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ORIGINAL RESEARCH

Antimicrobial sensitivity and resistance of *Salmonella* Enteritidis isolated from natural samples

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Abstract

Aim: To test the sensitivity of *S.* Enteritidis for selected antibiotics.

Materials and Methods: *S.* Enteritidis isolates obtained from different samples of chicken, mutton, turkey meat, faecal and cloacal samples of poultry and turkey, eggs, water and feed were subjected for sensitivity and resistance to selected antibiotics like- Chloramphenicol (30µg), Gentamicin (10 µg), Nalidixic Acid (30 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Amoxicillin (25 µg), Ampicillin (10 µg), Streptomycin (10 µg) and Sulfonamide (30 µg). Antimicrobial susceptibility of the isolates was established by the disk diffusion assay with MH (Muller-Hinton) agar in accordance with French National Antibiogram Committee Guidelines.

Results: The sensitivity of *S.* Enteritidis was 100% for ciprofloxacin followed by chloramphenicol and amikacin (96%), gentamycin (90%), amoxicillin (82%), streptomycin (80%), tetracycline (76%), nalidixic acid (68%), ampicillin (58%) and sulfonamide (10%). The resistance was highest for sulfonamide (76%) followed by ampicillin (32%), nalidixic acid (30%) and 6-20% for gentamycin, amoxicillin and tetracycline.

Conclusion: *S.* Enteritidis isolates were more sensitive to ciprofloxacin, chloramphenicol, amikacin, gentamycin, streptomycin, amoxicillin and tetracyclines and less sensitive to sulfonamides. Higher resistance was observed with sulfonamide followed by ampicillin and nalidixic acid.

Keywords : antibiotic resistance, antibiotic sensitivity, *S.* Enteritidis

Introduction

Salmonella food poisoning is one of the most common and widely distributed diseases in the world [1]. Salmonellosis outbreak was linked with wide variety of fruits, vegetables and juices [2]. Prior to 1998 *Salmonella* Typhimurium was more commonly isolated than *S.* Enteritidis; however in recent years *S.* Enteritidis has been the most common serotype isolated from food and has been particularly responsible for the overall increase in *Salmonella* infections in humans [3] and is one of the major public health problems in terms of socio-economic impact [4,5]. It is estimated that the annual economic cause due to food borne *Salmonella* infections in the U.S. are $2.4 billion [6]. Outbreaks are usually associated with ingestion of contaminated food of animal origin particularly avian products. *S.* Enteritidis has become prevalent in humans and poultry as a result of vertical and horizontal transmission [7].

Although the majority of infections result in asymptomatic or self limited disease in immunocompromised patients, neonates and elderly, antibiotic treatment is usually recommended. Resistance towards the traditional first line antibiotics is multi drug resistance (MDR) in Salmonella enteric [8]. Recently multidrug resistant (MDR) strains have emerged, presumably due to the extensive use of antimicrobial agents both in humans and animals [9]. In veterinary medicine antibiotics are used in livestock production, disease prevention and as growth promoting feed additives [10,11]. Indiscriminate and injudicious use of antibiotics in food animals should be monitored to reduce risk of MDR to humans [12]. Resistant strains of *Salmonella* to bacitracin, colistin and polymyxin-3 were isolated by Singh *et. al.*, [13] in north India from chicken eggs.

The use of antibiotics disrupts the normal flora of intestine, resulting in emergence of antibiotic resistant strains, which will limit the therapeutic options available for their treatment. The fatality rate for people infected with antibiotic resistant *Salmonella* strains is 21 times greater than for individuals infected with non antibiotic resistant *Salmonella* strains [14]. There is a need of continuous surveillance and sharing of antimicrobial susceptibility for Salmonella among countries of worldwide to ensure the effectiveness of control programmes [15].

In the present study we have studied the antimicrobial sensitivity and resistance to certain antibiotics.

Materials and Methods

A total of 235 samples (randomly collected) of

Table-1. Antibiotic sensitivity of *Salmonella* Enteritidis

Sr. No.	Antibiotic (µg)	Antimicrobial resistance, No. positive (%)		
		Sensitive	Intermediate	Resistant
1.	Chloramphenicol (30µg)	48(96%)	2(4%)	-
2.	Gentamycin (10µg)	45(90%)	2(4%)	3(6%)
3.	Nalidixic Acid (30µg)	30(60%)	5(10)	15(30%)
4.	Tetracycline (30µg)	38(76%)	2(4%)	10(20%)
5.	Ciprofloxacin (5µg)	50(100%)	-	-
6.	Amikacin (30µg)	48(96%)	2(4%)	-
7.	Amoxicillin (25µg)	41(82%)	1(2%)	8(16%)
8.	Ampicillin (10µg)	29(58%)	5(10%)	16(32%)
9.	Streptomycin (10µg)	40(80%)	5(10%)	5(10%)
10.	Sulfonamide (300µg)	5(10%)	7(14%)	38(76%)

chicken (25), turkey meat (20), mutton(25), eggs (25), feed (25), water (25), poultry and turkey faeces (25 and 20 respectively), poultry and turkey cloacal samples (25 and 20 respectively) were analyzed for the presence of *Salmonella spp*. both by cultural and PCR methods. The samples were collected and pre enriched in buffered peptone water, incubated at 37^{0}C for 16 h. After pre-enrichment 1 ml of each inoculum was transferred into selective broths (Himedia) including Tetrathionate (TT) broth, Selenite-F (SF) and Selenite cysteine (SC) broths while 0.1ml to Rappaport-Vassilidias (RV) broth. All broth inoculums were incubated at 42^{0}C for 18h except SC broth (incubation was at 37^{0}C for 18 h). All the enriched samples were subjected to PCR confirmation for *Salmonella spp.* infection using primers specific to *invA (invasion A*) gene sequence. PCR includes DNA extraction by Boiling and Snap chilling method from the selective broth cultures and then the DNA template was added to PCR reaction mixture and subjected to PCR assay (Initial denaturation, Final denaturation, Annealing, Initial extension, Final extension). The amplified product was tested for the presence of desired gene by using Agarose Gel Electrophoresis. The *Salmonella* positive samples by PCR method were further characterized for detection of *S.* Enteritidis strains using *sefA (Salmonella* Enteritidis *fimbrial A)* gene specific primers (amplification product: 310bp).

The *invA* gene (310bp) and *sefA* (389bp) genes were targeted for *Salmonella and S.* Enteritidis respectively. Out of 235 samples, 174 samples were positive for *Salmonella spp.*, out of which 126 were positive for *S.* Enteritidis by PCR technique. Fifty isolates of *S.* Enteritidis were subjected for antibiotic sensitivity test, using disk diffusion assay with Muller-Hinton agar in accordance with French National Antibiogram committee guide lines [16]. The antibiotics tested were - Amikacin (30µg), Amoxicillin (25µg), Ampicillin (10 µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Gentamycin (10µg), Nalidixic Acid (30µg), Streptomycin (10µg), Sulfonamide (300µg), Tetracycline (30 µg).

Results and Discussion

Antimicrobial resistance of *Salmonella* has double importance, firstly for the treatment of poultry in which they cause infections and secondly for human infections that they cause disease. One of the main factors for development of antimicrobial resistance is irrational use of antimicrobial drugs. In the present study (Table) *S.* Enteritidis isolates were highly sensitive to ciprofloxacin (100%) followed by amikacin (96%) and gentamycin (90%) which were almost similar to the results reported by Ammari *et. al.* [17], Okamoto *et. al.* [18] and Nunes et. al., [19]. The resistance of *S.* Enteritidis was zero for ciprofloxacin and amikacin, whereas the resistance to gentamycin was 6%, which are coinciding with the results of Pederson *et. al.* [20], Turkyilmaz *et. al.* [21] and Nunes *et. al.*, [19]. Very low level of resistance (0-1%) to gentamycin was reported by Breuil *et. al.* [22] and Vaz *et. al.*, [23], whereas Poppe *et. al.*, [24], Aksakal [25] and Aktar *et. al.*, [26] reported higher levels of resistance to gentamycin i.e 25.8%, 35% and 78.57% respectively.

The sensitivity and resistance of *S.* Enteritidis to chloramphenicol in this study was 96% and 0% respectively, which was almost similar to the sensitivity (99%, 99.6% and 100%) reported by Okamoto *et. al.* [18], Nunes et. al, [19] & Aktar, [26] respectively. Very low sensitivity (2.4%) and low resistance (0.4%) to chloramphenicol was reported by Poppe *et. al.* [24] and Nunes, [19] respectively. Moderate sensitivity (54.6%) and resistance (44.5%) was reported by Turkyilmaz *et. al.* [21]. The sensitivity of *S.* Enteritidis to amoxicilin in this study was 82%, whereas the resistance was 16%, which were similar to the results reported by Ammari *et. al.* [17]. The sensitivity of *S.* Enteritidis to streptomycin in this study was 80%, was almost similar to the sensitivity (83%) reported by Okamoto, [18] and more than the sensitivity (7.14%) reported by Aktar *et. al.*, [26]. The resistance to streptomycin was 10%, which was higher than the resistance (2.08% & 7%) reported by Vaz, [23]; Okamoto, [18] respectively and less than the resistance (64.28%, 92.85%) reported by Sultana, [27], Aktar, [26]. The sensitivity of *S.* Enteritidis to tetracycline in this study was 76%, was almost similar to the sensitivity (78%) reported by Okamoto *et. al.* [18] and more than the sensitivity (13.7% and 64.28%) reported by Nunes, [19] and Aktar, [26]. The resistance to tetracycline was 20%, which was higher than the resistance (8.7%) reported by Pederson *et. al.* [20], and less than the resistance (35-38%) reported by Aksakal [25] and Poppe *et. al.* [24]. Very low level of resistance was reported by Vaz, [23] (1.04%) and Nunes, [19] (2.1%). The resistance to tetracycline might be due to this antibiotic being one of the most commonly used

antibiotics for animal production.

The sensitivity of *S.* Enteritidis to nalidixic acid in the present study was 60%, which was much higher than the sensitivity (18% & 27%) reported by Okamoto *et. al.* [18] & Nunes, [19] respectively, and lower than the sensitivity (93.33% and 97.9%) reported by Ammari *et. al.* [17] and Nunes, [19]. The resistance to nalidixic acid (30%) in the present study was similar to the resistance reported by Cruchaga and Ecifita, [28], whereas low resistance of (1-4%) was observed by Breuil *et. al.* [22] & Nunes *et. al.*, [19]. High resistance than the present study was reported by Fasche, [29] (81%) and Kottwitz, [30] (41.5%). The sensitivity of *S.* Enteritidis to ampicillin in the present study was 58%, which was similar to the results reported by Okamoto *et. al.* [18] and Turkyilmaz *et. al.* [21] and less than the sensitivity (86.66%, 97.8% & 92.85%) reported by Ammari *et. al.* [17], Nunes, [19] & Aktar , [26]. The resistance to ampicillin was 32% which was higher than the resistance (2.1%, 12.1%, 14.3% and 25%) reported by Aksakal [25] and Poppe *et. al.* [24] Nunes, [19] and Makaya, [31] respectively and less than the resistance (41%) reported by Okamoto *et. al.* [18]. Poor sensitivity (10%) and higher resistance (76%) to sulfonamide were observed in the present study were similar to the results reported by Nogueria *et. al.* [32] and Okamoto *et. al.* [18] Nunes, [19]. Very poor resistance of *S.* Enteritidis to sulfonamides (1.7%) was reported by Poppe *et. al.* [24] and Pederson *et. al.* [20], whereas 20% resistance was reported by Aksakal [25], and 34.37% by Vaz *et. al.*, [23]. Very high resistance was reported by Carraminana *et. al.*, [33] (99%), and Cardoso *et. al.*, [34] (86.25%).

Indiscriminate use of antibiotics results in varied degrees of antibiotic resistance, which will increase the course of any infectious disease. The other antibiotics which were not studied in this study may be carried out in future.

Conclusion

S. Enteritidis isolates were more sensitive to ciprofloxacin, chloramphenicol, amikacin, gentamycin, streptomycin, amoxicillin and tetracyclines and less sensitive to sulfonamides. Higher resistance was observed with sulfonamide followed by ampicillin and nalidixic acid.

Author's contribution

MT had planned the study, helped in analysis and drafted the manuscript. RP analysed the results and had written the revised the manuscript, TRE collected the samples, and analysed the data. All the authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interests.

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