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Study of antibiogram profile of *Salmonella* spp. from diarrheic cases of pigs

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Abstract
The present study was designed to study the antibiogram profile of the *Salmonella* spp. from pigs. A total of 175 fecal samples collected from different pig farms which are located in Bidar, Hassan and Tumakuru districts of Karnataka In the present study, all twenty four *Salmonella* spp. isolates were subjected to antibiotic susceptibility test. About eight antibiotic agent's viz., Co-Trimoxazole (100%), Enrofloxacin (87.5%), Gentamicin (79.3%), Tetracycline (79.3%), Norfloxacin (54.2%), Ceftriaxon/ Tazobactam (50%), Chloramphenicol (45.8%), Amikacin (12.5%) showed complete sensitivity against *Salmonella* spp. isolates. Further, eight antibiotic agents viz., Amikacin (62.5%), Ceftriaxon/Tazobactam (50%), Neomycin (45.8%), Norfloxacin (25.1%), Chloramphenicol (25%), Gentamicin (20.7%), Enrofloxacin (12.5%), Amoxyclav (4.2%) showed intermediate sensitivity towards isolates. .*Salmonella* spp. isolates were found highly resistant towards Ampicillin (100%), Cefepime (100%), Piperacillin/ Tazobactam (100%), Erythromycin (100%) followed by Amoxyclav (95.8%), while moderately resistant towards Neomycin (54.2%) and lesser percent of isolates were resistant to Chloramphenicol (29.2%), Amikacin (25%), Tetracycline (20.7%), Norfloxacin (20.7%).

Keywords: Antibiogram, antimicrobial susceptibility, co-trimoxazole, enrofloxacin, gentamicin

Introduction
Pig rearing is one of the traditional activities in India carried out by rural folk. Among various livestock activities, piggery is most efficient way of meat production utilizing kitchen waste, vegetable waste etc. Though initially local breeds have been raised, now-a-days exotic pig breeding is popular and pork from such animal is having wide acceptance. Further, pig farming requires small investment on building and equipment's.

The World Health Organization (WHO) reports that the incidence and severity of cases of Salmonellosis have increased significantly (WHO, 2010) [27]. Strains resistant to a wide range of antimicrobials emerged in the 1990s and constitute a serious additional concern for public health (WHO, 2010) [27]. The economic impact of this zoonosis in commercial food production is also substantial and control of *Salmonella* spp.is becoming more challenging with the trend towards cheaper and faster food.

The vast majority (99.5%) of strains of *Salmonella* spp. isolated from humans and warm-blooded animals belong to subspecies I (Grimont, 2007) [8], while the other five subspecies II-V and *S. bongori* are primarily associated with cold-blooded animals and are only infrequently isolated from mammals (Foti *et al*., 2009 [6]; Nastasi A, 1999 [19]). *Salmonella* spp. are generally regarded as part of the normal intestinal flora of reptiles kept as pets (Warwick *et al*., 2001) [28] and reports suggests that wild terrestrial reptiles may be reservoirs of *Salmonella* spp. (Hidalgo-Vila *et al.*, 2007 [10]; Briones *et al.*, 2004 [2]). Moreover, amphibians, fish and even insects can be infected by *Salmonella* spp. (CDC, 2003 [3]; Mitscherlich, 1984 [18]; Greenberg *et al.*, 1970) [7].

According to WHO and the European Food Safety Authority (EFSA), all serotypes of *Salmonella enterica* are potentially hazardous to human health and thus regarded as pathogens (EFSA, 2010) [4]. However the majority of *Salmonella* spp. infections reported in humans and domestic animals are caused by relatively few of the more than 2500 serotypes.

In recent years problems related to *Salmonella* spp. have increased significantly due to emergence of multi drug resistant *Salmonella* spp. (WHO, 2010) [27]. Conventional antimicrobial agents, such as Ampicillin, Chloramphenicol, and Trimethoprim-Sulfamethoxazole have been the drug of choice in the treatment of Salmonellosis before the 1980s. However, multi-drug resistance, with rates of resistance to these antimicrobial agents of more than 50 percent has been reported in many areas of the world.

Materials and Methods

The material and methods used in the present study are presented in this chapter.

Antibiogram of *Salmonella* spp. isolates

The antibiotic susceptibility tests were performed as per method described by Bauer *et al*. (1966) [29] to find out the antibiotic resistance pattern of all *Salmonella* spp. isolates. *In vitro* antibiotic sensitivity test of the isolates was conducted by paper disc diffusion method using the discs supplied by HiMedia laboratories Pvt. Ltd, Mumbai (India). Isolates were subjected to antimicrobial sensitivity tests against 14 antibiotics as per the table 6.

Salmonella spp. isolates were grown in 5 ml nutrient broth (HiMedia) of 12 hrs at 37 ºC in incubator. The 1-2 ml grown broth culture were poured on Muller-Hinton agar plates (HiMedia) with micropipette. Left for 10 min. For pre-diffusion time. Then ethanol dipped and flamed forceps different antibiotic discs were placed on the agar surface at about two cm apart. The discs were slightly pressed with the forceps to make complete contact with the medium. The plates were incubated at 37 ºC for 18hrs. After the incubation period, the diameter of inhibition zones were measured and compared with interpretative chart provided by the manufacture and zones were graded as sensitive, intermediate and resistant.

Table 1: List of antimicrobial susceptibility discs used in antibiogram study of *Salmonella* spp. isolates

SL No	Antimicrobial agent	Symbol	Concentration (µg)	Diameter of zone of inhibition (mm)		
				Resistant	Intermediate	sensitive
1	Ampicillin	AMP	2	13	14-16	17
2	Cefepime	CPM	30	21	22-23	24
3	Gentamicin	HLG	120	14	15-16	17
4	Tetracycline	TE	30	11	12-14	15
5	Amikacin	AK	30	15	16-17	18
6	Enrofloxacin	Ex	5	19	20-21	22
7	Norfloxacin	Nx	10	19	20-21	22
8	Piperacillin/ Tazobactam	P/T	100	17	18-20	21
9	Co-trimoxazole	CoT	25	21	22-49	50
10	Chloramphenicol	C	50	17	-	17
11	Amoxyclav	AMC	30	13	14-17	18
12	Neomycin	N	30	18	14-17	18
13	Ceftriaxone/ Tazobactam	C/T	30/10	19	20-22	23
14	Erythromycin	E	15	13	14-22	23

Results

The present study was carried out with an objective to study the antibiogram profile of the *Salmonella* spp. from suspected c cases in pigs from different pig farms in Bidar, Hassan and Tumkuru districts of Karnataka. The result obtained during the programmer of research work was documented as follows.

Collection of samples

A total 175 samples were collected from different pig farms of Karnataka. The samples were processed in the Dept. of Veterinary Microbiology, Veterinary College and Bidar by standard protocols.

Antimicrobial sensitivity testing of *Salmonella* spp. isolates

The isolates were subjected for antibiogram test to know the antibiotic susceptibility pattern. The symbols and concentrations of antibiotics and their zone of inhibition in diameters for interpretation are given in Table 10. The overall antibiotic sensitivity pattern of *Salmonella* spp. isolates towards 14 antimicrobial agent's *viz*. Ampicillin, Cefapime, Gentamicin, Tetracycline, Amikacin, Enrofloxacin, Norfloxacin, Piperacillin/Tazobactam, Co-Trimoxazole, Chloramphenicol, Amoxyclav, Neomycin, Ceftriaxon/ Tazobactam and Erythromycin. The results are shown in table 10.

In the present study, all twenty four *Salmonella* spp. isolates were subjected to antibiotic susceptibility test. About eight antibiotic agent's *viz*., Co-Trimoxazole (100%), Enrofloxacin (87.5%), Gentamicin (79.3%), Tetracycline (79.3%), Norfloxacin (54.2%), Ceftriaxon/Tazobactam (50%), Chloramphenicol (45.8%) and Amikacin (12.5%) showed complete sensitivity against *Salmonella* spp. isolates. Further, eight antibiotic agents viz., Amikacin (62.5%), Ceftriaxon/ Tazobactam (50%), Neomycin (45.8%), Norfloxacin (25.1%), Chloramphenicol (25%), Gentamicin (20.7%), Enrofloxacin (12.5%), Amoxyclav (4.2%) showed intermediate sensitivity towards isolates.

Salmonella spp. isolates were found highly resistant towards Ampicillin (100%), Cefepime (100%), Piperacillin/ Tazobactam (100%), Erythromycin (100%) followed by Amoxyclav (95.8%), while moderately resistant towards Neomycin (54.2%) and lesser percent of isolates were resistant to Chloramphenicol (29.2%), Amikacin (25%), Tetracycline (20.7%), Norfloxacin (20.7%). All these results are being tabulated in Table 10 and plates with zone of inhibition shown in Plate 18.

The overall multiple drug resistance patterns of twenty-four *Salmonella* spp. isolates revealed that some *Salmonella* spp. isolates were resistant to two or more antibiotics. The overall multiple drug resistance patterns of 24 *Salmonella* spp. isolates revealed that maximum isolates were resistant towards four antibiotics used in the study followed by other some *Salmonella* spp. isolates are found resistant to six antibiotics (Table 10 and Plate 17).

Table 2: Antibiogram of the *Salmonella* spp. isolates

Sl. No.	Antimicrobial agent	Diameter of zone of inhibition (mm)		
		Sensitive	Intermediate	Resistant
1	Ampicillin	0	0	24(100%)
2	Cefepime	0	0	24(100%)
3	Gentamicin	19(79.3%)	5(20.7%)	0
4	Tetracycline	19(79.3%)	0	5(20.7%)
5	Amikacin	3(12.5%)	15(62.5%)	6(25%)
6	Enrofloxacin	21(87.5%)	3(12.5%)	0
7	Norfloxacin	13(54.3%)	6(25%)	5(20.7%)
8	Piperacillin/Tazobactam	0	0	24(100%)
9	Co-trimoxazole	24(100%)	0	0
10	Chloramphenicol	11(45.8%)	6(25%)	7(29.2%)
11	Amoxyclav	0	1(4.2%)	23(95.8%)
12	Neomycin	0	11(45.8%)	13(54.2%)
13	Ceftriaxone/Tazobactam	12(50%)	12(50%)	0
14	Erythromycine	0	0	24(100%)

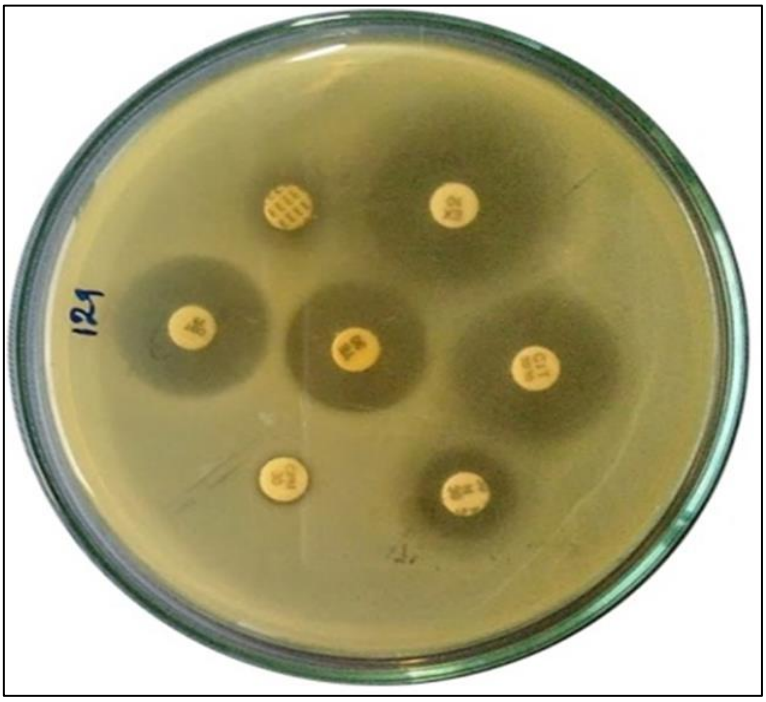

Plate 1: Antibiotic sensitivity pattern of *Salmonella* spp. isolates showing MDR pattern

Discussion

Antibiogram of *Salmonella* spp. isolates

Many pathogenic bacteria resistant to antimicrobial agents get transferred into man and animals directly from different food products. These circumstances may have a major impact on the degree for success in treating infectious diseases in man. Several recent examples demonstrated that use of antibiotics in all parts of the food production chain contributes to the increasing level of antibiotic resistance among the food-borne pathogenic bacteria. Modern industrialized food production adds extra emphasis on lowering the use of antibiotics in all parts of agriculture, animal husbandry and livestock farming because these food products are distributed to very large numbers of humans compared to more traditional smaller scale niche production. Antimicrobial resistance in the food born *Salmonella* spp. is becoming a problem worldwide in recent years. Studies have shown that serotypes of *Salmonella* spp. isolated from humans or from food production or processing facilities are resistant to one or more antibiotics (Miko *et al*., 2005 [16]; Sharma *et al*., 2005 [23]; Miranda *et al*., 2009 [17] and Enabulele *et al*., 2010) [5].

The antibiogram of twenty-four *Salmonella* spp. isolates subjected to antibiogram of aim with selected 14 antibiotics (Ampicillin, Cefapime, Gentamicin, Tetracycline, Amikacin, Enrofloxacin, Norfloxacin, Piperacillin/Tazobactam, Co-Trimoxazole, Chloromphenicol, Amoxyclav, Neomycin, Ceftriaxon/Tazobactam and Erythromycin) by disc diffusion method, showed variably resistant to the antibiotics tested. The pattern clearly indicates that the overall high percent of *Salmonella* spp. isolates were resistant to Ampicillin (100%), Cefepime (100%), Piperacillin/Tazobactam (100%), Erythromycin (100%) followed by Amoxyclav (95.8%), while moderately resistant to Neomycin (54.2%) and lesser percent of isolates were resistant to Chloramphenicol (29.2%), Amikacin (25%), Tetracycline (20.7%), Norfloxacin (20.7%). Highest sensitivity was shown towards Co-Trimoxazole (100%), following Enrofloxacin (87.5%), Gentamicin (79.3%), Tetracycline (79.3%), Norfloxacin (54.2%), Ceftriaxon/ Tazobactam (50%), Chloramphenicol (45.8%), Amikacin (12.5%). Eight antibiotic agents showed intermediate sensitivity towards Amikacin (62.5%), Ceftriaxon/ Tazobactam (50%), Neomycin (45.8%), Norfloxacin (25.1%), Chloramphenicol (25%), Gentamicin (20.7%), Enrofloxacin (12.5%), Amoxyclav (4.2%).

In present study, the sensitivity pattern towards Co-Trimoxazole (100%), Enrofloxacin (87.5%), Gentamicin (79.3%), Tetracycline (79.3%), Norfloxacin (54.2%), Ceftriaxon/Tazobactam (50%), Chloromphenicol (45.8%), Amikacin (12.5%) which are in accordance with Smith *et al*. (2010) [25]. Earlier Bourchrif *et al*. (2009), recorded 99 percent sensitivity of *Salmonella* spp. isolates to Ceftriaxone and 96 percent sensitivity in one study which is in close approximation of present findings. While lower sensitivity was reported by Selvaraj *et al*. (2010) [30] for Ceftriaxone (62.50%).

Sivasankaran *et al*. (2013) [31] observed 100 percent sensitivity to Ceftriaxone, Enrofloxacin, Gentamicin followed by Chloramphenicol (93.5%) and Tetracycline (95.2%).

The overall resistance pattern of *Salmonella* spp. isolates in the present study to Amikacin (62.5%) shows intermediate sensitivity in accordance with Valdezate *et al*. (2007) [26]. The observations in the study about sensitivity (45.8%) of *Salmonella* spp. isolates towards Chloramphenicol is analogous with the findings of Singh (2012) [24], Selvaraj *et al*. (2010) [30], Smith *et al*. (2010) [25] and Lambey *et al*. (2009) [15].

In present study, intermediate sensitivity to Neomycin (46.8%) and Gentamicin (20.7%) was in accordance with the findings of Selvaraj *et al*. (2010) [30], Kumar *et al*. (2009) [14] and Bouchrif *et al*. (2009) [1]. Here Co-trimoxazole shows 100 percent highest sensitivity with similar findings in Noor Zeba *et al*. (2015) [20].

In the present study *Salmonella* spp. were found 100 percent resistance was observed to Ampicillin, Cefepime, Piperacillin/ Tazobactam, Erythromycin followed by Amoxyclav similar findings were observed in Amoxyclav by Karthik *et al*., (2016) [12]. Kalambhe *et al*. (2016) [11] recorded two isolates shows resistance to Ampicillin and Co-trimoxazole. Erythromycin was 100 percent resistant to *Salmonella* spp. were observed by Paramesh, (2015) [21] and Lambey *et al*., (2009) [15]. Ampicillin resistant observed by Singh *et al*., (2012) [24]; Kaushik *et al*., (2014) [13] and Hasan *et al*. (2011) [9] recorded MDR showing Ampicillin, Co-trimoxazole and Chloramphenicol.

Based on the results obtained from antibiotic sensitivity tests, the conclusion can be derived is Co-Trimoxazole followed by Enrofloxacin, Gentamicin and Tetracycline are more effective against *Salmonella* spp. isolates and these findings can be used in treatment of *Salmonella* spp. infection in diarrheic cases of pigs.

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