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MULTI-DRUG RESISTANCE IN *SALMONELLA TYPHIMURIUM* FROM POULTRY IN HARYANA

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ABSTRACT: Non-typhodal Salmonellosis (NTS) is one of the main food borne infection of public health importance. *S.typhimurium* is one of the predominant serotype reported from NTS in addition *S.enteritidis*. Thirteen isolates of *S.typhimurium* collected from broilers showing diarrhea were subjected to antimicrobial sensitivity assay against ten antibiotics. All the isolates were found resistant against bacitracin, erythromycin, penicillin and amoxicillin clavulanic acid. Maximum resistance (100%) was found against azithromycin, ceftizoxime, ceftriaxone followed by neomycin and colistin. All the isolate were found resistant to more than four number of antibiotics used and represents multidrug resistant isolates. Five different type of resistotypes were observed out of which resitotype-3 was found predominantly in seven isolates. The multiple drug resistance in all isolates from poultry origin is unusual finding on one hand but it also poses potential threat to human health on the other side. Better diagnostic facilities and judicious use of antibiotics in poultry are recommended to be implemented.

KEYWORDS: Salmonella, antibiotics, broilers.

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1.INTRODUCTION

Salmonellosis is one of the major public health disease affecting humans and animals in developed and developing countries [1-6]. More than 2500 serovars of Salmonella spp. has been described [7] based on diversity of liposaccharide (O) antigen and flagellar (H) antigen [8]. Out of these, some serovars are host specific (*Salmonella gallinarum* and *S.pullorum*) [9] while other serovars have

broad host range (*S.typhimurium*, *S.enteritidis*) [10]. These later two are known to cause Non-Typhoidal Salmonellosis (NTS) in poultry and human, leading to severe gastroenteritis and mortality in severe cases [11-12]. Salmonella is a member of *Enterobacteriaecae* family, gram-negative rod-shaped bacteria, causes septicemia and enteritis commonly [26]. *S. typhimurium* and *S.enteritidis* are known to cause food poisoning in human and diarrhea in poultry leading to loss of production [13-15]. Multiple antimicrobial resistance is on rise in NTS and is rapidly become a threat to public health. Non-judicious use of antibiotics have resulted in rise in multiple drug resistant bacteria who survived the treatment [14]. The aim of present study was to investigate antibiotic susceptibility of *Salmonella typhimurium* in broilers in Haryana.

2. MATERIALS AND METHODS

Sample: A total of thirteen isolates of *S.typhimurium* maintained at Department of Biotechnology, DCR University of Science and Technology, Sonipat (Haryana), were selected for present study of antibiotic sensitivity testing. These bacteria were isolated from liver and blood of broiler at time of postmortem. The isolates were revived in nutrient broth (HiMedia) by incubating at 37ºC for 24h, followed by transferring on MacConkey Agar (HiMedia) (37ºC for 24h) and confirmed by gram's staining and biochemical test as per protocol of earlier reported protocol [16] with slight modification.

Antimicrobial Susceptibility testing

Purified colonies of revived *S. typhimurium* isolates were subjected to antibiotic susceptibility testing by disc diffusion method with slight modifications in protocol [17]. Briefly, a colony was transferred to and incubated in nutrient broth at 37ºC for 6-8 hours, followed by spreading on Muellar Hinton Agar (MHA) (HiMedia). Ten antibiotic disc (HiMedia) were selected and transferred to inoculated MHA plate and incubated for 24 h at 37ºC. The antibiotic disc (HiMeida) used were- Amoxicillin clavulanic acid (30µg)(Amc30), Ampicillin-Cloxacillin (10 µg) (Ax10), Azithromycin (15 µg) (Azx15) , Bacitracin (10 µg) (B10), Ceftrizoxime (30 µg) (Czx30), Ceftriaxone (30 µg)(Ctr30), Colistin (10 µg) (CL10), Erythromycin (15 µg) (E15), Neomycin (30 µg) (N30) and Penicillin-G (10 µg) (P10). The zone of inhibition (mm) were recorded and compared with the reference chart provided by manufacturer.

3. RESULTS AND DISCUSSION

All the *S. typhimurium* isolates were found resistant (100%) to bacitracin, erythromycin, penicillin and amoxicillin clavulanic acid followed by resistance against ampicillin-cloxacillin (4/13), colistin (2/13) and neomycin (1/13). *S. typhimurium* isolates were found to be most sensitive (100%) against azithromycin, ceftrizoxime and ceftriaxone followed by neomycin (12/13) and colistin (11/13). (Table 1).

Table 1: *S. typhimurium* isolates showing maximum resistance (%) and sensitivity (%)

	Antibiotic Code	Result	Percentage
most resistant antibiotics	B,E,P,AMC	13/13	**100%**
most sensitive antibiotics	AZM,CZX,CTR	13/13	100%
	N	12/13	92.3%
	CL	11/13	84.6%

Multiple drug resistance (resistance against more than 3 antibiotics) were recorded in all thirteen isolates, but one isolate among them was found resistant to six antibiotics. Five *S. typhimurium* isolates showed resistance against five antibiotics whereas seven isolates were found resistant to four antibiotics used (Table 2).

Table 2: Multiple antibiotic resistance pattern of *S .typhimurium* isolates

No. of isolates showing resistance	No. of antibiotics
1	6
5	5
7	4

Isolates were divided into five resitotypes (Table 3) based on combination of antibiotic resistance. Resitotype-3 (Amc, B, E, P) represented maximum number of isolates (7/13), followed by resistotype-5 (Amc, Ax, B, E, P) possessed by three isolates. Resistotype- 1, 2 and 4 were found least and was possessed by one isolate each.

Table 3: Resitotype pattern of thirteen *S. typhimurium* isolates

Resistotype	antibiotics found resistant	no.of resistant isolates
Resitotype-1	AMC,AX,B,CL,E	1
Resitotype-2	AMC,B,CL,E,P	1
Resitotype-3	AMC,B,E,P	7
Resitotype-4	AMC,B,E,N,P	1
Resitotype-5	AMC,Ax,B,E,P	3

DISCUSSION

Salmonella enterica infection in human and animals are being continuously reported throughout the world considering its public health importance and huge economic losses to agriculture. Among developed countries like USA and UK, national programs for control of Salmonellosis have been launched recently. European food safety Authority, have reported in year 2013 that NTS cases were

mostly caused by *S.enteritidis* (39.5%) followed by *S.typhimurium* (20.2%), leading to food borne illness in Humans. The transmission through animal derived food contaminated source was predominant [18]. Similar reports of both the above serotypes being predominant among NTS cases were also available from Canada by National Enteric Surveillance Program: NESP, (2014) [27] and USA by National Enteric Disease Surveillance: NEDS, (2013) [19]. In animals, reports of *S.enteritidis* and *S.typhimurium* causing NTS are widely available [20, 21]. In present study, *S.typhimurium* subjected to further analysis were isolated from different parts of Haryana state from broiler population. The presence of *S.typhimurium* from Haryana state were also reported previously [14]. To control Salmonella infection in animals, use of antimicrobials are generally preferred in broilers and vaccination in parents is also being administered at some places. Bacterial development of antibiotic resistance for survival can be intrinsic or acquired through exchange of DNA [14]. Intrinsic mechanism involves mutation of genetic material whereas acquired resistance may be through plasmid, integron or transposon [23]. In present study, resistance against ten antibiotics was studied belonging to four different class of antibiotics namely β- lactum (Amc, Ax, Czx and Ctr, P), aminoglycoside (N), polymixin (B,CL) and macrolides (E, Azt). Most isolates were found resistant against penicillin, bacitracin, erythromycin and amoxicillin Clavulanic acid. Veterinary pharmaceutic companies in India has come up with amoxicillin clavulanic acid and are being used in animals more recently. The resistance developed against amoxicillin clavulanic acid, necessitates cautious use of this antibiotic. This finding was in contrast to study [24], where maximum resistance was against tetracycline, streptomycin and kanamycin. In another study [25], resistance of *S.typhimurium* predominantly against tetracycline, sulfonamides, streptomycin, ampicillin and nalidixic acid was reported. Most of antibiotics found resistant in report from Haryana [13] were not used in present study to check status of less commonly studied antibiotics. Among both studies main difference was found in case of amoxicillin clavulanic acid, which was found to have developed less resistance by *S.typhimurium* by above worker from Malaysia, whereas in the present study ,all the isolates were found to have developed resistance. This suggest that in this part of India, bacteria have evolved better, may be due to non-judicious use of this antibiotic in recent past. From Kashmir, the other part of India [15], resistance pattern on 33 Salmonella isolates belonging to *S. gallinarum*, *S. enteritidis* and *S. typhimurium* from poultry origin were studied and found maximum resistance against cefopodoxime, nalidixic acid and sulphadiazine whereas amoxicillin-clavulanic acid was reported to be resistant in nearly 25% isolates only, which is also in contrast with present study. The local geographical conditions may also be one of the factors responsible for this different pattern of antibiotic resistance. All the isolates in present study were found sensitive against azithromycin, ceftizoxime and ceftriaxone followed by neomycin (12/13) and colistin (11/13). The neomycin sensitivity was found in agreement with report from Japan [24], suggesting its less use in poultry in both the countries. Third generation cephalosporin was reportedly found resistant in the

above mentioned study [24] from Japan, which was found in contrast to sensitivity shown in present study from *S. typhimurium* isolates of broilers of Haryana (India). This suggest that third generation cephalosporin may be in regular use in poultry in that part of Asian continent but may be used as last resort in Haryana (India). The present study was partially in agreement with report from Malaysia [25], where third generation cephalosporin were found most effective against *S. typhimurium*. The effectiveness of cefotaxime and cefpodoxime was found in treatment of *S. typhimurium* from poultry isolates in Kashmir (India) [15], which was partially in agreement with finding of present study where cephalosporin group antibiotics were also found useful in treatment of Salmonella infection. Reports related to study of Salmonellosis screening against azithromycin was not available for comparison to the investigator. As azithromycin is being used in Human in severe infections in India, it may be deliberately kept out by pharmaceutical companies from drugs for veterinary. Although all the *S. typhimurium* isolates in present study were found sensitive against azithromycin, it is not recommended here that it should be used in poultry sector but research to keep a check on the situation in current therapeutics is suggested. Multiple drug resistance was shown by all the isolates in present study, where *S. typhimurium* was found to be resistant against more than three antibiotics studied. This observation of multiple drug resistance was found in agreement with already available reports from different countries including India [15,24,25]. This suggests that use of multiple antibiotic are in regular use in different parts of Asia, either for therapeutic use or as feed additives in animals. The situation of disease caused by NTS and increasing multiple antibiotic resistance against them from poultry isolates, needs to be controlled by implementation of a national control program as being run by European Union, USA and Canada. The poor sanitation problem in developing country like India, may pose a serious threat to human population in event of possible spread of multiple drug resistance bacteria form animals. Five type of resistotype were observed in present study from thirteen *S. typhimurium* isolates where resistotype-3 (Amc, B, E, P) was found in maximum number of isolates (7/13) followed by resistotype-5 (Amc, Ax, B, E, P) observed in 3 isolates. Other resistotype- 1,2 and 4, were found in one number of isolate each. This was in partial agreement with observation of report from Japan [24] where six types of resistotype were found, all of them were resistant to three or more antibiotics. This suggests that a large of number of resistotype to *Salmonella* spp. are in circulation in environment in different parts of the world particularly Asian countries including India and Japan. The changes in agricultural practice has already in progress in India, where organic crop production is being taken up by some sections of farmers, considering importance of health rather than cost to input ratio. Eventually these farmers has shown profit also in organic farming, which needs to be studied and may be applied to poultry farming to discourage use of antibiotics.

4. CONCLUSION

S. typhimurium bacteria is of public health importance, one of the main etiological agent among NTS causing diseases in animals and human leading to gastroenteritis and even mortality in severe cases. Broilers used for meat purpose, are given a number of antibiotics in feed as well as for therapeutic use. The growing number of antibiotic resistant bacteria suggest that non-judicious use of antibiotics needs to be checked. Multiple drug resistance problem is now reported from different parts of world mainly from developing countries. The more studies on antibiotic sensitivity testing are required for better assessment of situation and to administer sensitive antibiotics in clinical cases particularly in broiler.

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CONFLICT OF INTEREST

This is declared that there is no conflict of interest known.

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