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Antibiogram Assay of *Salmonella* Gallinarum and Other *Salmonella enterica* Serovars of Poultry Origin in India

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ABSTRACT

The study was conducted from November 2008 to May 2009 to assess the antimicrobial sensitivity pattern of 22 *Salmonella* isolates viz., 15 *Salmonella* Gallinarum strains recovered from various sources of different regions of India including 3 reference strains and 5 other *Salmonella enterica* serovars (7 isolates) by testing with 16 different antimicrobials. All the isolates including the standard reference *Salmonella* strains were subjected to antimicrobial sensitivity test using 16 different antimicrobial agents by disc diffusion method. All the *Salmonella* Gallinarum isolates showed resistance to erythromycin and 86.7% of them were resistant to nalidixic acid. More than 53% of the Gallinarum isolates were either 100% resistant or less sensitive to the commonly used antimicrobials, kanamycin and tetracycline whereas about 93.3% of them were sensitive to gentamicin and amoxicillin/clavulanic acid. Antimicrobial sensitivity pattern for ciprofloxacin, ofloxacin, colistin and sulfa-trimethoprim was around 88.8 and 82% of the isolates were sensitive to enrofloxacin and chloramphenicol. Among other serotypes included in the study *S. typhimurium* showed maximum resistance against 6 antimicrobials followed by *S. kastrup* which was resistant to 5 antimicrobials. *S. typhimurium* was 100% sensitive only to ciprofloxacin. In overall, out of a total of 22 isolates tested for different antimicrobials 4/22 (18.2%) were resistant to at least one antimicrobial and the remaining 81.8% were resistant to at least two or more antimicrobials, supporting the fact for the emergence and widespread presence of multidrug resistant *Salmonella* species and the importance of the implementation of suitable measures to avoid indiscriminate use of antibiotics in food animals.

Key words: Antimicrobials, India, resistance, *Salmonella enterica, Salmonella* Gallinarum

INTRODUCTION

Salmonella organisms are responsible for a variety of acute and chronic diseases in poultry, animals and humans. Every year about one third of the food-borne disease outbreaks in human beings are attributed to salmonellae alone (Daniel *et al*., 2002). Contaminated poultry products are among the most important sources for food-borne outbreaks in humans and salmonellae are isolated more often from poultry and poultry products than from any other food animals (Braden, 2006; Habtamu *et al*., 2011; Kabir, 2010; Linam and Gerber, 2007). Therefore, infections of domestic poultry with salmonellae are expensive both for the poultry industry and for society as a whole.

There are reports of high prevalence of resistance in *Salmonella* isolates from countries such as Bangladesh (Khan *et al*., 2005), Bhutan (Dahal, 2007), Canada (Poppe *et al*., 2006), Ethiopia (Molla *et al*., 2003), France (Cailhol *et al*., 2005), India (Mandal *et al*., 2006; Singh *et al*., 2007), Korea (Lee *et al*., 2003), Mexico (Zaidi *et al*., 2006), Netherlands (Van Duijkeren *et al*., 2003), Senegal (Bada-Alambedji *et al*., 2006), Taiwan (Lauderdale *et al*., 2006) and the USA (Zhao *et al*., 2006). In recent years, antimicrobial resistance in *Salmonella* has assumed alarming proportions (Gyles, 2008) and the isolates were resistant to at least one of the 15 antimicrobials tested. It has been reported that livestock and their products can contribute to as much as 96% of the total *Salmonella* infection in humans. Antimicrobial resistance is a global public health problem (Ahmed *et al*., 2011). In developing countries like India, easy availability of a wide range of drugs coupled with inadequate health services result in increased proportions of drugs used as self-medication compared to prescribed drugs resulting in impending health problems and antimicrobial resistance. Therefore, the aim of this study was to assess the antimicrobial sensitivity pattern of *Salmonella* Gallinarum (*S*. Gallinarum) isolated from different regions of India and other *Salmonella enterica* serovars isolated from poultry during the study period.

MATERIALS AND METHODS

Bacterial stains: A total of 12 *S*. Gallinarum isolated from various sources in different regions of India and 3 reference *S*. Gallinarum strains were procured from the National Salmonella Centre, Indian Veterinary Research Institute, Izatnagar (Table 1). Additional 5 *Salmonella* serotypes (7 isolates) viz., *S. heidelberg*, *S. typhimurium*, *S. ayinde*, *S. essen* and *S. kastrup* isolated from poultry tissue samples and eggs in Bareilly district (Taddele *et al*., 2011), Uttar Pradesh, India were also included in this study.

Maintenance of strains: *S*. Gallinarum strains obtained from the National *Salmonella* Centre, IVRI, Izatnagar were checked for their biochemical profiles and inoculated on nutrient agar slants and kept at 4°C. Sub-culturing was done periodically to test the purity, morphological and biochemical characteristics of the strains.

Antimicrobial resistance testing: All the 22 *Salmonella* isolates including the 03 standard reference *Salmonella* strains were subjected to drug sensitivity test using sixteen (16) commonly used antimicrobial agents {ampicillin 10 µg (A10), amoxicillin/clavulanic acid 30 µg (Ac30), gentamicin 10 µg (G10), kanamycin 30 µg (K30), enrofloxacin 5 µg (Ex5), ciprofloxacin 5 µg (Cf5), erythromycin 15 µg (Em15), ofloxacin 5 µg (Of5), tetracycline 30 µg (T30), colistin 10 µg (Cl10), cephalexin 30 µg (Cp30), nalidixic acid 30 µg (Na30), chloramphenicol 30 µg (C30), amoxicillin 10 µg (Aml10), neomycin (N30) and sulfa-trimethoprim 25 µg (SXT25)} by disc diffusion method as described by Miles and Amyes (1996) and the interpretation was made as per the zone size interpretation chart provided by the manufacturer of antimicrobial discs (Himedia, India). The antibiogram profiles were categorized into four groups based on sensitivity pattern and results were scored as highly resistant (0), less sensitive (1), moderately sensitive (2) and highly sensitive (3) and the scored data was analysed statistically.

Table 1: Host and epidemiological sources of *S.* Gallinarum isolates

Isolate No.	Place of isolation	Source	Location on host
E-4627	Bangalore	Crocodile	-
E-4661	Dharwar	Human	Blood
E-4668	Hissar	Poultry	-
E-4678	Srinagar	Poultry	Faeces
E-4680	Srinagar	Poultry	Faeces
E-4684	Srinagar	Poultry	Faeces
E-4685	Srinagar	Poultry	Faeces
E-4686	Srinagar	Poultry	Faeces
E-4687	Srinagar	Poultry	Faeces
E-2424	Pune	Poultry	Liver
E-2638	Gorakhpur	Poultry	Heart blood
E-2639	Gorakhpur	Poultry	Spleen
E-75	Reference strain	-	-
E-76	Reference strain	-	-
E-402	Reference strain	-	-

RESULTS

Morphological and biochemical characterization of *Salmonella* isolates: The *Salmonella* Gallinarum isolates (15) and the serotypes (5) isolated from poultry showed typical cultural, morphological and biochemical characteristics of the genus and species. Subculturing was done periodically to test the purity, morphological and biochemical properties of the strains. All the isolates produced H_2S in TSI, were non-lactose fermenters, non-motile, positive in MR test, catalase test and negative with oxidase test and urease test and showed gas production from glucose.

Antimicrobial resistance testing for *S.* Gallinarum isolates: All the 15 *S.* Gallinarum isolates including the 03 reference strains were 100% resistant to erythromycin and 13 of them were resistant to nalidixic acid where as the two strains, E-4627 and E-4661, were less sensitive to this antimicrobial. Five (33.3%) isolates showed 100% resistance to neomycin and 8 (53.3%) were less sensitive to the same antimicrobial. Fourteen (93.3%) isolates were 100% sensitive to amoxicillin/clavulanic acid and gentamicin whereas strains E-4668 and E-4684 were moderately sensitive to amoxicillin/clavulanic acid and gentamicin, respectively. More than 53% of the isolates were either 100% resistant or less sensitive to the commonly used antimicrobials, kanamycin and tetracycline. The antimicrobials ciprofloxacin, ofloxacin, colistin and sulfa-trimethoprim were 88.8% effective where as enrofloxacin and chloramphenicol showed 82.2% efficacy against the *S.* Gallinarum isolates tested including reference strains. The reference strain, E-402, showed maximum resistance against ampicillin, erythromycin, tetracycline, cephalexin, nalidixic acid and neomycin and it was less sensitive to kanamycin, enrofloxacin, chloramphenicol and amoxicillin (Table 2 and Fig. 1). The other two reference strains (E-75 and E-76) showed resistance to three antimicrobials viz., erythromycin, nalidixic acid and neomycin. The overall sensitivity of isolates including reference strain to the tested antimicrobials was in the range of 0-98% (Fig. 1).

Table 2: Antimicrobial sensitivity pattern of *S.* Gallinarum isolates and reference strain

Strain	Resistant (-)	Less sensitive (+)	Moderately sensitive (++)	Highly sensitive (+++)
E-4627	Em15[3]	K30[4], T30[5], Na30[6], N30[7]	Cp30[8], Aml10[9]	A10[10], Ac30[11], G10[12], Ex5[13], Cf5[14], Of5[15], Cl10[16], C30[17], SXT25[18]
E-4661	Em15	K30, Na30, Aml10, N30	T30, Of5, C30	A10, Ac30, G10, Ex5, Cf5, Cl10, Cp30, SXT25
E-4668	Em15, Na30	Ex5, Of5, T30	A10, K30, C30, N30	Ac30, G10, Cf5, Cl10, Cp30, Aml10, SXT25
E-4678	Em15, Na30	A10, K30, T30, N30,	Ac30, Cl10, Cp30, Aml10	G10, Ex5, Cf5, Of5, C30, SXT25
E-4680	Em15, Na30, N30	K30, Cl10	A10, T30, Ex5, Aml10	Ac30, G10, Cf5, Of5, Cp30, C30, SXT25
E-4684	Em15, Na30	K30, T30, N30,	G10, Of5, Aml10, C30,	A10, Ac30, Ex5, Cf5, Cl10, Cp30, SXT25
E-4685	Em15, T30, Aml10, Na30	Cf5	K30, SXT25	A10, Ac30, G10, Ex5, Of5, Cl10, Cp30, C30, N30
E-4686	Em15, Na30	A10, Cf5, T30, N30, SXT25	K30, Ex5, Cp30, Na30	Ac30, G10, Of5, Cl10, C30, Aml10
E-4687	Em15, Na30	T30, N30, Aml10	K30, Cp30	A10, Ac30, G10, Ex5, Cf5, Of5, Cl10, C30, SXT25
E-75	Em15, Na30,N30	K30, T30,	Cl10, Cp30	A10, Ac30, G10, Ex5, Cf5, Of5, C30, Aml10, SXT25
E-76	Em15, Na30,N30	A10, K30, Ex5, Cp30, T30	Cl10, C30, Aml10	Ac30, G10, Cf5, Of5, SXT25
E-2424	A10, Em15, Na30	K30, N30	T30, Cp30, Aml10	Ac30, G10, Ex5, Cf5, Of5, Cl10, C30, SXT25
E-2638	Em15, Aml10, N30	A10, K30, Na30	T30, SXT25	Ac30, G10, Ex5, Cf5, Of5, Cl10, Cp30, C30
E-2639	Em15, K30, T30	Aml10, N30	Na30, C30, SXT25	A10, Ac30, G10, Ex5, Cf5, Of5, Cl10, Cp30
E-402	A10, Em15, T30, Cp30, Na30, N30	K30, Ex5, C30, Aml10,	Cf5, Of5	Ac30, G10, Cl10, SXT25

[3]Em15-erythromycin 15 µg, [4]K30-kanamycin 30 µg, [5]T30-tetracycline 30 µg, [6]Na30-nalidixic acid 30 µg, [7]N30 - neomycin, [8]Cp30-cephalexin 30 µg, [9]Aml10-amoxicillin 10 µg, [10]A10-ampicillin 10 µg, [11]Ac30-amoxicillin/clavulanic acid 30 µg, [12]G10-gentamicin 10 µg, [13]Ex5-enrofloxacin 5 µg, [14]Cf5-ciprofloxacin 5 µg, [15]Of5-ofloxacin 5 µg, [16]Cl10-colistin 10 µg, [17]C30-chloramphenicol 30 µg and [18]SXT25-sulfa-trimethoprim 25 µg

Table 3: Antimicrobial sensitivity pattern of *Salmonella* serotypes isolated dnring the present study

Strain	Resistance (-)	Less sensitive (+)	Moderately sensitive (++)	Highly sensitive (+++)
S. hiedelberg 1	Em15, Na30	T30, Cl10, N30	Ac30, K30, Ex5, Cp30, C30, Aml10	A10, , G10, Cf5, Of5, SXT25
S. hiedleberg 2	Em15, Na30	A10, K30, T30, Cl10, N30	C30, Aml10,	Ac30, G10, Ex5, Cf5, Of5, Cp30, SXT25
S. hiedleberg 3	Em15	K30, T30, Na30, N30	Aml10	A10, Ac30, G10, Ex5, Cf5, Of5, Cl10, Cp30, C30, SXT25
S. typhimurium	K30, Ex5, Em15, Of5, T30, N30,	A10, Cl10, Na30, Aml10	Ac30, G10, Cp30, C30, SXT25	Cf5
S. ayinde	N30	K30, Em15, Na30,	Cf5, T30, Aml10	A10, Ac30, G10, Ex5, Of5, Cl10, Cp30, C30, SXT25
S. essen	T30	A10, K30, Em15, Na30, N30,	Aml10	Ac30, G10, Ex5, Cf5, Of5, Cl10, Cp30, C30, SXT25
S. kastrup	K30, Em15, T30, Na30, N30	A10, Ex5, C30, Aml10	Ac30, G10, Cf5, Of5, SXT25	Cl10, Cp30,

Antimicrobial resistance testing for 5 *Salmonella* serotypes: Among the five *Salmonella* serotypes (*S. heidelberg*, *S. typhimurium*, *S. ayinde*, *S. essen* and *S. kastrup*) tested *S. typhimurium* showed resistance to 6 antimicrobials viz., kanamycin, enrofloxacin, erythromycin, tetracycline, ofloxacin and neomycin and it was highly sensitive only to ciprofloxacin. *S. kastrup* was also resistant to kanamycin, erythromycin, tetracycline, nalidixic acid and neomycin. The remaining isolates were resistant to at least one antimicrobial and were sensitive to at least 5 antimicrobials (Table 3 and Fig. 2). Four antimicrobials (gentamicin, ciprofloxacin, cephalexin and sulfa-trimethoprim) were the most effective antimicrobials showing about 90.5% efficacies (Fig. 2).

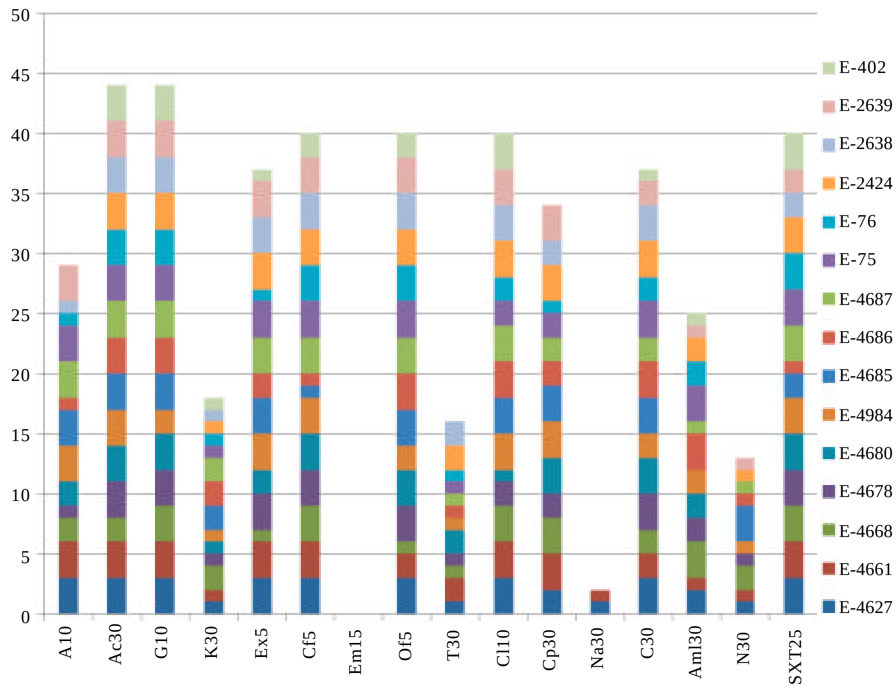

Fig. 1: Stacked column showing the sensitivity of *S*. Gallinarum isolates and reference strains to different antimicrobials tested

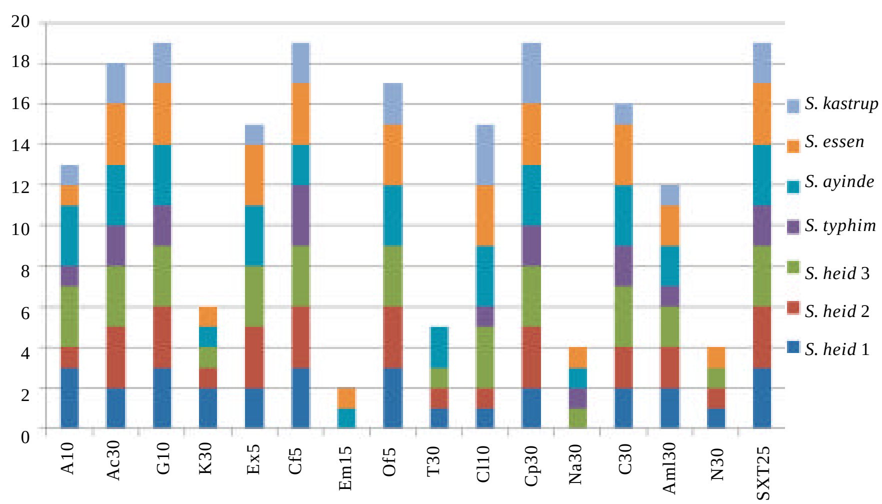

Fig. 2: Stacked column showing the sensitivity of different *Salmonella* serotypes to different antimicrobials tested

DISCUSSION

The clinical management of salmonellosis in man and animals is mainly based on antimicrobials and fluid therapy which is quite expensive; and indiscriminate use of antimicrobials may lead to emergence of multiple drug resistant strains which are threat to human and animal population.

The emergence of multidrug resistance among *Salmonella* spp. is an increasing concern. *Salmonella* strains of avian origin are also often resistant to variety of antimicrobials approved for poultry including tetracycline, oxytetracycline, penicillin, aminoglycosides, sulfisoxazole and fluoroquinolones (Kabir, 2010; Oliveira *et al*., 2006; Parveen *et al*., 2007). Multiple drug resistance against 6-8 drugs has been reported in several *Salmonella* serotypes of human and animal origin in India by several investigators (Mandal *et al*., 2006; Prakash *et al*., 2005; Singh *et al*., 2007). Emergences of multiple drug resistant *Salmonella* strains have often been alleged to be responsible for frequently occurring outbreaks and hyperendemicity of salmonellosis in India. The present study was undertaken to test the antimicrobial sensitivity pattern of *S*. Gallinarum isolates and other 5 *Salmonella* serotypes.

In the present study, all *S*. Gallinarum isolates including reference strains were found to be 100% resistant to erythromycin and 13 (86.7%) of them were resistant to nalidixic acid. The resistance pattern for erythromycin was in accordance with previous findings of Khan *et al*. (2005) in Bangladesh; whereas the resistance to nalidixic acid is in close agreement with the prevalence rate of 92-96% reported from India (Lakshmi *et al*., 2006) and 96% from Bhutan (Dahal, 2007). High prevalence of nalidixic acid resistance among poultry isolates (89%) was also reported from France (Cailhol *et al*., 2005). Sensitivity to amoxicillin/clavulanic acid and gentamicin has been observed in 93.3% of the Gallinarum isolates which was in close harmony with previous observations (Dahal, 2007; Gaedirelwe and Sebunya, 2008; Lee *et al*., 2003). The *Salmonella* isolates in India from 1996-99 and 2001 were reported to be 100% chloramphenicol sensitive and sensitivity as high as 79% was reported in 2000 (Mandal *et al*., 2004). The present study also showed chloramphenicol sensitivity of around 82%. This re-emergence of chloramphenicol sensitivity could be attributed to the limited use of the antimicrobial during the last decade in India (Khan and Shukla, 2004; Achla *et al*., 2005; Mohanty *et al*., 2006). However, a report by Khan *et al*. (2005) from Bangladesh showed less sensitivity of *Salmonella* isolates to chloramphenicol.

On an average the antimicrobial pattern for ciprofloxacin, ofloxacin, tetracycline, colistin and sulfa-trimethoprim observed against *S*. Gallinarum isolates in this study was in close agreement with earlier reports of Lee *et al*. (2003) in Korea on the same organism but the pattern for ampicillin, enrofloxacin and kanamycin did not correlate exactly with their results, which may be due to molecular heterogeneity of the strains and use of different branded antimicrobials. In addition, a particular antimicrobial is effective at the time of introduction to the market but later it may become ineffective due to inadvertent use for long duration in sub optimal doses. A resistance to trimethoprim-sulfamethoxazole among poultry isolates has been reported from India (Prakash *et al*., 2005), Mexico (Zaidi *et al*., 2006), Senegal (Bada-Alambedji *et al*., 2006) and USA (Zhao *et al*., 2006). However, sensitivity to sulfa-trimethoprim was around 89% in this study.

Among the other *Salmonella* isolates 5/7 (71.4%) were resistant and 2/7 (28.6%) of them were less sensitive to erythromycin. Resistance to nalidixic acid was observed in 3/7 (43%) of these isolates and the remaining 4/7 (57%) were less sensitive to this antimicrobial. Reports from Bangladesh (Khan *et al*., 2005) and France (Cailhol *et al*., 2005) have showed the same observation with regard to the resistance pattern to these antimicrobials.

Out of a total of 22 isolates tested for different antimicrobials 4/22 (18.2%) were resistant to one antimicrobial and the remaining 81.8% were resistant to at least two or more antimicrobials. The maximum resistance was observed in *S*. Gallinarum reference strain E-402 and *S*. *typhimurium*, which were resistant to 6 antimicrobials followed by *S*. *kastrup*, which showed resistance to 5 antimicrobials. The other *S.* Gallinarum reference strains, E-75 and E-76, were resistant to three antimicrobials. *S. typhimurium* was sensitive only to ciprofloxacin. Antimicrobial resistance has been reported to be more common in *S. typhimurium* than the other serovars in India (Prakash *et al.*, 2005).

Advances to prevent and control salmonellosis in the food animal industry by various means such as improved biosecurity, vaccination, use of competitive exclusion products and the introduction of novel immuno-potentiators with limited success has necessitated the use of antimicrobial chemotherapy in the treatment and control of salmonellosis (Zhao *et al.*, 2007). The occurrence and proliferation of antibiotic-resistant *Salmonella* in environmental samples, poultry and other animals and humans may be due to the use of medicated feeds, the practice of dipping hatching eggs in solutions containing antimicrobial agents, routine inoculation of day-old poults with antibiotics and treatment of other animals and humans with antibiotics (Kabir, 2010). The widespread use of antimicrobials for therapeutic purposes in food animals and as growth promoters in animal feeds have been implicated in promoting emergence and spread of antimicrobial resistance among salmonellae (Humphrey, 2001; White *et al.*, 2001), through mutation and acquisition of resistance encoding genes (Fluit, 2005). The circumstances in India may be aggravated due to easy accessibility of antimicrobials at cheaper price and their extensive use in poultry production (Prakash *et al.*, 2005; Singh *et al.*, 2007). The other major contributing factor is widespread availability of different brands and uncontrolled use of these antimicrobials.

CONCLUSION

Present study showed for the emergence and widespread prevalence of multidrug resistant *Salmonella* species in poultry. Therefore, measures need to be focussed to avoid indiscriminate use of antibiotics in food animals and quality control of antibiotics should be initialized to prevent uncontrolled use of antibiotics. *Surveillance*, identification and antibiotic sensitivity of the prevalent *Salmonella* serotypes in the country would help devise suitable prevention and control programme for this important poultry pathogen having food-borne zoonosis.

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