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Short communication

# Prevalence and antimicrobial resistance of *Salmonella* enteritidis and other salmonellas in the eggs and egg-storing trays from retails markets of Coimbatore, South India

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**Abstract**

A study was conducted to determine the incidence of *Salmonella enterica* serovar Enteritidis and other *Salmonella* serovars on eggshell, egg contents and on egg-storing trays. A total of 492 eggs and 82 egg-storing trays were examined over a period of 1 year from different retail outlets of a residential area of Coimbatore city, South India. *Salmonella* contamination was recorded in 38 of 492 (7.7%) eggs out of which 29 was in eggshell (5.9%) and 9 in egg contents (1.8%). Around 7.5% of the egg-storing trays were also found to be contaminated with *Salmonella*. Serotyping of the *Salmonella* strains showed that 89.7% of the strains from eggshell, 100% of the strains from egg contents and 71.4% of the strains from egg-storing trays were *Salmonella* Enteritidis. Other serovarvars encountered were *S.* Cerro, *S.* Molade and *S.* Mbandaka from eggshell and *S.* Cerro from egg-storing trays. Seasonal variations in the prevalence pattern were identified with, a higher prevalence during monsoon months followed by post-monsoon and premonsoon. Further examination of the *Salmonella* strains was carried out by testing their antimicrobial sensitivity against 10 commonly used antimicrobials. Results revealed high prevalence of multiple antimicrobial resistance among these strains suggesting possible prior selection by use of antimicrobials in egg production.


*Keywords:* *Salmonella* enteritidis; Egg; Antimicrobial resistance

## 1. Introduction

Salmonellosis is a most prevalent foodborne disease in many countries world-wide and it has been estimated that approximately 1.4 million cases were reported annually in the developed nations such as USA (Mead et al., 1999). The organism has been isolated from a range of foods in almost every country in which it has been investigated. Typical foodstuffs from which *Salmonella* has been isolated include swine and poultry-meat, poultry products and dairy products. (Gebreyes et al., 2000; Rajashekara et al., 2000). *Salmonella* Enteritidis is considered to be an important human pathogen worldwide and there has been a dramatic increase in human *S.* Enteritidis infections in the last decade. The consumption of eggs or egg containing foods has been associated with high percentage of many human *S.* Enteritidis outbreaks.

In recent years, concern about poultry, meats and other foodstuffs contaminated with foodborne pathogens has gained considerable attention because of the increased incidence of antimicrobial-resistant bacteria associated with human illness (CDC, 1997; Davies et al.,

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1999; Breuil et al., 2000). A high prevalence of antimicrobial resistant *Salmonella* in broiler chicken and foods of animal origin has been reported earlier from India (Hatha and Lakshmanaperumalsamy, 1995; Suresh et al., 2000). During the last few years, the National Egg Co-ordination Committee (NECC), Govt. of India has taken steps to promote egg as a source of good quality protein and the consumption of egg has gone up considerably. There are mass production centres in various parts of the country especially in southern states, Tamil Nadu and Andhra Pradesh. Though the consumption has been promoted, no effective steps are taken to monitor the quality of the egg reaching the market and no guidelines have been prescribed for the storage of eggs in retail markets. The present study has been taken up primarily to assess the level of *Salmonella* contamination in egg with special reference to *S.* Enteritidis, as it has been reported that egg-associated salmonellosis is a pandemic (Rodrigue et al., 1990). Further characterization of the isolated *Salmonella* strains was also carried out and the seasonality in incidence pattern has been investigated.

## 2. Methods

### 2.1. Sample collection

Eggs were collected randomly from different outlets, in a residential area of Coimbatore city, over a period of 1-year (June 1997–May 1998). A total of 492 eggshells, 492 egg content and 82 egg-storing trays were analysed for the presence of *Salmonella*.

### 2.2. Bacteriological methods

Unwashed eggs were collected in sterile polythene bags and transported to the laboratory. The surfaces of egg trays were swabbed on the spot and the samples were transported to laboratory in buffered peptone water (BPW, Himedia, Bombay). Aseptic procedures were strictly adopted during collection of samples. To study the seasonal variation in the prevalence of *Salmonella*, the study period was divided into premonsoon (February–May), Monsoon (June–September) and post-monsoon (October–January).

Swab technique was used to sample the shell surface of the intact eggs and egg-storing trays. Sterile cotton swabs dipped in sterile BPW were used to swab the entire surface area of the eggshell and the area to keep one egg in an egg-storing tray. For isolation of *Salmonella*, modified method of Hatha and Lakshmanaperumalsamy (1997) was used, the preenrichment medium lactose broth was replaced with BPW. The swabs were directly inoculated into 10 ml BPW in screw-capped bottles and incubated at 37 °C for 24 h.

In order to collect the egg contents, eggs were surface sterilized by immersion in 70% alcohol for 2 min, air dried in a sterile chamber for 10 min then cracked with a sterile knife. Each egg's content was mixed thoroughly and 25 ml of the mixed egg content was inoculated into 225 ml of BPW and incubated at 37 °C for 24 h. After preenrichment, 1 ml of the cultures of all sample types were transferred to 9 ml of tetrathionate broth (Himedia, Bombay) and to 9 ml of selenite cystine broth (Himedia, Bombay) and incubated at 37 °C for 24 h for selective enrichment. The cultures were then streaked onto xylose lysine deoxycholate (Himedia, Bombay) agar, brilliant green agar (Himedia, Bombay) and hektoen enteric agar (Himedia, Bombay) and incubated at 37 °C for 24–48 h. The plates were observed for typical *Salmonella*-like colonies, and two colonies per plate were picked, purified and subjected to primary biochemical screening which involved reactions on triple sugar iron (Himedia, Bombay) agar, lysine iron agar (Himedia, Bombay), indole production in tryptone broth (Himedia, Bombay) and urea splitting ability in Christesen's urea agar (Himedia, Bombay). Cultures that matched typical reactions of *Salmonella* in preliminary screening were further subjected to carbohydrate utilization involving lactose, sucrose, dulcitol and salicin and further confirmed by slide agglutination test using polyvalent O sera (Wellcome laboratories, England). The confirmed strains were serotyped at National *Salmonella* and *Escherichia* Centre, Govt. of India Central Research Institute, Kasauli, Himachal Pradesh.

### 2.3. Antimicrobial sensitivity testing

Antimicrobial susceptibility tests were carried out by the disk diffusion method (Bauer et al., 1966). Antimicrobials used, and their concentrations are as follows: Ampicillin (10 mcg); Chloramphenicol (30 mcg); Ciprofloxacin (10 mcg); Gentamycin (10 mcg); Kanamycin (30 mcg); Nalidixic adid (30 mcg); Neomycin (30 mcg); Polymixin B (300 units); Sulphamethoxazole (25 mcg) and Tetracycline (30 mcg). All the antimicrobial disks were purchased from Himedia, Bombay.

Pure cultures of *Salmonella* serovars were enriched in brain–heart infusion broth at 37 °C for 6–8 h. These cultures were then streaked over Mueller Hinton agar plates (Himedia, Bombay) using a sterile cotton swab. The antibiotic discs were dispensed using a disc dispenser (Himedia, Bombay) sufficiently separated from each other so as to avoid overlapping of inhibition zones. After 30 min the plates were inverted and incubated at 37 °C for 16–18 h. Results were recorded by measuring the diameter of the inhibition zones and compared with the interpretive chart of performance standards for antimicrobial disk susceptibility tests, supplied by the Himedia laboratories,

Bombay and classified as resistant, intermediate and sensitive.

## 3. Results and discussion

### 3.1. Incidence of Salmonella in eggs and egg-storing trays

The results of *Salmonella* incidence in marketed eggs and commercial egg-storing trays are shown in Table 1. *Salmonella* contamination was recorded in 6.1% of eggshells and 1.8% of egg contents. *Salmonella* contamination was also recorded in 7 (8.5%) of 82 egg trays. The incidence levels of *S.* Enteritidis in eggshell reported earlier were variable. In Spain, Perales and Audicana, (1989) reported around 1% *Salmonella* contamination. In the United Kingdom prevalence levels were reported to be varying from zero (Mawer et al., 1989) to 7% (Humphrey, 1994a, b, Evans et al., 1998). The prevalence level in the present investigation is slightly higher than these observations. It may be noted that an average consumer in India is not very aware of the consequences of food-poisoning and often the producers and retailers take advantage of the situation. The eggs are not well scrubbed and they are sold fresh from the production facility. This results in the presence of faecal matter on the eggshell and resultant presence of pathogen if the hen is excreting *Salmonella*. Also the eggs are stored at room temperature, which again helps mesophiles to multiply fast if they get access to egg content through cracks which can develop during transportation and handling.

The results clearly indicate that *S.* Enteritidis was the most frequently isolated (86.7%) serovar on the eggshell along with other 3 serovars, such as *S.* Cerro (6.7%), *S.* Mbandaka (3.3%) and *S.* Molade (3.3%). In the present investigation, egg contents were contaminated only by *S.* Enteritidis and other serovars of *Salmonella* spp. isolated from the eggshells were not recovered in the egg contents. The results of this study suggests that not all the eggs with shell contamination had their contents contaminated but all the eggs contaminated with *S.* Enteritidis in their content had contamination on their shells. The contamination recorded in eggs content was only by *S.* Enteritidis and no other serovars were isolated from the egg content. This is indicative of transovarial route of contamination by *S.* Enteritidis. One of the eggshells was found to have both *S.* Enteritidis and *S.* Mbandaka. It is reported that the contamination of egg contents with *S.* Enteritidis is predominantly the result of infection of the reproductive tissue rather than passage through the shell after lay (Humphrey, 1994a, b). There was no association between shell contamination and the presence of *S.* Enteritidis of egg content laid by naturally infected hens (Mawer et al., 1989; Humphrey et al., 1989, 1991).

Table 1
The prevalence of *Salmonella* in eggshell, egg content and the egg-storing trays and the serovars encountered

| Source | No. of sample analysed | No. of samples tested positive | Serovars encounterd |
|---|---|---|---|
| Eggshell | 492 | 30 (6.1)[a] | *S.* Enteritidis (86.7)[a]<br>*S.* Cerro (6.7)<br>*S.* Molade (3.3)<br>*S.* Mbandaka (3.3) |
| Egg content | 492 | 9 (1.8) | *S.* Enteritidis (100) |
| Egg storing tray | 82 | 7 (7.5) | *S.* Enteritidis (71.4)<br>*S.* Cerro (28.6) |

[a]Values in the parenthesis indicate the percentage of incidence.

### 3.2. Seasonal variation in incidence pattern

Variation was recorded in the prevalence of *Salmonella* in different seasons of the study period (Table 2). The results show that incidence of *Salmonella* was higher in the monsoon and post-monsoon seasons. The results were subjected to significance testing using chi-square which revealed that there were no significant variation in the incidence levels during different seasons ($P<0.233$). Feachem (1974) and Goyal et al. (1977) reported that a reduced temperature is preferred by many pathogens. Survival of *Salmonella* on eggshells is enhanced by high relative humidity and low temperature (Lancaster and Crabb, 1953; Baker, 1990), which is well-marked feature during the monsoon and post-monsoon months. High temperature results in the reduced prevalence during the premonsoon. Baker (1990), who observed that *Salmonella* on eggshells die rapidly at high temperatures during storage reported similar results. High temperature ranging from 28 to 34 °C is a characteristic feature of premonsoon seasons in the study area, compared to 20–25 °C during post-monsoon season. Prevalence of *Salmonella* in egg content did not follow the seasonal variation trend as recorded in eggshells and increased prevalence was recorded in the premonsoon season, possibly due to higher levels of multiplication in higher temperatures.

### 3.3. Antimicrobial resistance patterns

The results of antimicrobial resistance of *Salmonella* strains isolated from both eggs and egg-storing trays are given in Table 3. The results revealed that all the strains from egg and egg-storing trays were resistant to ampicillin, neomycin, polymyxin-B and tetracycline. Though none of the *Salmonella* strains from eggs and

Table 2
Seasonal variation in the prevalence of *Salmonella* in eggs and egg-storing tray

| Season | Percentage incidence of *Salmonella* in | | |
|---|---|---|---|
| | Eggshell | Egg content | Egg-storing tray |
| Premonsoon | 20.69 | 22.22 | 28.57 |
| Monsoon | 44.83 | 33.33 | 42.86 |
| Post-monsoon | 34.48 | 44.44 | 28.57 |

Table 3
Percentage of antimicrobial resistance among the *Salmonella* strains from egg and egg-storing trays

| Antimicrobials | Egg ($n^a = 39$) | Egg trays ($n = 7$) |
|---|---|---|
| Ampicillin | 100 | 100 |
| Chloramphenicol | 0.0 | 0.0 |
| Ciprofloxacin | 8.9 | 0.0 |
| Gentamicin | 0.0 | 0.0 |
| Kanamycin | 31.1 | 45.5 |
| Nalidixic acid | 40 | 45.5 |
| Neomycin | 100 | 100 |
| Polymyxin-B | 100 | 100 |
| Sulphamethoxazole | 4.4 | 18.2 |
| Tetracycline | 100 | 100 |

[a]$n$ = Number of strains of Salmonella.

Table 4
Antibiotic resistance patterns of different *Salmonella* serovars from the eggshell, egg contents and egg-storing tray

| Source | *Salmonella* Serovar | Resistance pattern | % of occurrence |
|---|---|---|---|
| Eggshell | *S.* Enteritidis ($n = 26$) | AKNaNPT (1)[a] | 3.33 |
| | | ANaNPST (1) | 3.33 |
| | | ANaNPT (8) | 26.67 |
| | | AKNPT (7) | 23.33 |
| | | ACiNPT (2) | 6.67 |
| | | ANPT (7) | 23.33 |
| | *S.* Cerro ($n = 2$) | AKNPT (2) | 6.67 |
| | *S.* Molade ($n = 1$) | ACiNPT (1) | 3.33 |
| | *S.* Mbandaka ($n = 1$) | ACiNPT (1) | 3.33 |
| Egg content | *S.* Enteritidis ($n = 9$) | AKNaNPT (1) | 11.11 |
| | | ANaNPST (1) | 11.11 |
| | | ANaNPT (2) | 22.22 |
| | | AKNPT (3) | 33.33 |
| | | ANPT (2) | 22.22 |
| Egg tray | *S.* Enteritidis ($n = 5$) | AKNaNPST (1) | 14.28 |
| | | AKNaNPT (2) | 28.56 |
| | | ANPT (2) | 28.56 |
| | *S.* Cerro ($n = 2$) | ANPT (2) | 28.56 |

A—Ampicillin, Ci—Ciprofloxacin, K—Kanamycin, Na—Nalidixic acid, N—Neomycin, P—Polymixin-B, T—Tetracycline.
[a]Figures in the parenthesis indicate the number of serovars with similar resistance pattern.

egg-storing trays were resistant against chloramphenicol and gentamicin, they differed in their resistance level to ciprofloxacin as 8.9% of the strains from eggs were resistant and none of those from egg-storing trays showed any resistance. There was no significant variation in resistance levels among *Salmonella* strains from eggs and egg-storing trays.

In poultry, the antimicrobial tetracycline is used in day-old poults and chicks as a single injection, or administered via the drinking water to control infection by *Salmonella*, *Escherichia coli* and *Mycoplasma* (Lucas et al., 1970; Cassell, 1981; Hamdy et al., 1982). Ampicillin-resistant strains isolated from eggs were in higher proportion than from the broiler chickens and environmental samples from India (Suresh et al., 2000). Resistance to ciprofloxacin and sulphamethoxazole was recorded relatively at lower proportions. Ciprofloxacin is a fluoroquinolone antimicrobial that is increasingly and successfully used for the treatment of septicemic salmonellosis in humans (Brown et al., 1994; Griggs et al., 1994). Ciprofloxacin resistance in human and veterinary *Salmonella* isolates has occasionally been found (Brown et al., 1994). All the strains isolated from egg and egg-storing trays were found to be resistant to neomycin, tetracycline and ampicillin. Tetracycline has been used to treat day-old chickens, which might have resulted in the emergence of tetracycline resistant *Salmonella* in the layer and broiler flocks (Ekperigin et al., 1983; Williams, 1984).

The resistance patterns encountered among *Salmonella* serovars from different sources is represented in Table 4. The results indicated six different patterns of resistance among the *Salmonella* strains from egg and 3 different patterns among the *Salmonella* strains from egg-storing trays. The *Salmonella* strains having similar level of resistance and resistance pattern indicates their origin from a common source. The logical interpretation of the results of the MAR index is that all *Salmonella* stains isolated in the study showed that the strains might have originated from environments where antimicrobials are often used. Poultry, one of the major reservoirs of *Salmonella* species, are considered to be a high-risk source of *Salmonella* species. There is a large body of literature reviewed by Novick (1981) demonstrating that the sub-therapeutic and therapeutic use of antimicrobials in the mass production of poultry, eggs and pork has promoted the emergence and maintenance of MAR pathogenic bacteria in the environments of these animals. Also ongoing infection with *S.* Enteritidis and use of medication at breeder level could considerably increase the prevalence of multiple resistant *S.* Enteritidis in poultry rearing environment.

Control measures, which could be applied at a number of points from farm to home which might limit the risk to public health from infection of laying hens with *S.* Enteritidis and the associated contamination of eggs have been recommended by several authors. On-farm measures such as regular testing of birds, faecal

material, applying serological testing methods to detect *S.* Enteritidis, eliminating environmental contamination before replacement of new birds, providing contamination free feed, frequent egg collection and improved hatchery hygiene, oral administration of cultured caecal contents from an appropriate donor bird to re-establishing a mature gut flora and chlorination of drinking water could be practised to reduce *Salmonella* contamination. The specific recommendations considering the Indian scenario involves better temperature control of eggs at retail and catering outlets, thorough cleaning of the shell eggs before marketing and monitoring the availability of cracked eggs from retail stores merits due consideration. The results of the present study indicate that *S.* Enteritidis contaminated eggs are common in the Indian retail market. The multiple antimicrobial-resistant nature of the organism adds to the gravity of the problem. Also the reuse of egg trays and use as play things by children could pose potential risk. As the results from this single investigation are not sufficient for formulating standards by the regulatory agencies, more large-scale studies are required to quantify the situation on a national basis. Along with the promotion of the egg as complete food consumer awareness programmes related to health risk may also be imparted by the regulatory agencies of government of India.

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