

## Isolation, Identification and Antibiotic Susceptibility of *Salmonella* Species from Seafood Sold in Local Markets of Goa

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### Abstract

Present short work was designed to study incidences of *Salmonella* species in the raw seafood from local markets and fish collection jetties of Goa. Study observed 12% (24/200) of the raw sea foods (9.5% fish, 2% prawns and 0.5% crabs) contaminated with *Salmonella* spp. All the 24 isolates were tested for antimicrobial susceptibility by disc diffusion assay. Isolates were found resistant to trimethoprim-sulfamethoxazole (100%), ampicillin (41%) and tetracycline (25%). All the isolates were sensitive to ciprofloxacin, chloramphenicol, streptomycin and norfloxacin. Intermediate phenotypes were observed against nalidixic acid, kanamycin and gentamicin. The study shows occurrence of antibiotic resistant *Salmonella* species in retail fish markets and collection jetties in Goa, India.

### Introduction

Seafood has high nutritional value and seafood products are increasingly popular worldwide. The biological agents which are involved in sea food contamination includes bacteria, viruses and parasites which can cause range of symptoms like mild gastroenteritis to life threatening diseases (Amagliani et al. 2012). Bacterial contamination of seafood products are being a major risk factor for transmission of pathogens (Iwamoto et al. 2010).

*Salmonellae* are Gram-negative, facultative anaerobes, non-spore forming rods that belong to the family *Enterobacteriaceae*. Salmonellosis has two distinct syndromes; it can be detected as systemic disease or gastroenteritis. Gastroenteritis is more often associated with food borne transmission (Knodler 2014). *Salmonella* has been highlighted as the potential outbreak causing infection in European countries (EFSA 2010), US (CDC 2014) and other

countries worldwide including India (Kumar et al. 2009). *Salmonella* serovars are widely present and can enter in water bodies through wild animals, domestic stock, poor sanitation and inappropriate disposal of human and animal waste (Amagliani et al. 2012). Salmonellosis constitutes a major public health burden and represents a significant cost to society in many countries. Very few countries report data on the economic cost of the disease. In case of USA, an estimated 1.4 million nontyphoidal *Salmonella* infections were reported annually resulting in 168,000 visits to physicians, 15,000 hospitalizations and 580 deaths (Voetsch et al. 2004). Vast range of sea food products were reported with *Salmonella* infections worldwide which includes molluscs, mussels, oysters, and shrimps (Iwamoto et al. 2010). Nearly 10% of import and 2.8% of domestic raw sea food were positive for *Salmonella* (Heinitz et al. 2000). Particularly in India, Kumar et al. (2009) has studied *Salmonella* extensively and food type such as Finfish, Shrimps, Clams, Crab, Lobster,

Octopus, Squid Mussel and Oyster have been reported to be contaminated with *Salmonella*. In Mangalore, Indian seafood associated food poisoning outbreaks were observed causing 34 persons to be infected (Antony et al. 2009).

Goa which lies on the West coast of India is a smallest Maritime time State with a coastline of 104 Km. It has 48 Fishing villages along the coast line however Fish landing takes place at 88 Centres. The continental shelf of Goa has been estimated at 10,000 square meters. Approximately, 40,000 to 50,000 people are dependent on fish harvesting, processing and marketing in Goa (Gaonkar 2008). In Goa (India), seafood is highly preferred due to ease of availability. These raw seafood are sold locally without any further processing in order to minimise bacterial load. Therefore, occurrence of bacterial pathogens such as seafood can't be denied. In this preliminary study we made an attempt to determine the incidences of *Salmonella* species among the fresh seafood catch and seafood that gets sold locally in state of Goa.

## Material and Methods

### *Sample collection*

A total of 200 raw seafood samples comprising fish (143), prawns (47) and crabs (10) were collected in sterile zip-lock bags. Samples were collected from two jetties (Vasco and Betim) and two local fish markets (Panjim and Mapusa) of Goa. Samples were transported to the laboratory in sterile containers at 4°C and processed immediately.

### *Isolation and Identification*

Isolation of *Salmonella* species was carried out according to the method defined by United States Department of Agriculture (USDA 2014). Accordingly, the samples were washed with sterile water and macerated by stomacher in sterile bags. Approximately one gram of macerated sample was inoculated into 10 ml of buffered peptone water and incubated at  $35 \pm 2^\circ\text{C}$  for 22-26 h. The enriched broth was transferred (0.5 ml) into 10 ml tetrathionate broth (TT) broth. The samples were incubated at  $42 \pm 0.5^\circ\text{C}$  for 22-24 h. A loopful of enriched broth was streaked on Hektoen Enteric agar (HKE) and incubated at 37°C for 24 h. Green colonies with dark

centres were suspected as *Salmonella* spp. Presumptive isolates were stored at 4°C in Nutrient broth. These presumptive *Salmonella* isolates were further confirmed by biochemical tests. Isolates were tested for Gram staining, catalase activity, oxidase activity L-lysine decarboxylase and  $\alpha$ -galactosidase (ONPG) activity. Biochemical tests such as Voges Proskauer and Indole were performed and typical growth on TSI and Urea agar (Christensen) slants were observed.

### *Antimicrobial susceptibility testing (AST)*

A panel of 10 antibiotics namely Ampicillin, Trimethoprim-Sulfamethoxazole, Ciprofloxacin, Chloramphenicol, Streptomycin, Tetracycline, Norfloxacin, Nalidixic acid, Kanamycin and Gentamicin were selected for AST which are generally used in human diseases prophylaxis to treat bacterial infections. For tests, two to three typical colonies of each isolate from XLD or HE agar were enriched in 5 ml of BHI broth at 37°C for 12-18 h. After incubation growth was pelleted by centrifugation at 5000g for 5 min and adjusted to 0.5 McFarland with 0.85 % NaCl. Adjusted bacterial suspension was spread evenly over Muller Hinton agar plates with sterile cotton swabs. Antimicrobial discs (Hi-Media, Mumbai) were placed by keeping appropriate distance between discs. The plates were immediately kept in the refrigerator at 4°C for 10-15 min in order to diffuse the antimicrobial concentration evenly before the initiation of bacterial growth phases. Later the plates were incubated at 37°C for 18 to 24 h. The zones of inhibition were measured and compared with Clinical and Laboratory Standard Institute (CLSI) standards.

## Results and discussion

Fishery sector has been playing very important role in improving the socioeconomic status of over 14 million fishers in the country, whose livelihood is depending on fishing and allied activities (Gaonkar 2008). This is emerging as a viable sector contributing towards employment generation in fishing and allied activities, supplementing food supply, raising nutrition level and earning foreign exchange through exports. The Marine Fisheries resources of Goan waters provide valuable food and support livelihoods to fishermen and their families (Gaonkar 2008). The fresh catch obtained by fisherman is sold locally without any effort for microbicidal treatments. On the other hand recent reports suggest that such

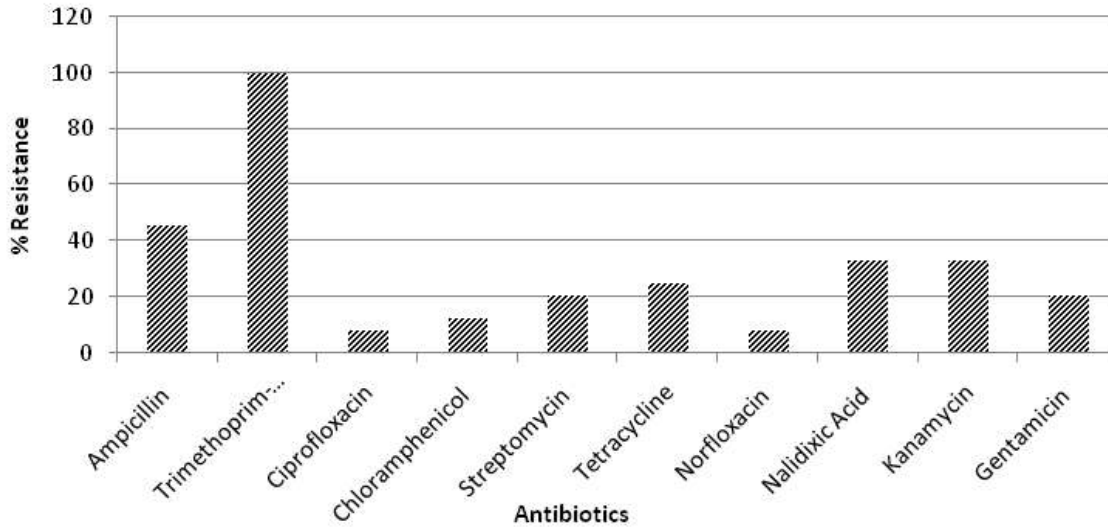


Fig. 1: Antibiotic resistance profile of *Salmonella* species obtained from seafood

seafood may possess bacterial pathogens contamination (Poharkar et al., 2014; Poharkar et al., 2013). Therefore it is necessary to understand the incidences of pathogens in such seafoods.

In this study, an attempt was made to observe the incidences of *Salmonella* species among the fish catch from coast areas of Goa and local market. To determine the incidences, a total of 200 samples comprising of fish (143), prawns (47) and crabs (10) were collected and processed for isolation of *Salmonella* species. The isolation and identification was performed as per method defined by USDA. Accordingly, 24 samples were found to contain *Salmonella* species. Of these 24, 19 were from fish, 4 from prawns and 1 from crab. Several previous studies indicates such incidences of *Salmonella* from seafood (Amagliani et al. 2012). *Salmonella* species are widely present in nature. The main source for the addition of *Salmonella* species to waterbodies are domestic waste, wild animals and direct disposal of sewages (Amagliani et al. 2012). Once *Salmonella* gets introduced to the environment it can survive for longer period (Winfield & Groisman 2003). Such longer persistence ability of *Salmonella*, in water bodies increases the chances of contamination of associated biota such as fish. Contamination of seafood is commonly observed and therefore frequently reported. In addition, several types of fish have been reported to contain *Salmonella* in their intestinal content, which may spread to organs (Nesse, Løvold, et al. 2005; Nesse, Refsum, et al. 2005). Huss et al. (2000) have classified seafoods in different categories, in which raw seafood such as fish have been show at most risk. Goa is known for tourism and therefore several different types of dishes are prepared which utilise raw sea foods. Therefore,

there is obvious increased risk of *Salmonella* infection. Also, several seafood gets served as semi-cooked dishes which may lead to gastrointestinal disease. One of the reasons for such gastrointestinal diseases could be occurrence of *Salmonella* in such foods.

Emerging antibiotic resistance among pathogenic bacteria is concerning matter. Pathogens obtained from humans, animals, food as well as environment has been shown to possess antibiotic resistance (Castanon 2007). Isolates obtained in this study were checked for the commonly used antibiotics. All the isolates were found to be resistant to sulphamethoxazole-trimethoprim (Fig. 1). Generally, *Salmonella* species are sensitive to sulphamethoxazole-trimethoprim and very few reports show the resistance to sulphamethoxazole-trimethoprim. A recent study with *Salmonella enterica* obtained from dairy products from Egypt observes 91.5% resistant isolates to sulphamethoxazole-trimethoprim (Ahmed et al. 2014). Comparatively, several other studies which attempts sulphamethoxazole-trimethoprim sensitivity analysis with *Salmonella* species shows lower range (11-35%) resistant isolates (Adesiyun et al. 2014; Ertas Onmaz et al. 2014; Onyango et al. 2014). Ampicillin and tetracycline were not effective against 41% and 25% of the isolates. Occurrence of antibiotic resistant *Salmonella* in seafood is concerning. Further study is necessary to determine the genetical constituent causing antibiotic resistance.

This preliminary study shows prevalence of the antibiotic resistant *Salmonella* spp. in the raw seafood that sold in the markets of Goa. More study is necessary to carry out on all other markets and jetties to have overall incidences occurring across Goa. Also, a study with sea and estuarine water may help

to trace out the source. Microbiological quality control and surveillance programs should be carried out in order to restrict or minimise the incidences of *Salmonella*. Also, fisherman and seafood processing personnel should make aware for the incidences of the Salmonellosis and its consequences. Also, generation of area specific antibiogram will help in prudent treatment of food borne salmonellosis. Efforts should be taken for the implementation of hazard analysis and critical control points to reduce the incidence of *Salmonellas* pecies in seafood.

### Acknowledgement

The authors are thankful to Director, ICAR Research Complex for Goa, Ela, Old Goa, for providing necessary facilities to carry out this research work.

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