 8%), gentamicin (27, 24%), kanamycin (37, 33%), nalidixic acid (10, 9%), furazolidone (97, 87%), streptomycin (33, 30%), sulphamethoxazole (91, 82%), tetracycline (48, 43%), and trimethoprim (5, 4.5%).

As shown in Table 2, the majority of the *Salmonella* isolates (99, 89.2%) were resistant to more than one antimicrobial drug as follows: to seven (2.7%), six (5.4%), five (19.8%), four (18.9%), three (28.8%), two (13.5%), and one (10.8%) drug. Multiple drug resistance (MDR), defined as resistance to three or more antimicrobials, was seen in 84 (75.7%) of the *Salmonella* isolates. MDR was recorded in strains of all *S. enterica* serovars to a variable extent, including *S.* Abortusequi (58.9%), *S.* Weltevreden (100%), *S.* Dumfries (100%), *S.* Tshiongwe (100%), *S. I.* 4,5,12:r,i:1,5 (91.7%), *S.* Bovismorbificans (66.7%), *S.* Drogana (100%), *S.* Lagos (100%), *S.* Richmond (100%), *S.* Typhimurium (83.3%), *S.* Newport (100%), *S.* Paratyphi B var Java (84.6%), and *S.* Saintpaul (100%) (Table 2). MDR was not found in the three strains of *S.* Kottbus (Table 2).

Although 111 isolates of 14 serovars belonged to 51 resistotypes, 43% belonged to six major resistotypes (Table 3). Resistotype 13 (resistant to furazolidone, sulphamethoxazole and tetracycline) was the most common, followed by resistotype 19 (resistant to nalidixic acid, sulphamethoxazole and tetracycline), resistotype 28 (resistant to furazolidone, streptomycine, sulphamethoxazole and tetracycline), and resistotype 40 (resistant to furazolidone, gentamycin, kanamycin, streptomycine, sulphamethoxazole and tetracycline) comprising 11, 8, 8 and 7 strains of different serovars, respectively.

Discussion

In absence of regulations regarding the use of antimicrobials in domestic animals in India, there is no restriction on use of antimicrobials intended for human use and choice of drug often depends on economic availability of the medicine [25,26]. In equids, penicillins, tetracyclines, gentamicin, cotrimoxazole, cefotaxime, ampicillin and kanamycin are often the drugs of choice, but many veterinarians also use quinolones, third-generation cephalosporins, and new generation aminoglycosides [25,26]. MDR in *Salmonella* isolates and other bacteria is increasingly common in clinical as well as non-clinical samples of equine origin in India [26,27] and it might be associated with indiscriminate drug use of antimicrobials in animals and potentially in human beings in India [25,26].

Although use of sulfonamides, furazolidone and tetracycline is minimal in equids [25, 26, 28], the majority of *Salmonella* isolates of equine origin were resistant to sulfamethoxazole (82%), furazolidone (87%) and tetracycline (43%). This pattern of MDR might be attributed to widespread use of these antimicrobials as empirical therapy of febrile syndromes in humans and other animals, as well as to the use of these antimicrobials as growth-enhancers in animal production [25,26,29,30]. Resistance to sulfamethoxazole in MDR *Salmonella* has been prevalent for three decades among human isolates in India [30, 31] and communicability of *Salmonella* between human and animals [32, 33] might be responsible for the similar pattern in *Salmonella* isolates from equids and from other animals. Resistance to similar drugs is reported in *Salmonella* isolated from food, water [21,22] and in different animals [34] in India.

Horse meat is rarely consumed in India and neighbouring countries, but spread of salmonellosis from horses carrying MDR *Salmonella* to other livestock reared for meat or milk or other products and even to persons caring for horses is a possibility facilitating circulation of MDR strains [35]. Animals scavenging on dead horses, or dogs and other pet animals fed on horse meat, may also acquire

Salmonella infection from contaminated horse meat/

Table 2. Multiple drug resistance (MDR) among *Salmonella* of equine origin in India.

***Salmonella* Serovars**	**Numbers Tested**	**Number of drugs resisted**							**% Of isolates showing resistance to ≥3 drugs(MDR)**
		7	**6**	**5**	**4**	**3**	**2**	**1**	
S. Abortusequi	45	1	3	7	6	9	11	8	58.9
S. Weltevreden	1	1	0	0	0	0	0	0	100.0
S. Dumfries	2	0	0	1	1	0	0	0	100.0
S. Tshiongwe	1	0	0	0	1	0	0	0	100.0
S.*I.* 4,5,12:r,i:i,5	12	1	0	2	6	2	1	0	91.7
S. Bovismorbificans	3	0	0	0	2	0	0	1	66.7
S. Drogana	8	0	0	1	1	6	0	0	100.0
S. Lagos	4	0	0	0	0	4	0	0	100.0
S. Kottbus	3	0	0	0	0	0	0	3	0.0
S. Richmond	1	0	0	0	1	0	0	0	100.0
S. Typhimurium	6	0	0	0	1	4	1	0	83.3
S. Newport	7	0	1	5	0	1	0	0	100.0
S. Paratyphi B Java	13	0	2	5	1	3	2	0	84.6
S. Saintpaul	5	0	0	1	1	3	0	0	100.0
Total	111	3	6	22	21	32	15	12	75.7
Percent resistant	100	2.7	5.4	19.8	18.9	28.8	13.5	10.8	75.7

flesh and may have a significant role in the spread of salmonellosis in the environment [32,33]. Prevalence of *Salmonella* isolates from equids resistant to drugs used in human patients and also in the strains belonging to serovars often causing salmonellosis in human but not in equids (*S*. Paratyphi B) indicates the similar possibility of the circulation of MDR strains in the environment and among horses, one of the natural hosts for this dreaded pathogen. Moreover, the possibility of the spread of MDR *Salmonella* infection through horse meat exported to the consumer countries cannot be excluded, and one such outbreak of MDR *S*. Newport (the serovar also present in Indian horses) recently occurred in France [36]. *Salmonella* serovars similar to those in equids of India have also been reported frequently from meat and excretions of horses in other countries [32,33,35-38].

Apart from resistance to sulfamethoxazole and furazolidone, *S. Abortusequi* isolates were more often resistant to ciprofloxacin, gentamicin, kanamycin and streptomycin but rarely to tetracycline (Table 1). Ciprofloxacin, gentamicin, kanamycin, and streptomycin are commonly used and tetracyclines are rarely used drugs in equids in India [25,26]. The presence of *Salmonella* Abortusequi, being a host-adapted *Salmonella* rarely isolated from other animals and sources [12], indicated that empirical drug use affects the emergence of drug resistance in *Salmonella* [30,39,40] even in cases when the causal agent is not directly hit by the treatment. Resistance to chloramphenicol in only *S*. Paratyphi B and *S*. Saintpaul, common serovars in human patients in India, further indicates that *Salmonella* probably circulates in different animals and humans; and empirical drug use resulting in emergence of resistant strains in one host might be responsible for similar resistance in other hosts where the concerned drug is not used. Chloramphenicol is rarely used in animals, but it is the most commonly used antibiotic in humans for treatment of typhoid and paratyphoid fever [30,31].

Nalidixic acid and ciprofloxacin resistance in isolates of *S*. Abortusequi, *S*. Typhimurium, *S*. Paratyphi B and *S*. Weltevreden is also significant because it is associated with higher MICs for other fluoroquinolones rendering them clinically ineffective in spite of sensitivity shown by disc diffusion method *in vitro* [30,41]. Ciprofloxacin and enerofloxacin are the commonly used drugs in equids in India [25,26] which might have selected for resistance to fluoroquinolones.

Predominance of MDR among *Salmonella* isolates from equids is of particular importance because of the apparent risk to humans acquiring zoonotic *Salmonella* through contact with horses. Although there is no microbiologically proven link between antimicrobial resistance and virulence for humans in zoonotic *Salmonella*, increased rates of hospitalisation have been reported for patients with infections with MDR *S.*Typhimurium [29]. Multiple drug resistance to empirically used drugs is anticipated; however, resistance to chloramphenicol and tetracyclines, rarely used in horses, is surprising because emergence of drug-resistant *Salmonella* is thought to be due to selective pressure from the use of antimicrobials. Maintenance of drug resistance

Table 3. Resistotypes (R-types) among *Salmonella* isolates of equine origin in India.

R-type	Resistant to antimicrobials	Nos.	Serovars of *Salmonella* isolates showing resistance pattern
1	F	7	*S.* Abortusequi (7)
2	Sm	4	*S.* Abortusequi (1), *S.* Kottbus (3)
3	T	1	*S.* Bovismorbificans (1)
4	A, F	1	*S.* Abortusequi (1)
5	F, G	1	*S.* Abortusequi (1)
6	F, K	4	*S.* Abortusequi (4)
7	F, Sm	4	*S.* Abortusequi (4)
8	G, Sm	1	*S.* Abortusequi (1)
9	Sm, T	1	*S.I.* 4,5,12:ri:i,5 (1)
10	Na, Sm	3	*S.* Typhimurium (1), *S.* Paratyphi B var Java (2)
11	A, Ac, F	1	*S.* Saintpaul (1)
12	F, K, S	1	*S.* Abortusequi
13	F, Sm, T	11	*S.* Abortusequi (1), *S.* Drogana (6), *S.* Lagos (2), *S.I.* 4,5,12:r,i:i,5 (2)
14	F, S, T	2	*S.* Lagos (2)
15	F, G, Sm	1	*S.* Newport (1)
16	F, K, Sm	6	*S.* Abortusequi (6)
17	F, Na, Sm	1	*S.* Typhimurium (1)
18	G, Na, Sm	1	Abortusequi (1)
19	Na, Sm, T	8	*S.* Paratyphi B var Java (3), *S.* Typhimurium (3), *S.* Saintpaul (2)
20	A, Ac, Ce, F	1	*S.* Bovismorbificans (1)
21	A, Ac, Sm, T	1	*S.* Richmond (1)
22	A, F, Sm, T	1	*S.I.* 4,5,12:r,i:i,5 (1)
23	A, Na, Sm, T	1	*S.* Paratyphi B var Java (1)
24	Cf, Na, Sm, T	1	*S.* Typhimurium (1)
25	F, G, S, Sm	3	*S.* Abortusequi 92), *S.* Tshiongwe (1)
26	F, K, Na, Sm	3	*S.* Abortusequi (3)
27	F, Na, Sm, T	1	*S.* Saintpaul (1)
28	F, S, Sm, T	8	*S.I.* 4,5,12:ri:i,5 (5), *S.* Bovismorbificans (1) *S.* Dumfries (1), *S.* Drogana (1)
29	F, K, S, Sm	1	*S.* Abortusequi (1)
30	A, Ce, F, K, Sm	1	*S.* Abortusequi (1)
31	A, Cf, Na, Sm, T	1	*S.* Paratyphi B var Java (1)
32	A, F, S, Sm, T	1	*S.I.* 4,5,12:r,i:i,5 (1)
33	A, Ce, S, Sm, T	1	*S.* Drogana (1)
34	A, F, Na, Sm, T	3	*S.* Paratyphi B var Java (3)
35	Ce, C, Na, Sm, T	1	*S.* Saintpaul (1)
36	Ce, F, Na, Sm, T	1	*S.* Paratyphi B var Java (1)
37	Cf, F, K, Na, Sm	2	*S.* Abortusequi (2)
38	Cf, F, G, K, Sm	1	*S.* Abortusequi (1)
39	F, K, Na, S, Sm	1	*S.* Abortusequi (1)
40	F, G, K, S, Sm	7	*S.* Abortusequi (1), *S.* Dumfries (1), *S.* Newport (5)
41	F, K, S, Sm, T	1	*S.I.* 4,5,12:r,i:i,5 (1)
42	F, K, S, Sm, Tm	1	*S.* Abortusequi (1)
43	Cf, F, K, Na, Sm, Tm	1	*S.* Abortusequi (1)
44	F, G, K, Na, S, Sm	1	*S.* Abortusequi (1)
45	Cf, F, G, K, Na, Sm	1	*S.* Abortusequi (1)
46	F, G, K, S, Sm, T	1	*S.* Newport (1)
47	A, Ac, C, F, T, Tm	1	*S.* Paratyphi B var Java (1)
48	A, Ac, Cf, K, F, S, Sm	1	*S.* Abortusequi (1)
49	A, Cf, F, Na, Sm, T	1	*S.* Paratyphi B var Java (1)
50	Ce, F, G, K, S, Sm, T	1	*S.* Abortusequi (1)
51	A, Ac, F, S, Sm, T, Tm	1	*S.I.* 4,5,12:r,i:i,5 (1)

A, ampicillin; Ac, ampicillin+cloxacillin; Ce, cefotaxime; C, chloramphenicol; Cf, ciprofloxacin; G, gentamicin; K, kanamycin; Na, nalidixic acid; F, furazolidone; S, streptomycin; Sm, sulphamethoxazole; T, tetracycline; Tm, trimethoprim.

traits, even in absence of antimicrobial selection pressure, might be due to either the ability of *Salmonella* to build up multidrug resistance as an integral part of its genome [29] or to recent acquisition of MDR strains by equids from other animals and human beings.

Cefotaxime resistance in only 5% of isolates of *Salmonella* of equine origin supports recommending this drug as the safest choice for treatment of salmonellosis in equids [28]. Low levels of resistance to cefotaxime in isolates of *Salmonella* from equids might be attributed to either less use of this third-generation cephalosporin in equids [26,28] or in other animals and human beings probably due to its comparatively high cost compared to broad-spectrum and cheaper fluoroquinolones [42].

The study concludes that MDR is common among *Salmonella* isolates of equine origin in India. Detection of MDR in more than 75% of *Salmonella* strains, even in the serovars rarely causing any disease in equids, indicated that the horse is a natural host of *Salmonella*. Resistance to antimicrobial drugs not used in equids also suggests that either *Salmonella* maintains the MDR gene pool on chromosomes even in absence of selection pressure, or that *Salmonella* is frequently communicable between different host species or that both of the factors required in MDR development and maintenance might be important.

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