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Research Note

Identification of *Salmonella enterica* subsp. *enterica* serovar Paratyphi B dT$^+$ strains from seafood of Cochin retail markets

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Among the major food borne pathogens, *Salmonella* belonging to Enterobacteriaceae family continues to be the leading cause of food related infections all over the world. Every year, the global burden due to salmonellosis is on the rise and Centers for Disease Control and Prevention (CDC) estimates nearly1.35 million cases of salmonellosis, with 26,500 hospitalizations and 420 deaths in United States (CDC, 2021). Among the 2579 serotypes of *Salmonella* reported, other than S. typhi and S. Paratyphi, all are collectively called as non-typhoidal *Salmonella* (NTS). NTS mostly causes invasive self-limited mild infections in healthier population, but can be fatal in children (< 5 years), old age persons (> 65 years) and immunocompromised individuals. Within *Salmonella* enterica subsp. enterica, S. Typhi and S. Paratyphi A, B and C are the etiological agents respectively for typhoid and paratyphoid infections, collectively called as enteric fever. For NTS, food animals act as the potential reservoir, while for the typhoidal strains human beings remain as the single restricted host. Typhoidal strains of *Salmonella* spreads mainly through the consumption of food contaminated with the faeces of an infected person. In recent decades, S. Paratyphi A has emerged as the predominant cause of enteric fever and reports on infections due to S. Paratyphi B and C are currently rare. There are reports of outbreaks due to S. Paratyphi B infection in many countries (Denny et al., 2007; Hassan et al., 2018).

Till now, the lead acetate test described by Alfredsson et al. (1972) is accepted as the "gold" standard to biotype S. Paratyphi B. Based on this test, S. Paratyphi B can be broadly classified in to "classic" (d-tartrate -) and "java" (d-tartrate +) based on their ability to utilize dextrorotatory [L(+)]-tartrate (*d*-tartrate) as the source of carbon. In human beings, generally dT$^-$ strains of S. Paratyphi B elicit enhanced pathogenicity with typhoidal symptoms, while dT$^+$ strains exhibit less severe gastroenteric disease. People having infections with dT$^+$ strains of S. Paratyphi B often experience mild symptoms such as diarrhoea, fever, vomiting and abdominal cramps *etc* within 12 to 72 h of exposure. Mostly such infections are self-limiting, but rarely cause meningitis and septicaemia in infected people. There is limited data on the occurrence of *S. enterica* subsp. *enterica* serovar Paratyphi B dT$^+$ in seafood from India. The intention of this study was to identify the dT$^+$ biotype of S. Paratyphi B isolated from seafood of Cochin region, India.

A total of 470 fresh seafood samples were collected from retail markets in and around Cochin and screened for the presence of *Salmonella* sp as per BAM, USFDA (Andrews & Hammack, 2001). Serotyping at National *Salmonella* Centre (Veterinary), ICAR-Indian Veterinary Research Institute (IVRI), Bareilly revealed 39 strains as S. Paratyphi B with antigenic formulae 1,4,[5],12: b:1,2. from seafood items *viz*., *Rastrelliger kanagurta, Nemepteres japonicus, Tilapia mossambica, Sphyraeno jello, Metapenaeus dobsonii*. Lead acetate test was carried out for all confirmed strains of S. Paratyphi B as described by Alfredsson et al. (1972). Bacterial peptone water, pH 7.4 with potassium sodium tartrate tetrahydrate (SRL, India) at a final concentration of 1% inoculated with young bacterial inoculum at 0.5 MacFarland concentration was incubated aerobically at 37^{o}C without shaking. One set of tubes were incubated for three days while the

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other set for 6 days. After the incubation period, the *d*-tartrate utilization by the cultures was assessed by the addition of saturated aqueous lead acetate solution. The precipitate formed was homogenized by brief mixing. The formation of a small precipitate after 1 to 2 h of lead acetate addition were confirmed as dT^+ strains of S. Paratyphi B while bulky precipitate formation was considered as $dT^{''}$ strain. Strains which change the inoculated media colour from brilliant blue to a yellow/green upon incubation also indicates dT^+ character. Tube without bacterial inoculum was taken as control.

In the present study, all the 39 strains of S. Paratyphi B changed the colour of broth from blue to yellow on completion of incubation time after addition of lead acetate solution. All the strains produced whitish precipitate at bottom of the tube with turbid supernatant and were confirmed as S. Paratyphi d-tartrate (+) (*ie.,* S. Paratyphi B biovar java). Identical results were obtained on third as well as sixth day of incubation indicating that three days of incubation was sufficient for confirmation of the d-tartrate (+) strains. All the strains were confirmed as dextrorotatory tartrate-positive (*S. enterica* subsp. *enterica* serovar Paratyphi B dT^+), formerly called *S. enterica* subsp. *enterica* serovar Java. This study demonstrates that, the seafood collected from retail markets of Cochin region harbours d-tartrate (+) strains of S. Paratyphi B with 1.06% prevalence. This is a matter of serious concern since the d-tartrate (+) strains possess high risk for seafood handlers and consumers in causing gastroenteritis without pyrexia (Chart et al., 2003). Recently, the importance of dT^+ strains of S. Paratyphi B increased worldwide and emerged as the 11th most frequently reported human salmonellosis serovar in the EU during 2014 (Hassan et al., 2018). Even though they are predominant poultry serotype (Van Pelt et al., 2003), their sporadic occurrence was reported from other sources such as goat's milk cheese (Desenclos, 1996), alfalfa sprouts (Gaulin et al., 2002), fish aquariums (Stratton et al., 2001; Levings et al., 2006), turtles (Harris et al., 2009), sprouted nut butter spread (CDC, 2015), *etc.* Hassan et al., 2018 reported a multi-state food-borne outbreak in USA caused by dT^+ strains of S. Paratyphi B. Frozen raw tuna imported from Indonesia consumed raw as sushi was responsible for the outbreak. In India, salmonellosis caused by S. Paratyphi B dT^+ strains are quite uncommon. The symptoms of NTS infections in human beings is more or less similar to S. Paratyphi B dT^+ infections which may lead to mis-reporting or under reporting. This jeopardizing scenario calls for efficient identification and detection of this serovar. Data regarding the prevalence of biotype of S. Paratyphi B from seafood is important for epidemiological studies as well as for setting up of food safety standards. There had been increasing incidence of *S. enterica* subsp. *enterica* serovar Paratyphi B dT^+ isolates in poultry and poultry products (Dorn et al., 2001). Recently Tartrate + S. Paratyphi B has been isolated from fish and seafood (Miko et al., 2002; Levings et al., 2006; Hassan et al., 2018). Their presence in seafood from fish markets in Cochin reflects the contamination that might have happened in seafood chain either from poultry/animal sources or from handling by infected persons.

In conclusion, the presented data underline the significance of proper surveillance programmes across various disciplines to formulate and establish better tool for effective control and management of S. Paratyphi B infections. Regular disinfection and cleaning of seafood markets are inevitable to prevent the occurrence and spread of S. Paratyphi B. The concerned government departments should create and emphasize suitable awareness among the seafood handlers regarding the hygienic practices to be followed in seafood markets so as to ensure public health safety. The quality testing of ice and water used in seafood markets also should be implemented as a mandatory step to keep the contamination to a minimal level.

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