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Poultry as a possible source of non-typhoidal *Salmonella enterica* serovars in humans in Bangladesh

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ABSTRACT

We investigated *Salmonella enterica* isolates from human clinical cases of gastroenteritis to determine the distribution of non-typhoidal *Salmonella* serovars in the human population, and compared them to isolates originating from poultry by serotyping, phage typing, plasmid profiling, pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) to evaluate the potential role of poultry in human non-typhoidal salmonellosis in Bangladesh. Nine different serovars were identified among the human isolates of which *Salmonella* Paratyphi B var Java (*S.* Java), *S.* Kentucky, *S.* Enteritidis, *S.* Virchow and *S.* Weltevreden also were commonly isolated from poultry. The poultry isolates belonging to *S.* Java, *S.* Kentucky and *S.* Enteritidis were indistinguishable from human isolates or genetically closely related, based on PFGE profiles and MLST. *S.* Kentucky clone ST198 and *S.* Java clone ST43 both well-known cause of human infections were also isolated from poultry.

1. Introduction

Gastroenteritis attributable to non-typhoidal *Salmonella* represents a global public health burden with about 93.8 million cases, 80.3 millions of which are food-borne, resulting in 155,000 deaths annually (Majowicz et al., 2010). There are >2500 serovars of *Salmonella enetrica* (Grimont and Weill, 2007). Most non-typhoidal *Salmonella* serovars (motile serovars) are zoonotic and ~10% of them can be isolated from poultry (Gast, 2007). To reduce the burden of non-typhoidal human cases, developed countries have a structured surveillance for their source identification and mitigation. Mandatory reporting of human clinical cases of salmonellosis is absent in Bangladesh, and only a few reports on enteric fever are available (Brooks et al., 2005; Ram et al., 2007). No reports have been published concerning the magnitude of non-typhoidal cases, their causative serovars and sources. Monitoring of livestock including poultry is absent in most resource-limited countries including Bangladesh, making people here more vulnerable to the exposure to various non-typhoidal *Salmonella*-contaminated meat products and eggs. However, local emergence and circulation of any new non-typhoidal zoonotic serovars of *Salmonella*, or a particular genotype within a serovar, either in humans or in animals, may be of public health concern, as dissemination over long distances may take place by human travels or related trades (Weill et al., 2006; Aarestrup et al., 2007; Le Hello et al., 2011).

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Poultry meat and eggs constitute an important source of animal protein for people in Bangladesh and poultry can be a sustainable reservoir for non-typhoidal human *Salmonella* infections. Unlike the developed world, poultry farms in Bangladesh are mostly of FAO-defined production type III—small scale commercial operation (Food and Agriculture Organization, 2007) with compromised biosecurity. In the absence of any existing surveillance, no data have been available on possible zoonotic *Salmonella* serovars circulating in poultry in Bangladesh until recently where two cross-sectional surveys involving layer and broiler farms, and an observational study on two breeder farms were performed (Barua et al., 2012, 2013). In these studies the isolation of *S.* Kentucky, *S.* Virchow, *S.* Paratyphi B var Java (in short: *S.* Java), *S.* Enteritidis and *S.* Weltevreden documented that poultry in Bangladesh indeed harbor *Salmonella* serovars with a major potential for causing human infections. Here, we describe the serovars of *Salmonella* isolates from human non-typhoidal clinical cases, their detailed molecular characteristics and relatedness to those of poultry origin to explore the potential role of poultry in their emergence and circulations.

2. Materials and methods

2.1. Salmonella isolates

In Bangladesh, there is only one laboratory named Clinical Microbiology Laboratory (CML) at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr, b) where screening for enteric pathogens (including *Salmonella*) is performed from stool samples of human cases of gastroenteritis. These samples are predominantly from patients with gastroenteritis complainants, such as diarrhea, vomiting, abdominal cramp and fever, enrolled at the Dhaka Hospital (DH) of icddr, b. The annual load of all kinds of clinical patients including those suffering from gastroenteritis at DH is about 110,000 (www.icddrb.org/what-we-do/hospitals/dhaka-hospital). CML receives all types of clinical samples referred from patients attending the Dhaka Hospital of icddr, b as well as from patients referred from other clinics and hospitals in Dhaka for microbiological investigations. After presumptive diagnosis of *Salmonella* following standard microbiological and biochemical methods serotyping of these isolates are done with group specific commercially available antisera (Denka Seiken, Japan). Isolates which react either with D or C group are subsequently tested with commercially available *S.* Typhi and *S.* Paratyphi A/C serotype specific antisera to verify whether they belong to any typhoidal serovars. All *Salmonella* positive isolates belonging to typhoidal or non-typhoidal serovars are grown in trypticase soy broth containing 0.3% yeast extract (TSBY) and stored at −70 °C after addition of 15% glycerol for further uses. From this *Salmonella* strain collection, representative serotypes of *Salmonella* species were transferred to the Enteric and Food Microbiology Laboratory (EFML) for further studies at the molecular level. We collected 57 non-typhoidal *Salmonella* isolates comprising 50 from the period 2009–2010, 4 from 2003 and 3 from 2011 from EFML. All these isolates were further reexamined at the Department of Microbiology, Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh, and shipped to the Department of Veterinary Disease Biology, University of Copenhagen (DVDB-KU), Denmark by a professional courier service.

Upon receiving at DVDB-KU, the isolates were screened to ensure the purity by culturing them on novobiocin (Oxoid Ltd., England) supplemented Modified Semisolid Rappaport Vassiliadis (MSRV) agar (Oxoid Ltd., England) followed by growing on brilliant-green phenol-red lactose sucrose (BPLS) agar (Merck, Germany) and subsequently identified biochemically and confirmed serologically by agglutination test with anti-*Salmonella* polyvalent serum (SSI, Copenhagen, Denmark) and kept at −80 °C using 15% glycerol until characterized further.

We compared the human isolates with 79 poultry-originating isolates of which 34, 22, 17, 5 and 1 belonged to the serovars *S.* Kentucky, *S.* Virchow, *S.* Paratyphi B var Java, *S.* Enteritidis and *S.* Weltevreden, respectively, and representing the predominant serovars circulating in poultry in Bangladesh in 2009–2010 (Barua et al., 2012, 2013). These strains originated from naturally pooled fecal samples from infected poultry farms, which subsequently were serotyped and characterized by plasmid profiling and pulsed-field gel electrophoresis (PFGE), as described previously (Barua et al., 2012, 2013).

2.2. Serotyping of human isolates

All the *Salmonella* isolates isolated from human stool samples were serotyped at Statens Serum Institut, Copenhagen, Denmark based on somatic (O) and flagellar (H) antigens by agglutination tests with *Salmonella* antisera (SSI Diagnostica, Hillerød, Denmark) and assigned to different serovars according to the White–Kauffmann–Le Minor Scheme (Grimont and Weill, 2007).

2.3. Phage typing of the isolates belonging to S. Typhimurium and S. Enteritidis serovars

Phage typing of all the *S.* Typhimurium ($n = 19$) and *S.* Enteritidis ($n = 6$) isolates from humans and *S.* Enteritidis isolates from poultry ($n = 5$) was conducted at National Food Institute in Denmark following standard procedures (Anderson et al., 1977; Ward et al., 1987). Isolates showing a reaction which did not conform to any recognized phage types in the typing scheme were assigned as "reacted but did not conform" (RDNC) and strains that did not react with any of the typing phages were designated as "untypeable" (UT).

2.4. Plasmid profiling of human isolates

Plasmid isolation of all human isolates was done following the method of Kado and Liu (1981) with some modifications, described elsewhere (Olsen, 2000). The plasmid size was determined by calculating the plasmid mobilities relative to reference plasmids in *Escherichia coli* V517 (Macrina et al., 1978) and *E. coli* 39R861 (Threlfall et al., 1986), according to the size estimation method described by Rochelle et al. (1985).