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Anti-microbial sensitivity pattern of *Salmonella* species from animal and environmental source

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Abstract

Salmonella spp. is one of the most important zoonotic pathogens transmitted among humans, animals, and birds. In recent times, there is a high risk of emergence of the multi-drug-resistant strains due to the similarity of antibiotic classes used to treat animals and humans. Hence, the present study was aimed at performing antimicrobial susceptibility testing using Kirby-Bauer's disc diffusion method and to determine the anti-microbial sensitivity pattern. *Salmonella enterica* isolates showed 100% resistance to Penicillin-G and varied degree of resistance to rest of the selected class of anti-microbials. *Salmonella typhimurium* isolates showed multiple antibiotic resistance index of >0.2 for Ampicillin and Penicillin-G. Antibiotic resistance have a profound effect on future treatment options in food animals and humans, which is a major public health concern.

Keywords: Antimicrobial susceptibility testing, multi-drug resistant, *Salmonella*

1. Introduction

Salmonella is an important zoonotic agent with major public health as well as food safety concern and occurs worldwide. Salmonellosis is a hyperendemic disease in India that affects both humans, animals and birds (Raichowdhuri *et al.*, 1983) ^[1] and exhibits clinically as typhoidal and nontyphoidal Salmonellosis (Hendriksen *et al.*, 2011) ^[2]. Typhoidal *Salmonella* are restricted to humans with few serotypes viz. Typhi, Sendai, and Paratyphi A, B, and C, whereas Non-typhoidal *Salmonella* (NTS) includes thousands of serotypes like Typhimurium, Enteritidis *etc.*, with broad host range such as cattle, swine, poultry, wild birds (gulls) and pets which acts as common source of foodborne illness in humans.

Anti-microbial resistant (AMR) and multi drug resistance (MDR) by *Salmonella* serotypes which are involved in infection and intoxication in humans, animal and bird population is a well-established fact. Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant (AMR) *Salmonella* associated with consumption of contaminated milk and meat products (Zewdu *et al.*, 2009) ^[3] through mutation, acquisition of resistance encoding genes and irrational use of antimicrobials in food animals (Fluit, 2005) ^[4]. Excretion of *Salmonella* with feces can contaminate water, soil, other animals and feed. Therefore, this study was conducted to identify the anti-microbial resistance pattern among *Salmonella* species from animal and environmental sources at Shivamogga district of Karnataka state.

2. Materials and Methods

A total of 9 different isolates comprising of 6 *Salmonella typhimurium* and 3 *Salmonella enterica* spp., isolated from different sources viz. feces of animal and bird origin, cloacal swabs, soil, and floor swabs and were confirmed using PCR and were maintained in glycerol stock at Department of Veterinary Public Health and Epidemiology, Veterinary College-Shivamogga, Karnataka. These confirmed isolates were revived and selected for the present study.

2.1 Antimicrobial susceptibility test of *Salmonella* isolates

Antimicrobial susceptibility testing (AST) was done using Kirby-Bauer's disc diffusion method (Bauer *et al.*, 1966) ^[5] with break-point interpretations according to Clinical and

Laboratory Standards Institute (CLSI, 2018). Confirmed pure *Salmonella* isolates of the present study were subjected for AST against different antibiotics discs (Hi-media®).

2.1.1 Preparation of Mueller-Hinton agar

Mueller-Hinton agar (MHA) was made according to the instructions of the manufacturer (Hi-media®). The MHA media was autoclaved at 121 °C for 15 minutes and allowed to cool in room temperature for about 40 °C to 50 °C. Later, about 12-15 ml of molten agar was poured into petri plates to get an agar bed of about 4 mm thickness.

2.1.2 Inoculum preparation

Pure colonies of *Salmonella* isolates of 24 hours old culture on nutrient agar were inoculated to tube containing sterile normal saline solution, vortexed and the suspension was compared and adjusted to 0.5 McFarland standards (McFarland Standards Set, Hi-Media®). Freshly prepared suspensions were used within 15 minutes of their preparation.

2.1.3 Inoculation onto MHA plates

After the adjustment of the turbidity, sterile cotton swab was dipped into the inoculum, rotated several times and the excess amount of inoculum was removed by pressing the swab to the inside wall of the tube. Using the swab, the culture was inoculated by spreading onto entire MHA agar surface and ensuring uniform distribution of the inoculum by rotating the agar plate. Finally, the rim of the agar plates was swabbed, and entire procedure was done under biosafety cabinet aseptically. To absorb any excess moisture present on the agar surface, lid of the agar plates was left partly open for 3-5 minutes before applying the antibiotic discs.

2.2 Selection of antimicrobials for AST

Different class of antimicrobials were selected to perform AST as shown in the Table 1.

2.3 Application of the discs to inoculated agar plates

Selected antimicrobial discs (Hi-media®) were placed onto inoculated agar plate using sterile forceps. Each disc was placed over the agar surface, pressed down and each disc was not closer than 24 mm from center-to-center and made sure that the surface of each disc was in complete contact with the agar surface. Within 15 minutes after applying the discs, plates were kept inverted in incubator at 37 °C for 24

hours.

3. Results and discussion

In the present study, *Salmonella enterica* isolates showed 100% resistance to penicillin-G and for other antimicrobials like ampicillin, cefotaxime, ceftriaxone, ceftazidime, doxycycline, kanamycin, nalidixic acid and tetracycline (Fig. 1) showed resistance of 66.66%, 33.33%, 33.33%, 33.33%, 66.66%, 33.33%, 33.33% and 33.33%, respectively (Fig. 2).

Salmonella typhimurium isolates were found resistant to penicillin-G (100%) and showed varied degree of resistance for ampicillin (66.66%), cefotaxime (16.66%), ceftriaxone (33.33%), ceftazidime (16.66%), doxycycline (33.33%), gentamicin (33.33%), nalidixic acid (16.66%) and tetracycline (16.66%) (Table 2 and Fig. 3).

Improper use of antimicrobials in the animal production systems has led to the cause of emergence of antimicrobial resistant (AMR) *Salmonella* when organism mutates or acquires a resistance gene (Zhang *et al.*, 2006) [6]. This property of the *Salmonella* has failed to respond to the many antimicrobials, which results into multidrug resistance that leads to prolonged illness and reduces the effectiveness of treatment (Mitema *et al.*, 2001) [7]. These AMR *Salmonella* are transmitted to humans through consumption of animal origin products and emerged as major public health issue worldwide (Marshall *et al.*, 2011) [8].

Similar to the present study, Suresh *et al.* (2019) [9] showed that highest *Salmonella* isolates were resistant to amikacin (61.90%), followed by ampicillin, tetracycline, ceftriaxone, cefotaxime, gentamicin, nalidixic acid, sulfamethoxazole, ciprofloxacin, doxycycline hydrochloride, chloramphenicol and streptomycin with percentage of 52.30%, 61.90%, 52.30%, 38.09, 33.33%, 28.57%, 28.57%, 23.80%, 19.04%, 19.04% and 9.52%, respectively.

The study of Velasquez *et al.* (2018) [10] showed that *Salmonella* spp. isolated from poultry farm were 63% (12/19) resistant to tetracycline, followed by 26% (5/19) resistant to streptomycin and 21% (4/19) to nalidixic acid. The present study showed similarity with the study conducted by Dusadee *et al.* (2019) [11] that showed common resistance of *Salmonella* isolates from broiler against nalidixic acid (49.54%), followed by ampicillin (30.28%), tetracycline (27.52%), amoxicillin (26.61%), ciprofloxacin (23.85%), norfloxacin (19.27%), chloramphenicol (4.59%), trimethoprim/sulfamethoxazole (4.59%), and ceftazidime (1.83%).

Table 1: List of antimicrobial discs used for AMST of *Salmonella* isolates

Sl. No.	Antimicrobial discs	Code	Disc content
1.	Ampicillin	AMP	10 µg
2.	Azithromycin	AZM	15 µg
3.	Cefotaxime	CTX	5 µg
4.	Ceftriaxone	CTR	30 µg
5.	Ceftazidime	CAZ	30 µg
6.	Chloramphenicol	C	30 µg
7.	Ciprofloxacin	CIP	5 µg
8.	Co-trimoxazole	COT	25 µg
9.	Doxycycline	DO	30 µg
10.	Gentamicin	GEN	10 µg
11.	Kanamycin	K	30 µg

Table 2: Antibioqram of *Salmonella* species/subspecies/serotypes isolated from different eco-epidemiological regions of animal interface

Sl. No.	Antimicrobial discs	Code	Disc content	MAR index	Sensitive		Resistant	
					No. of isolates	%	No. of isolates	%
Salmonella typhimurium (n = 6)								
1.	Ampicillin	AMP	10 µg	0.25	2	33.33	4	66.66
2.	Azithromycin	AZM	15 µg	NA	6	100	0	0
3.	Cefotaxime	CTX	5 µg	0.0625	5	83.33	1	16.66
4.	Ceftriaxone	CTR	30 µg	0.125	4	66.66	2	33.33
5.	Ceftazidime	CAZ	30 µg	0.0625	5	83.33	1	16.66
6.	Chloramphenicol	C	30 µg	NA	6	100	0	0
7.	Ciprofloxacin	CIP	5 µg	NA	6	100	0	0
8.	Co-trimoxazole	COT	25 µg	NA	6	100	0	0
9.	Doxycycline	DO	30 µg	0.125	4	66.66	2	33.33
10.	Gentamicin	GEN	10 µg	0.125	4	66.66	2	33.33
11.	Kanamycin	K	30 µg	NA	6	100	0	0
12.	Levofloxacin	LE	5 µg	NA	6	100	0	0
13.	Nalidixic acid	NA	30 µg	0.0625	5	83.33	1	16.66
14.	Penicillin-G	P	10 units	0.375	0	0	6	100
15.	Streptomycin	S	10	NA	6	100	0	0
16.	Tetracycline	TE	30	0.0625	5	83.33	1	16.66
Salmonella enterica species (n = 3)								
1.	Ampicillin	AMP	10 µg	0.125	1	33.33	2	66.66
2.	Azithromycin	AZM	15 µg	NA	3	100	0	0
3.	Cefotaxime	CTX	5 µg	0.0625	2	66.66	1	33.33
4.	Ceftriaxone	CTR	30 µg	0.0625	2	66.66	1	33.33
5.	Ceftazidime	CAZ	30 µg	0.0625	2	66.66	1	33.33
6.	Chloramphenicol	C	30 µg	NA	3	100	0	0
7.	Ciprofloxacin	CIP	5 µg	NA	3	100	0	0
8.	Co-trimoxazole	COT	25 µg	NA	3	100	0	0
9.	Doxycycline	DO	30 µg	0.125	1	33.33	2	66.66
10.	Gentamicin	GEN	10 µg	NA	3	100	0	0
11.	Kanamycin	K	30 µg	0.0625	2	66.66	1	33.33
12.	Levofloxacin	LE	5 µg	NA	3	100	0	0
13.	Nalidixic acid	NA	30 µg	0.0625	2	66.66	1	33.33
14.	Penicillin-G	P	10 units	0.187	0	0	3	100
15.	Streptomycin	S	10 µg	NA	3	100	0	0
16.	Tetracycline	TE	30 µg	0.0625	2	66.66	1	33.33

Note: 1) Intermediate zone of inhibition is considered as resistance for calculation of MAR index

NA: Not applicable



Fig 1: Antimicrobial susceptibility pattern of *Salmonella* species/subspecies on Mueller-Hinton agar plate
Plate A, B and C showing zone of inhibition for different antimicrobial discs

4. Conclusion

The present study indicated that the results should be taken as precautionary warning for the spread of multidrug resistance. Contact with animal feces, feeds, water sources,

agriculture etc. may be cause of the transmission of resistant genes from various vectors to food producing animals.

Salmonella strains pose significant health problems worldwide by showing resistance against most beta-lactam antibiotics, which were previously used for treatment *Salmonella* infections.

5. Acknowledgement

There was no conflict of interest.

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