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Isolation, serotype diversity and antibiogram of *Salmonella enterica* isolated from different species of poultry in India

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ABSTRACT

Objective: To study the occurrence and serotype diversity of *Salmonella* isolates in different species of poultry (chicken, emu and duck) and determine their resistance pattern against various antibiotics of different classes.

Methods: About 507 samples comprising 202 caecal contents and 305 fecal samples from chicken, emu and duck were processed for isolation of *Salmonella enterica*. Salmonellae were isolated and detected by standard protocol of ISO 6579 Amendment 1: Annex D. Genetic confirmation was also made by using *16S rRNA* genus specific PCR. Serotype specific PCR was also done to detect the most common serovars *viz*. *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Gallinarum. All obtained isolates were subjected to a set of 25 antibiotics to study their antibiogram by using Baeur–Kirby disk diffusion method.

Results: Out of 507 samples processed, 32 isolates of *Salmonella enterica* (18 from caecal contents and 14 from faecal samples) were obtained, of which 24 belonged to 6 different serovars, 6 were untypeable and 2 were rough strains. *Salmonella* Enteritidis was the most predominant serotype (9), followed by *Salmonella* Typhimurium (5), *Salmonella* Virchow (4), *Salmonella* Gallinarum (3), *Salmonella* Reading (2) and *Salmonella* Altona (1). Antibiotic resistance pattern was maximum (100%) to oxacillin, penicillin and clindamycin, followed by ampicillin (68.75%), tetracycline (65.62%), nalidixic acid (56.25%) and colistin (46.87%). High sensitivity of isolates was recorded for chloramphenicol (96.87%) followed by meropenem (84.37%).

Conclusions: Occurrence of high proportion of serovars in our study which can cause serious gastroenteritis in humans is a matter of concern. *Salmonella* Altona has been detected for the first time in India from poultry. This serotype is known to cause serious outbreaks of gastroenteritis in humans. Multidrug resistant isolates were recovered at high percentage which can be attributed to non-judicious use of antibiotics both in prophylaxis and treatment regimen. This observation draws serious attention as poultry serves as an important source of transmission of these multidrug resistant *Salmonella* serovars to humans.

1. Introduction

Salmonellosis is one of the important bacterial diseases which affect diverse number of hosts worldwide [1]. Poultry are the important reservoir of many zoonotically important pathogens, of which *Salmonella* is of prime importance [2]. Salmonellosis in poultry is an important area of study as it not only affects the poultry industry but can also occur in humans by consumption of contaminated poultry meat and eggs [3]. Poultry comprises a number of species which include chickens, ducks and emus. Salmonellosis has been endemic in poultry industry of India [4]. Several researchers have reported variable prevalence rates of *Salmonella* infection in different parts of India [5,6]. Diverse number of serovars of *Salmonella* has been reported from poultry worldwide. More than 53 serovars have been reported from India and this number is on ever increasing [7]. Various serovars like *Salmonella* Enteritidis (*S.* Enteritidis), *Salmonella* Typhimurium (*S.*

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Typhimurium), *Salmonella* Virchow (*S.* Virchow) and *Salmonella* Newport are important nontyphoidal causes of human salmonellosis caused by consumption of contaminated poultry products. *Salmonella* Gallinarum (*S.* Gallinarum) and *Salmonella* Pullorum are the only two host-specific true pathogens of poultry birds and they affect the poultry industry to a great extent resulting in huge economic losses in terms of morbidity and mortality. Isolation and identification of *Salmonella* are very tedious and take several days before coming to the final conclusion. There has been great demand in terms of quick and sensitive detection of *Salmonella* from poultry in order to take timely therapeutic and prophylactic measures. Several PCR-based assays have been developed for rapid detection of *Salmonella* sp. [8,9]. Various serotype-specific PCR have also been developed for some common serovars to reduce time and cost in processing isolates by conventional serotyping which is very much labor intensive and time-consuming [10].

In the past few decades, emergence of antibiotic resistance among different species of bacteria was on the rise [11]. This problem poses great threat to public health in case of zoonotically important bacteria transmitted from food animals. In this context, contaminated poultry products serve as an important threat to public health as it is an important reservoir of salmonellae. Irrational use of antibiotics as growth promoters in poultry is an important factor that has favored the selection of resistant bacteria in fecal microflora of poultry [12].These resistant strains are easily passed to human through food chains resulting in serious consequences in terms of treatment failure and rapid outbreaks of resistant salmonellae.

The present study was conducted to detect and determine the diversity of various serovars prevalent in poultry birds and associated public health risk in various regions of Rajasthan, India. The work will also help to know the status of antibiotic resistance pattern among various *Salmonella* isolates so as to aid in suggesting proper and effective therapeutic measures.

2. Materials and methods

2.1. Sampling

A total of 507 samples comprising 305 fecal samples and 202 caecal contents from different species of poultry (Table 1) were collected from March 2013 to August 2014. Freshly voided fecal samples were collected in sufficient amount in sterile test tubes by cotton swabs while caecal contents were taken from various slaughtered birds and transferred to laboratory as soon as possible on ice.

2.2. Isolation

Samples were homogenized in sterile phosphate buffer solution (pH 7.2) by stirrer to avoid contamination. Homogenized samples were centrifuged at 1500 r/min for 15 min to settle the coarse fecal matter. Supernatant was taken in fresh sterile tube to process according to guidelines of standard revised protocol for *Salmonella* isolation ISO 6579 Amendment 1: Annex D [13]. However, due to the limitation of this protocol in detection of only motile serovars, we also processed samples in less inhibitory selective broth of selenite cystine for recovery of nonmotile serovars. The protocol involved initial enrichment of supernatant in buffered peptone water (1:10) for 16 h at 37 °C. Three drops of each pre-enriched samples were placed separately on modified semi solid Rappaport-Vassiliadis (MSRV) agar and incubated at 41.5 °C for 24 h. After incubation, plates were observed for production of grey-white, turbid zone extending from point of inoculation (Figure 1). A loopful of culture was taken from the border of the opaque zone formed on MSRV and streaked on xylose lysine deoxycholate agar and Hektoen enteric agar. Plates were incubated at 37 °C for 24 h and observed for typical colonies of *Salmonella*. For detection of nonmotile *Salmonella*, pre-enriched samples were inoculated in selenite cystine broth and incubated at 37 °C for 24 h. Selective plating was done similarly to above. All suspected colonies were purified and preserved on nutrient agar slants.

Table 1
Detail of samples collected from different poultry species.

Type of samples	Poultry species			Total
	Chicken	Duck	Emu	
Fecal samples	232	38	35	305
Caecal contents	202	–	–	202
Total	434	38	35	507

2.3. Biochemical characterisation

All suspected colonies were subjected to different biochemical tests by HiSalmonella™ identification kit (Himedia, Mumbai, India). The kit contained 12 biochemical tests *viz.* methyl red, Voges–Proskauer, urease, hydrogen sulphide production, citrate utilization, lysine, o-nitrophenyl β-galactoside, lactose, arabinose, maltose, sorbitol and dulcitol. Also, isolates were inoculated in triple sugar iron agar slants to observe the triple sugar iron reaction.

2.4. Latex agglutination test

All suspected colonies were subjected to polyvalent latex agglutination test for preliminary identification by using

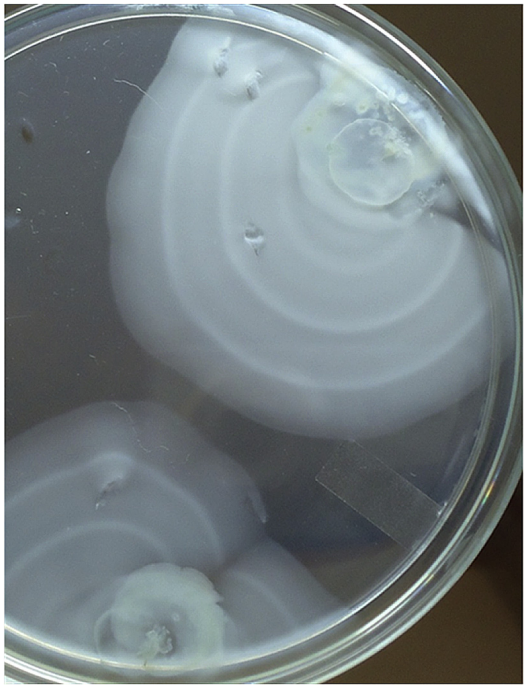

Figure 1. Grey-white, turbid opaque zone growth of tentatively positive sample of *Salmonella* sp. extending from point of inoculation on MSRV medium.