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Salmonella enterica serovars Takoradi, Tananarive and Uganda from foods in Tripura, their virulence & antimicrobial resistance

Sir,

The presence of *Salmonella* in foods has been considered a public health issue, as the pathogen might be transmitted from the contaminated foods into the human food chain. The zoonotic pathogen *Salmonella enterica* is a leading cause of foodborne infection in humans[1,2]. Currently, the number of outbreaks due to *S. enterica* infections is increasing mostly due to contaminated foods of animal origin[3].

The species *S. enterica* is highly diverse with about 2,600 serovars[4]. Based on the infectivity, they are classified as typhoidal (TS) and non-typhoidal *Salmonella* (NTS) serovars. Control of NTS is challenging as they have multiple sources and virulence factors. Gastroenteritis caused by the NTS is self-limiting, but it also instigates significant health risks due to its invasive nature[5]. The incidence of invasive non-typhoidal *Salmonella* (iNTS) has been increasing in several countries, especially Malawi[6-8]. Globally, the infections and deaths caused by NTS have been projected to be about 78 million and >59,000, respectively[9].

NTS control requires a 'One Health Approach', as involving multiple sectors such as public health, animal welfare, and food and environment protection remains essential. The NTS infections in India are on the rise[10]. Several virulence factors in TS and NTS encoded on the *Salmonella* pathogenicity islands (SPIs) play an important role in invasion into the host cell, multiplication and spread[11].

Since 2020, the Indian Council of Medical Research (ICMR) has conducted an ongoing project on systematic laboratory-based surveillance of foodborne enteric disease and related outbreaks in four North-east Indian States. Between January and April 2023, we screened 1004 food samples collected from 36 places covering 4 different regions of Tripura. During the surveillance period, there was no flux of foreign travellers. In this ongoing study, we identified three *S. enterica* serovars that were not reported from India. These *Salmonella* strains were first identified by biochemical tests[12], followed by serotyping using *Salmonella* somatic (O) and flagellar (H) antisera (SSI Diagnostica, Denmark) according to the Kauffmann–White scheme[4]. Antimicrobial susceptibility was determined by disk diffusion testing[13]. To determine the important virulence encoding genes, these strains were subjected to PCR analysis following previously published methods[14,15].

S. enterica serovars Takoradi, Tananarive and Uganda were identified from market samples of mutton, sweet and fish, respectively, collected between January and April 2023 in Tripura. The serotype scheme, antimicrobial resistance profile and virulence-encoding genes detected by the PCR assay are shown in Table. *S.* Takoradi and *S.* Tananarive were resistant to tetracycline. All these serovars showed reduced susceptibility to azithromycin but were susceptible to fluoroquinolones (ciprofloxacin, norfloxacin, ofloxacin), meropenem, ceftriaxone, sulfamethoxazole/trimethoprim, chloramphenicol and cefotaxime. The emergence of reduced susceptibility to azithromycin is a cause of concern. This drug has been used to treat enteric infections in India. Many of the TS and NTS clinical strains have shown resistance to azithromycin[16,17].

All the three serovars harboured *orgA,* (oxygen-regulated gene for host recognition and invasion), *invE/A* (SPI-1, for host cell invasion), *ttrC* (tetrathionate reductase, fitness advantage), *ssaQ* (SPI-2 T3SS; secretion system apparatus protein), *mgtC* (SPI-3 intracellular proliferation inside macrophages, Mg^{2+} transporter), *misL* (SPI-3, T5SS-secreted (autotransported) adhesins, involved in intramacrophage survival), *spi4R* and *spi4D* (encodes a T1SS), *sopB* (SPI-1, SPI-5-T3SS secreted effector *Salmonella* outer protein B for bacterial internalization),

Table. Detection of antimicrobial resistance and virulence encoding genes in *S*. Takorandi, *S*. Tananarive and *S*. Uganda

Sample ID	Source	*Salmonella enterica* serovar (O:H antigen formula)	Antimicrobial susceptibility test			Virulence encoding gene*
			Resistance	Intermediate	Susceptible	
TRP/NTH/ RAWMEAT/1829	Mutton	Takoradi (8:i;1,5)	*TET*	AM, S, AZM, D, CAZ	NA, CIP, NOR, OFX, MEM, CR, SXT, C, CTX	*invE/A, orgA, ttrC, ssaQ, mgtC, misL, spi4R, spi4D, sopB, pipA, hilA, stn*
TRP/STH/ SWEET/1966	Sweet	Tananarive (6,8:y;1,5)	*TET*	AM, S, AZM, D	NA, CIP, NOR, OFX, MEM, CR, SXT, C, CAZ, CTX	*invE/A, orgA, ttrC, ssaQ, mgtC, misL, spi4R, spi4D, sopB, pipA, hilA, stn*
TRP/DHL/ RAWFISH/1104	Fish	Uganda (3,10:l,z_{13};1,5)		AM, S, AZM, D, NA, TET	CIP, NOR, OFX, MEM, CRO, SXT, C, CAZ, CTX	*invE/A, orgA, ttrC, ssaQ, mgtC, misL, spi4R, spi4D, soupB, pipA, pefA, hilA, stn*

*All the serovars were negative for *spvC*. AM, ampicillin; AZM, azithromycin; C, chloramphenicol; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; D, doxycycline; MEM, meropenem; NA, nalidixic acid; NOR, norfloxacin; OFX, ofloxacin; S, streptomycin; SXT, sulfamethoxazole/trimethoprim; TET, tetracycline

pip (SPI-5, pathogenicity island protein for intestinal mucosal fluid secretion and inflammation), *hilA* (SPI-1-T3SS, hyperinvasive locus the expression of invasion) and *stn* (*Salmonella* enterotoxin). In addition to these potential virulence genes, the *S*. Uganda serovar had *pefA* that encodes fimbria-associated virulence. The possession of these virulence encoding genes would suggest that the NTS serovars investigated in the current study have the ability to cause enteric salmonellosis in humans. Many of these genes have been reported to be present in *S*. Typhi. In this study, all the serovars were negative for the plasmid virulence factor encoding gene *spvC*. Some of the studies indicate importance of this gene for the survival and proliferation of NTS inside the reticuloendothelial cells and associated with bacteraemia in humans[18,19].

To our knowledge, *S. enterica* serovars Takoradi, Tananarive and Uganda have not yet been reported from India. *S*. Takoradi has been reported from poultry slaughterhouses in Korea[20], and birds in Scotland[21]. *S*. Tananarive has been isolated from humans[22] and food animals (*https://www.fao.org/4/i1547e/i1547e00.pdf, https://www.bvmj.bu.edu.eg/issues/25-2/8.pdf*). *S*. Uganda caused a foodborne outbreak in China[23] and was also identified from turkeys in Canada[24], cattle and mink in USA[25,26], camels in Nigeria[27], and ducks in Trinidad[28].

Our findings highlight the risks associated with new *Salmonella* serovars with virulence potential and antimicrobial resistance in foods. Considering the public health risks associated with food contamination, constant surveillance and stringent control measures are needed at the production level and also along the food chain.

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Goutam Chowdhury[1,#], Hritusree Guha[3,#], Asish K. Mukhopadhyay[1], Madhuchanda Das[4], Venencia Albert[4], Samaresh Das[2], Nilanjan Chakraborty[1], Shanta Dutta[1], Tapan Majumdar[3] & Thandavarayan Ramamurthy[1]

[1]ICMR-National Institute of Cholera and Enteric Diseases, [2]Centre for Development of Advanced Computing (CDAC), Kolkata, [3]Department of Microbiology, Agartala Government Medical College, Tripura, & [4]Indian Council of Medical Research, New Delhi, India
For correspondence:
drtapan1960@gmail.com

[#]Equal Contribution

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