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Research Article

PHENOTYPIC DETERMINATION OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA TYPHIMURIUM* OF ANIMAL ORIGIN

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ABSTRACT

The present study was conducted for determination of phenotypic antimicrobial resistance in *Salmonella Typhimurium*. All the isolates (n=12) were subjected to *in vitro* antimicrobial resistance study against 25 antimicrobial agents belonging to 10 antimicrobial groups. Antibiogram revealed 100% resistance towards cloxacillin, metronidazole and chloramphenicol followed by ceftriaxone sulbactum, ceftriaxone tazobactum, gemifloxacin and least resistance was recorded against amikacin, ampicillin, amoxicillin and gatifloxacin. On the other hand, all the isolates were susceptible for enrofloxacin, ciprofloxacin, enoxacin, levofloxacin, norfloxacin and pefloxacin. Remarkable sensitivity of flouroquinolones amongst all the antimicrobial groups tested points to the selective potential of fluoroquinolones in treating salmonellosis in all animal species.

Keywords: Antimicrobial resistance, *Salmonella typhimurium*, Serotyping

Salmonella is Gram-negative facultative intracellular pathogen of major zoonotic significance. The organism is widespread in the intestines of most of the wild and domestic birds, reptiles and mammals, including humans and domestic livestock. Depending on the serotype and host, it causes a variety of diseases ranging from acute gastroenteritis to systemic infections. The pattern of distribution differs for individual Salmonella serotypes. S. Typhimurium infects all animal species and has a worldwide distribution, while distribution of serotypes like S. choleraeusuis (porcine), S. dublin (bovine) and S. enteritidis (poultry) matches the distribution of the hosts to which they are adapted (Ekperigin and Nagaraja, 1998). Salmonella infection is usually acquired through direct contact with faeces and indirectly from contaminated food or inanimate objects, and usually can be controlled using antibiotics.

Antibiotics are amongst the most commonly prescribed drugs in veterinary medicine and indiscriminate therapeutic usage leads to potential development of resistance. Hence, microorganisms are frequently exposed to the selective pressure of antibiotics leading to the development of resistance. Resistance to antimicrobial agents in bacteria is mediated by several mechanisms, including changes in bacterial cell wall permeability, energy-dependent removal of antimicrobials via membrane-bound efflux pumps, modification of the site of drug action, and destruction or inactivation of antimicrobials (Chen et al., 2004).

Antibiotic resistance in the bacteria may be acquired through the acquisition of resistant genes by horizontal gene transfer or the mutation of existing genes. Acquired resistance occurs in strains of organism that would normally be susceptible to drug and it becomes problematic when with the drug usage, the growth increases. This loss in susceptibility may be due to mutational or transmissible resistance. Mutational resistance arises spontaneously in a few cells in the large dividing population, whereas transmissible resistance is through the transfer of genetic material thereby conferring resistance, from resistant to susceptible bacteria (Aarestrup, 2005).

The emergence of strains showing multi-resistance to several antimicrobial drugs, referred to as multidrug resistant (MDR), is a public health concern. Different mechanisms of resistance may exist against the same drug in different strains of pathogens (Leclercq and Courvalin, 2002).

To generate baseline data in future risk assessment of antimicrobial resistance, a number of surveillance systems on the local, continental or global scale have been initiated. Consequently, the prevalence and molecular basis of antimicrobial resistance in Salmonella strains from humans, livestock and food have also been investigated in several countries. This paper describes the determination of phenotypic antimicrobial drug resistance in Salmonella typhimurium isolates obtained from cattle, buffalo, primate and emu (domestic and wildlife origin).

MATERIALS AND METHODS

A total of twelve Salmonella typhimurium isolates (n=12) from cattle, buffalo, primate and poultry, were identified on the basis of morphology of colonies on Hektoen Enteric (HE) agar, Brilliant green agar (BGA), MacConkey Lactose agar (MLA), Xylose Lysine Deoxycholate agar (XLD agar) and Tryptone Soya Agar (TSA). Biochemically, all the isolates were positive for methyl red, nitrate reduction and citrate utilization whereas all the isolates were negative for urease, lysine decarboxylation, gelatin liquefaction, indole and Voges-Proskauer.

These isolates were sent to National Salmonella and Escherichia Centre, Central Research Institute, Kasauli (Himachal Pradesh) for serotyping. All isolates (n=12) from wild animals and livestock found to be Salmonella typhimurium with antigenic structure 4,2:i:1,2. Out of twelve, four isolates were from cattle, five from buffalo, two from primate and one from poultry (emu). The isolates were maintained as glycerol suspension (20% v/v) at –80^{0}C for long-term preservation and further study.

In-vitro drug sensitivity of Salmonella isolates

The antimicrobial susceptibility testing of isolates

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Table 1
Antimicrobial resistance pattern of *Salmonella* isolates (n=12)

S. No	Antibiotic group	Antibiotic	Concentration (µg)	% Resistance
1.	Tetracyclines	Tetracycline TE	10	75
		Oxytetracycline O	30	66.7
2.	Sulphonamides	Sulphadiazine SZ	100	25
		Trimethoprim TR	10	25
3.	Quinolones	Nalidixic acid NA	30	33.3
4.	Penicillins	Ampicillin AMP	10	8.3
		Amoxycillin AMX	10	8.3
		Cloxacillin COX	10	100
5.	Penicillin/ β lactamase inhibitor combination	Amoxycillin/ Sulbactum AMS	30/15	0
6.	Flouroquinolones	Enrofloxacin EX	10	0
		Ciprorofloxacin CIP	5	0
		Enoxacin EX	10	0
		Gemifloxacin GEM	5	33.3
		Norfloxacin NX	10	0
		Levofloxacin LE	5	0
		Gatifloxacin GAT	5	0
		Pefloxacin PF	5	0
7.	Aminoglycosides	Gentamicin GEN	10	16.7
		Amikacin AK	30	8.3
8.	Cephalosporins	Ceftriaxon CTR	30	16.7
		Cefaperazon CPZ	75	16.7
		Ceftriaxon/ Sulbactum CIS	30/15	58.3
		Ceftriaxon/ Tazobactum CIT	30/10	41.7
9.	Nitroimidazoles	Metronidazole MT	5	100
10.	Macrolides	Chloramphenicol CHL	30	100

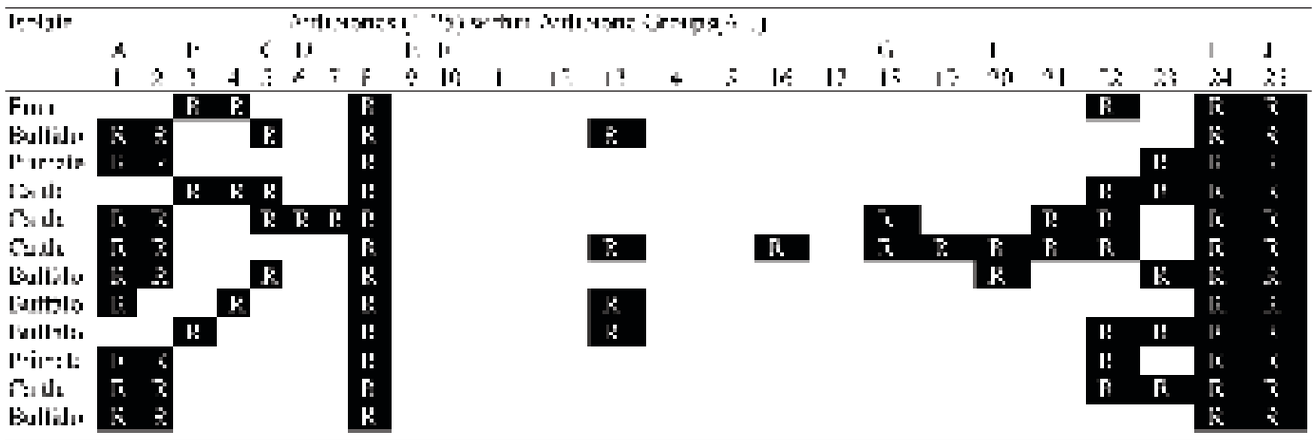
was done on Mueller-Hinton agar (HiMedia) using the disc diffusion method (Quinn et al., 1994). The antibiotic discs along with their concentration is given in the Table 1. Antibiotics, for which interpretive criteria was not available as per CSLI guidelines, breakpoints of antibiotic in similar group were used. Phenotypic antibiotic resistance pattern was interpreted as per guidelines of Magiorakos et al. (2012). Isolates resistant to three or more antibiotics belonging to different groups were classified as multidrug resistant (MDR). Among MDR isolates, isolates susceptible to only two antibiotics belonging to two different groups were considered extreme drug resistant (XDR), while resistance to all the antibiotics was considered as pan-drug resistant (PDR). To remain conservative in our estimates of resistance; isolates exhibiting intermediate zones of inhibition were interpreted as sensitive.

RESULTS AND DISCUSSION

All the isolates (n=12) from wild animals and livestock were found to be of Salmonella Typhimurium with antigenic structure 4,2:i:1,2 on serotyping done from National Salmonella and Escherichia Centre, Central Research Institute, Kasauli (Himachal Pradesh. The antimicrobial susceptibility testing was done (Table 1) for all the twelve Salmonella typhimurium isolates and revealed resistance in descending order to cloxacillin, metronidazole and chloramphenicol (100%), tetracycline (75%), oxytetracycline (66.67%), ceftriaxone-sulbactum (58.34%), ceftriaxone-tazobactum (41.67%), gemifloxacin and nalidixic acid (33.34%), sulphadiazine and trimethoprim (25%), cefaperazone, ceftriaxone and gentamicin (16.67%), amikacin, ampicillin, -amoxicillin and gatifloxacin (8.34%), whereas no resistance was found towards amoxicillin- sulbactum, ciprofloxacin, enrofloxacin, enoxacin, levofloxacin, norfloxacin and pefloxacin.

Phenotypically, resistance was more in Salmonella

Table 2
Determination of Multi Drug Resistance (MDR) isolates on the basis of *in vitro* sensitivity



A-Tetracyclines (1-Tetracycline; 2-Oxytetracycline), B-Sulphonamides (3-Sulphadiazine; 4-Trimethoprim)
C-Quinolones (5- Nalidixic acid), D-Penicillins(6-Ampicillin; 7-Amoxycillin; 8-Cloxacillin)
E-Penicillin/ β lactamase inhibitor combination (9-Amoxycillin/ Sulbactum)
F-Flouroquinolones(10-Enrofloxacin; 11-Ciprorofloxacin; 12-Enoxacin; 13-Gemifloxacin; 14-Norfloxacin; 15- Levofloxacin; 16-Gatifloxacin; 17-Pefloxacin), G-Aminoglycosides (18-Gentamicin; 19-Amikacin)
H-Cephalosporins (20- Ceftriaxone; 21-Cefaperazone; 22-Ceftriaxone-Sulbactum; 23-Ceftriaxone-Tazobactum),
I-Nitroimidazoles (24-Metronidazole), J-Macrolides (25-Chloramphenicol)

isolated from cattle (60% of resistance to 10 antimicrobial groups tested) and buffalo (40%) than in primate (30%) and emu (30%). These isolates showed different resistance phenotypes.

In the strains isolated from buffaloes, maximum resistance was observed against tetracycline, macrolides, quinolones and nitroimidazoles. In addition to these antibiotics, strains isolated from primate and poultry also showed resistance to sulphonamides. Whereas in cattle, resistance towards aminoglycosides and cephalosporins was noticed in addition to above reported antibiotics. These differences could be related to the different antibiotic regimes used for the different antimicrobial agents in livestock species (Schwarz and Chaslu-Dancla, 2001; McEwen and Fedorka-Cray, 2002 and Van den Bogaard et al., 2001). Within each animal species, there was significant difference in the phenotypic resistance pattern. The least resistance was noticed in wildlife isolates as compared to livestock because of very less or no antibiotic usage in wildlife. Within livestock isolates, the maximum resistance was found in clinical case of cattle with chronic diarrhea due to the reason for excessive use of antibiotics.

The phenotypic resistance was seen in all the isolates showing resistance to one or more antibiotics. All the isolates were found to be multidrug resistance (Table 2) and among them none was found to be extreme drug resistant. No pan-drug resistant isolate was recorded.

In agreement to our study, Gopee et al. (2000) reported higher antimicrobial resistance (98.0%) in Salmonella isolates. The phenotype resistance in present study was higher for tetracyclines, nitroimidazoles, macrolides and betalactam group which is similar to the findings of Koochakzadeh et al. (2015) and Keen et al. (2007) who also observed higher resistance for tetracycline and betalactam group. Farias et al. (2015) performed antimicrobial sensitivity of Salmonella spp. isolated from feces of wild animals, environment and feed samples and found that antimicrobial resistance was highest against streptomycin (11.8%) and tetracycline (11.8%) followed by ampicillin (8.8%), gentamicin (8.8%) and kanamycin (8.8%). In accordance with the present study, Keen et al. (2007) also observed resistance to one or more antibiotics in all the salmonella isolates from different animals from various zoo animals.

There is remarkable sensitivity of flouroquinolones amongst all the antimicrobial groups tested. It points to the selective potential of fluoroquinolones, in treating salmonellosis in all animal species. In conclusion, the data presented supports the exposure assessment within the scientific risk analysis of antimicrobial drug resistance and highlights the need for the prudent use of antimicrobials in animal husbandry.

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