



## Molecular detection and antibiogram of virulent *Salmonellae* isolated from chicken meat

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### ABSTRACT

*Salmonella* has emerged as one of the most common and frequent pathogen associated with the food poisoning. In the present study, a total of 240 chicken meat samples were collected from retail markets of Shivamogga region; where, 13 samples were found positive for *Salmonella* species by genus specific PCR targeting *invA* virulent gene, giving an overall prevalence of 5.4%. Retail outlets (slaughter-cum-retailing units) that traditionally processed chicken meat showed higher prevalence of 7.5% (n=120); whereas, retail chicken meat originated from organized abattoirs showed prevalence of 3.3% (n=120). Among traditional retail outlets, those retail units processing multiple animal species showed higher prevalence (8.3%, n=60) of *Salmonella* spp. compared to those that processed chicken meat alone (6.6%, n=60). All the *Salmonella* species (n=13) isolated from the chicken meat possessed virulence (*invA*) gene indicating their potential pathogenic mechanisms and also showed varying degree of susceptibility against common antimicrobials (multiple antibiotic resistance index of 0.1176 to 0.3529). High prevalence rate of *Salmonella* spp. has important public health concern and underscores a need for intense surveillance and monitoring of retail chicken meat and implementation of food quality assurance systems across the chicken supply chain.

**Key words:** Antibiogram, Chicken meat, Prevalence, Retail market, *Salmonella*, Virulence

*Salmonellae* are Gram negative bacteria belonging to the family Enterobacteriaceae. Of the several pathogens, *Salmonella* species appear to be the commonest cause of food poisoning not only in the developing countries but even the industrialized nations (Costa *et al.* 2012). *Salmonellae* predominantly occur in poultry (chicken meat and eggs) compared to other animal origin foods such as pork, beef and mutton; retail chicken meat gets *Salmonella* species either from birds (farming), slaughter processing (carcasses contamination) or distribution (retailing) of the meat (WHO 1992). Koushik *et al.* (2014) reported 23.7% of *Salmonella* prevalence in chicken meat. According to a study, about 22.8 million cases of food acquired

*Salmonellosis* have been reported in the South East Asian region with 37,600 deaths annually (Van *et al.* 2012).

*Salmonella* species pose immense public health risk worldwide and poultry continues to be the major contributor (Kabir 2010). Human transmission occurs by the way of consumption of raw or undercooked poultry (meat or eggs), contaminated water or through occupational exposures (Bailey and Cosby 2003). Most importantly, consumption of the poultry meat originating from poor processing facilities potentially leads to increased incidence of such foodborne diseases (Pui *et al.* 2011). *Salmonella* species predominantly infect mucosal surfaces and their invasion propensities lead to chronic conditions such as reactive arthritis, Reiter's syndrome and ankylosing spondylitis (Bunning *et al.* 1988, Smith 1994). Although, cultural isolation is considered as gold standard for isolation and identification of *Salmonella*, molecular techniques like PCR are increasingly being used for rapid detection and confirmation of *Salmonella* species.

Apart from the public health risk by the way of foodborne diseases, *Salmonella* in the boiler chicken meat have serious economic and trade consequences. Keeping these considerations in view, a cross-sectional survey was carried out to generate the baseline data on the prevalence of *Salmonella* species in the retail chicken meat and to

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characterize the isolates for their virulence properties using polymerase chain reaction (PCR) and also to study the antibiogram profile of *Salmonella* isolates.

#### MATERIALS AND METHODS

Chicken meat samples (240) were collected from retail markets located in Shivamogga (13.9333° N, 75.5667° E; Karnataka state). Based on its origin, sample were categorized as *organized* (meat obtained from organized abattoirs) or *unorganized* (traditional slaughter processing) retail units. Also, depending on number species retailed, the outlets were designated as *single* (chicken only) or *multiple* (sheep and goat) species retail outlets (Table 1). Samples were transported to the laboratory under chilled conditions (<4°C) and subjected for isolation of *Salmonella* species using the standard ISO 6579:2002 method. Virulence specific invasion gene (*invA*) was amplified using polymerase chain reaction (PCR) for determining the pathogenic potential of *Salmonella* isolates. For this, DNA from pure cultures was isolated using the snap-chill method (Salehi *et al.* 2005) followed by PCR using the primers reported by Rahn *et al.* (1992) viz. 5'-GTG AAA TTA TCG CCA CGT TCG GGC AA-3' (forward) and 5'-TCA TCG CAC CGT CAA AGG AAC C-3' (reverse) so as to get a specific product of 284 bp size (Nagappa *et al.* 2007). Antimicrobial susceptibility of the isolates was determined against 17 antimicrobials using the Kirby-Bauer's disc diffusion method (Bauer *et al.* 1966, Clinical and Laboratory Standards Institute-CLSI, 2012) and their maximum antimicrobial resistance (MAR) index was calculated as described by Yildirim *et al.* (2011).

#### RESULTS AND DISCUSSION

##### *Prevalence of Salmonella species in retail chicken meat:*

A total of 13 samples were found positive for *Salmonella* species giving an overall prevalence of 5.4%. Higher prevalence of *Salmonella* species (7.5%) was observed in 'unorganized retail meat' compared to the 'organized retail meat' (3.3%). Further, traditional retails that processed multiple species showed higher prevalence of *Salmonella* spp. (8.3%) compared to those retails that processed chicken species alone (Table 1).

Prevalence of *Salmonella* spp. in chicken meat has been found to be highly variable (4.2 to 83.3%) as evident by the studies conducted by different researchers (Pangloli *et al.* 2003, Schönenbrücher *et al.* 2008, Greig and Ravel 2009, Minami *et al.* 2010, Moon 2011). Reason for such variation in the prevalence of *Salmonella* species in chicken meat could be attributed to the geographic location, type and nature of slaughter processing units, level of hygiene/sanitation followed across the supply chain and the management practices (Arslan and Ayla 2010). Differences in the prevalence of *Salmonella* between organized and unorganized retails observed in this study could be attributed to the improved sanitary practices followed during processing, storage and handling of chicken meat in the organized sector than the traditional system of chicken meat

Table 1. Prevalence of *Salmonella* in retail chicken meat

| Retail meat type        | Origin of meat                                | Species retailed (No. of samples positive) | (No. of samples analyzed) (%) |
|-------------------------|---|--|-------------------------------|
| Organized retail meat   | Centralized processing in organized abattoirs | One (chicken) (n=120)                      | 4 (3.3)                       |
| Unorganized retail meat | Traditional processing at retail outlets      | One (chicken) (n=60)                       | 4 (6.6)                       |
|                         |   | Multiple (chicken + sheep + goat) (n=60)   | 5 (8.3)                       |

production (Morris and Wells 1970, Sharma *et al.* 1995, Corry *et al.* 2002). Most importantly, cross-contamination occurring between species due to handling, equipment, water and processing environment contributes to the increased contamination especially when multiple species are processed at a single retail unit.

*Detection of invA gene in Salmonella isolates using PCR:* Pathogenic *Salmonella* species possess virulence mechanisms; invasion is a property that confers virulence making an isolate pathogenic to the host. Amplification of 284 bp product specific to *invA* gene shows virulence property of the isolate. To produce the clinical disease in human consumers, the isolate must be virulent; hence such pathogenic determinants must be detected in the *Salmonella* species isolated from the field samples. All the 13 *Salmonella* isolates recovered from the retail chicken meat in the present study showed pathogenicity associated virulence (*invA*) gene (Fig. 1).

The *invA* gene based PCR approach has become an

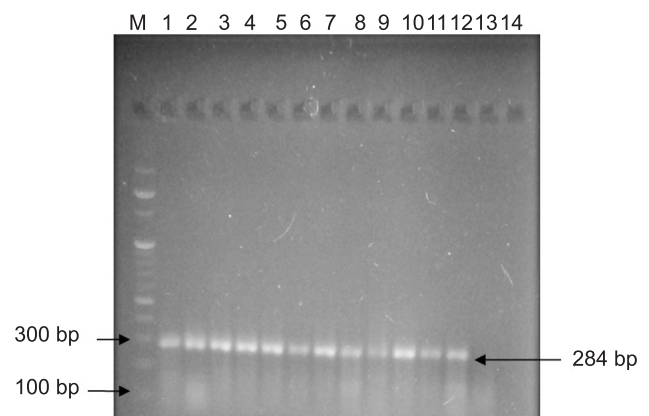


Fig. 1. Polymerase chain reaction (PCR) amplification of *invA* gene from *Salmonella* isolates. M, 100 bp ladder; lane 1, *S. Typhimurium*; lane 2, 7R; lane 3, 20R; lane 4, 28R; lane 5, 36T; lane 6, 40T; lane 7, 42R; lane 8, 57R; lane 9, 111T; lane 10, 120T; lane 11, 165R; lane 12, 171R; lane 13, NTC and lane 14, blank.

international standard for the confirmation of members of the genus *Salmonella*; hence, detection of *invA* gene further confirms the *Salmonella* species that were earlier confirmed by a battery of classical biochemical and sugar fermentation reactions (Malorny *et al.* 2003).

The *invA* gene has been proved as virulence or pathogenicity marker in *Salmonella* isolates (Makino *et al.* 1999, Feretti *et al.* 2001, Malorny *et al.* 2003, Nagappa *et al.* 2006, Wolffs *et al.* 2006); nevertheless, other virulence markers viz. 16S rRNA, *rfbE*, *fliC*, *virA*, *spvC*, *inT* and *fliO* have also been targeted for this purpose (Khan *et al.* 2000, Agarwal *et al.* 2002). Presence of *invA* gene specific 284 bp PCR amplicon in all the 13 retail chicken meat isolates suggested their potential pathogenic nature. Further, the results of PCR were in concordance with the cultural or biochemical confirmations.

**Antimicrobial susceptibility and MAR index of *Salmonella* isolates:** Antibiogram of 13 *Salmonella* isolates against 17 antimicrobials showed complete sensitivity against seven antibiotics viz. amikacin, cefotaxime, ceftriaxone/tazobactam, chloramphenicol, ciprofloxacin, enrofloxacin and gentamicin. Sensitivity of *Salmonella* isolates against some antimicrobials was variable i.e. ceftazidime (84.6%), streptomycin (84.6%) and cotrimaxazole (76.9%). However, majority of isolates showed resistance to polymixin-B (76.9%) followed by nalidixic acid (69.2%), ampicillin (61.5%), ampicillin-sulbactam/colistin/tetracycline (53.8%), cefadroxil (23.0%), streptomycin (15.3%) and ceftazidime (7.6%).

Bacteria are usually under selection pressure due to the widespread use of antimicrobials in food animal production and exposure to the sub-therapeutic doses lead to the emergence of resistance among *Salmonella* species (Wray *et al.* 1986, WHO 1988). Varying degree of susceptibility has been observed among *Salmonella* species against commonly used antimicrobials (Itoh *et al.* 1997, Salehi *et al.* 2005, Van *et al.* 2012). Moon (2011) found resistance to ciprofloxacin in 56.25% isolates and similar studies have also showed 9.6% and 35% resistance (Malorny *et al.* 2003, Mayrhofer *et al.* 2004); however, all the isolates in this study were sensitive to ciprofloxacin. Dione *et al.* (2009) also reported resistance against sulfamethoxazole/trimethoprim (75.9%), tetracycline (74.7%) and streptomycin (73.9%) in *Salmonella* isolates. Similarly, Minami *et al.* (2010) reported resistance to tetracycline and streptomycin in *Salmonella* isolates. When compared to these studies, lower rate of resistance was observed to tetracycline (53.84%) and streptomycin (15.39%) in our study. Ellerbroek *et al.* (2010) also reported resistance against tetracycline (92.2%), chloramphenicol (41.5%) and ciprofloxacin (49.4%) in *Salmonella* species isolated from the chicken meat. The pattern of antimicrobial susceptibility observed in this study emphasized need for rational use of antimicrobials in poultry production practices.

The MAR index evaluates health risk associated with the field isolate (Kruperman 1983). MAR index >0.2 indicates acquisition of resistance by such bacterial against several

antimicrobials. The MAR index of *Salmonella* isolates ranged from 0.1176 to 0.3529 (mean 0.2578, median 0.2941). Highest MAR index of 0.3529 indicated resistance against six antimicrobials; however, these indices were low as compared to the observations made by Yildirim *et al.* (2011) (range 0.214–1.0, average 0.593). Higher MAR index indicates selective acquisition of resistance in the bacterial isolate which has serious concerns on the public health.

Cross-sectional survey showed *Salmonella* species in 5.4% of retail chicken meat samples. Level of occurrence was higher in retail chicken meat originating from unorganized traditional type processing than organized abattoirs. The unorganized retailers that processed multiple animal species showed higher prevalence compared to those retailers that processed chicken meat alone. Unorganized traditional retail outlets must promptly adhere to the hygienic codes of slaughter and processing of the chicken meat in order to reduce the prevalence of *Salmonella*. Source of contamination to the meat though could be attributed to the prevailing practices of production and processing, it needs to be further investigated. All the *Salmonella* isolates had virulence (*invA*) gene indicating their pathogenic nature and showed varying degree of antimicrobial susceptibility with multiple antibiotic resistance index ranging from 0.1176 to 0.3529. Multi drug resistant *Salmonella* spp. from chicken meat and the MAR indices had led to the conclusion that injudicious usage of multiple antibiotics in the modern poultry practice is prevalent and could be a major contributing factor for MDR.

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