Salmonella among humans of Asian countries

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Abstract: This review aims at comprehensively reporting the impact of Salmonellosis in humans of developing Asian countries, the mode of dissemination, the various methods of serotyping, drug-resistance and the distribution of different Salmonella serotypes in these countries. The review focusses mainly on developing Asian countries which have a big population, higher risk of dissemination of the pathogen due to poor standards of public hygiene and sanitation, and also a high rate of arriving and departing travellers. Moreover, most of these countries lack a proper continuous surveillance and control system to monitor the epizootiology of *Salmonella* serovars. Various serotypes that were prevalent in Asian countries included the following: *S. weltevreden*, *S. rissen* and *S. typhimurium, S. anatum*, *S. rissen, S. stanley* and *S. enteritidis*. A high degree of serotypes distribution was found among the different countries. Resistance to multiple drugs was present in many serovars. The reported results indicate an alarming rate of human Salmonellosis in developing Asian countries, highlighting the importance of developing stringent surveillance and control programs aiming at control of this pathogen.
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1. Introduction

Salmonella is "a facultative anaerobe, gram negative flagellated rod-shaped bacterium which measures about 2-3 x 0.4-0.6 µm in size"[1,2]. This common foodborne pathogen is mainly determined by the consumption of fresh fruits and vegetables. Around 22 million cases of typhoid fever and almost 6 million additional cases of paratyphoid fever are reported every year around the world. In the USA alone, about 400 cases of typhoid fever and 100 cases of paratyphoid fever are reported annually [3]. Salmonellosis is often associated with self-limiting gastroenteritis. *Salmonella* infection can lead to invasive and focal infections that can be severe in populations of children, elderly people, and immunocompromised patients [4]. While human Salmonellosis is a major health concern, avian Salmonellosis is of equally serious concern as contaminated poultry happens to be one of the most common sources of transmission of the pathogen to humans. Avian Salmonellosis is an important disease acting as a major obstacle for the development of the poultry industry, especially in the developing countries of Asia and Africa. Contaminated poultry meat and eggs are known to be major contributors of food-borne outbreaks in humans and poultry, compared to any other food [5,6]. Infections with enteric Salmonella are a worldwide hazard to both man and animals [7], with around "1.028 million cases, 19,000 hospitalizations, and 400 deaths in the United States being reported every year"[8].

Salmonellosis

Salmonellosis in humans is a worldwide problem but has been often associated with travel to Asian countries. Cases are detected mainly among travellers to southern Asia "(6–30 times higher than for all other destinations)". Other high risk geographic areas are 'East and Southeast Asia, Africa, the Caribbean, and Central and South America" [9]. *S. enterica* serotype Paratyphi A can be the cause of high risk of infection among those visiting Southeast Asia, with strains that are highly resistant to the antibiotic nalidixic acid or exhibit multidrug-resistance "(resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole)" [10]. It has been reported that the risk of contracting typhoid fever is fluctuating from one region to another. It is important to consider the fact that the risk of contracting the disease is time dependant, increasing with the increase in the duration of the visit. However, some travellers, visiting endemic places, were infected even during short visits of less than one week [11].

Age of patient, serotype and the region are important factors that determine the ability of

Salmonella to cause invasive infection. The *S. Typhi* and *S. Paratyphi* A are highly invasive in both children and adults [12]. Certain serovars of Non typhoidal *Salmonella* [NTS], such as S. choleraesuis, *S. dublin*, and S. virchow, are more potent in causing invasive salmonellosis than other serovars. Based on the species of individual *Salmonella* serovars, they are either grouped as "host adapted such as *S. typhi* in humans, S. choleraesuis in pigs, *etc*." or "non host adapted such as *S. typhimurium*, *S. enteritidis*, *etc*." [13]. In humans, salmonellosis causes four major disease patterns namely: "enteric fever, gastroenteritis, bacteremia and other complications of nontyphoidal Salmonellosis as well as chronic carrier state" [14]. The number of cases reported every year is not precise because many small scale or sporadic outbreaks are not reported and, in general, only large outbreaks are investigated. Information about Salmonellosis is not easy to find in many countries. The fact of unreporting is shown in the following quoted statement:"Asia, Africa and South and Central America with only between 1- 10% of cases being reported "[15,16].

Hendriksen *et al*., 2011 [17], reported on the distribution of the 15 most frequently identified human serovars of Salmonella from 2001 to 2007 in laboratories from 37 countries that were part of the World Health Organization Global Foodborne Infections Network. During the study period, the distribution of various *Salmonella* isolates from humans was "serovar Enteritidis was 43.5% (range: 40.6% [2007] to 44.9% [2003]), and Salmonella serovar typhimurium was 17.1% (range: 15.0% [2007] to 18.9% [2001])". Other Salmonella serovars reported were *S.* newport, *S.* virchow (mainly observed in Asian, European, and Oceanic countries), *S.* hadar (profound in European countries), and *S.a*gona (intensely in Latin and North American and European countries". The proportion in variation of serovar distribution was higher within regions, rather than between different countries. The data pointed at the complex nature of the global epidemiology of *Salmonella,* and the mandatory requirement for enhancing surveillance for those serovars which have been implicated in outbreaks. This review aims at focusing on Salmonella serovars obtained from selected Asian countries and the extent of damage caused by the pathogen in those areas.

Salmonella serotypes in developing Asian countries

Foodborne diseases are considered to be of great worldwide concern to the public health and to development [11]. The industrialization, large scale-food production, reduction in trade barriers, and human migration are important factors contributing to the high incidence and the degree of severity of foodborne diseases in the whole world [18]. Poor hygienic conditions in developing countries favor the spread of *Salmonella,* resulting in serious health hazards. *Salmonella* serotyping serves a number of important goals such as detection of widespread outbreaks, identifying the sources and causes of outbreaks, monitoring trends of dissemination of the pathogen over time, and attribution of human disease to various zoonotic sources [19]. This kind of surveillance not only serves as a key factor in preventing foodborne disease outbreaks, but also increases awareness among "health authorities, food producers, food regulators, and consumers". In Asia, studies conducted from 2000 to 2002 in countries such as "Japan, Korea, and Thailand together reported *S.* enteritidis as the most common human serotype, followed by *S.* weltevreden being the second most common serotype in 2000 and 2001 but dropping to fourth in 2002, being surpassed by *S. rissen* and *S. typhimurium*. In 2002, *S. enteritidis* accounted for 38% of human isolates but only 7% of nonhuman isolates". The most common nonhuman serotypes reported in Asia were "*S. anatum*, *S.* rissen, and *S.* stanley" [9].

Epidemiological studies of pathogens are a major contribution towards controlling their spread and dissemination. The technique of typing pathogens is a crucial factor in any epidemiological investigation. It is not absolutely necessary that serotypes reported by a region circulate locally, but could be acquired by travel or imported foods. Intraregional comparisons are limited by the fact that there are no universal case definitions and surveillance systems in all countries worldwide [9]. Moreover the source from which the *Salmonella* are reported may vary from country to country, thereby not providing a comprehensive picture. Sometimes, the overall incidence of the strains, considering animal and human sources are underreported.

Salmonella serovars typing methods

Numerous methods for typing strains of *S. enterica* serovar typhi have recently been developed. Phage typing and isoenzyme analysis that were the classical typing methods are now being increasingly supported by "molecular techniques, such as pulsed-field gel electrophoresis (PFGE), ribotyping, random amplification of polymorphic DNA (RAPD), and DNA fingerprinting using the mobile genetic element IS*200*". The use of Amplified Fragment Length Polymorphism (AFLP) is a recent development for distinguishing the *S. enterica* serovar typhi strains [20]. Liu *et al*., 2003 [21] conducted a serotyping study on *Salmonella* from various Asian countries including "Singapore, Indonesia, India, Bangladesh, Malaysia, and Nepal". Three VNTR loci from *S. enterica* serovar typhi strain CT18 were characterized and were used as molecular markers to distinguish the

isolates based on their country of origin. The study showed the existence of substantial genetic heterogeneity at the VNTR loci among the different *S. enterica* serovar typhi isolates based on their location. The use of genotypic methods has helped in revealing more genetic variability in recent times [22]. In a study on 73 Vietnamese and 217 Hong Kong strains, using molecular methods such as plasmid profile, fingerprinting analysis, ribotyping, and total-DNA fingerprinting indicated not only a high level of genetic heterogeneity among isolates from different countries, but also within the same country [23]. *Salmonella* typhi is known to have significant genome plasticity [24].

Moehario in 2009, [25] conducted a study on genetic fingerprints using RFLP and pulsed-field gel electrophoresis (PFGE) of *S.typhi* isolated from various geographic areas spanning west to east across Indonesia. A total of 33 *Spe*I digested *S.* Typhi chromosomal DNA gave 22 schizotypes, 20 pulsotypes, and 12 subtypes indicating genomic diversity and the presence of more than one clone of *S. typhi*. Cluster analysis showed four clusters, three of which were associated with geographic area. This study confirmed the presence of endemic strains in localized geographic areas, as well as the movement of one strain type throughout the archipelago. The migration of strain types proven by this study shows the ease with which the pathogen spreads, inducing a public health risk to travellers.

Acquisition of multi drug resistance to a wide range of antimicrobials by the pathogenic strains make them more potent and virulent. In a cross-sectional sample of 381 serovar typhi strains from 8 Asian countries namely: "Bangladesh, China, India, Indonesia, Laos, Nepal, Pakistan, and central Vietnam", collected between 2002 to 2004 [18], reported various degrees of multidrug resistance (16 to 37%) and nalidixic acid resistance (5 to 51%). Those eight Asian countries included in this study are responsible for around 80% of the world's cases of typhoid fever. These results strongly establish the high level of drug resistance throughout the whole of Asia. Ochiai *et al*., 2005 [26] conducted a one year-surveillance study to investigate the incidence of typhoid and paratyphoid cases in specific high risk populations of "Karachi, Pakistan; Calcutta, India; North Jakarta, Indonesia; and Hechi City, China". During the surveillance period in the four sites specified above, "285 *S.* typhi episodes and 84 *S.* paratyphi A episodes were documented. The *S.* paratyphi A caused 14% of enteric fever episodes in Indonesia, 15% in Pakistan, 24% in India and 64% in China. Pakistan recorded the highest incidence of *S.* typhi (394/100,000/year), while China reported the lowest *S.* typhi (15.2/100,000/year). In countries like China and India considered most populated countries in the world, *S.* paratyphi A was involved in episodes of enteric fever that were difficult to distinguish them clinically from typhoid fever episodes. Although the same treatment regimens may be successfully applied to curb both organisms, Both *S.* paratyphi A as well as *S.* typhi should be controlled in the future to prevent enteric fever in Asia, particularly bearing in mind the emergence of drug-resistant strains [27].

*S.*paratyphi A caused the rise in the incidence rate of enteric fever in many Asian countries, accounting for 50% of *Salmonella*-bloodstream isolates among patients with enteric fever. Based on that, concerns have been raised regarding the efficacy of typhoid fever vaccine on enteric fever rates [28].

There are more than 2,435 known serotypes of *Salmonella* of which in India alone, 209 serovars have been documented, with many that are human pathogens [29]. Salmonellosis is a direct occupational anthropozoonotic disease causing extensive economic losses and posing a serious human health hazard. *Salmonella* are widely distributed in nature and cause a spectrum of diseases in man, animal and birds. Poultry eggs, meat and their products are the most common means of dissemination of *Salmonella* to humans. In India Salmonellosis is hyper-endemic and there is an urgent need to strengthen the monitoring and surveillance of salmonellosis using suitable diagnostic tools so as to prevent and control its occurrence [30].

A study by Murugkar *et al*., 2005 [31] was carried out on humans of Northeastern India for *Salmonella* isolation, serotyping, phage typing and antibiogram patterns. The results obtained included ninety five isolates of *Salmonella enterica*belonging to five serotypes- *"S. typhimurium*, *S. enteritidis*, *S. gallinarum*, *S. paratyphi* B and *S. bareilly*" with an overall prevalence rate of 14.40 per cent. Phage typing studies revealed that *S. typhimurium* could be "distributed among four phages- DT003, DT004, DT096 and DT193", while all the *S. enteritidis* isolates "belonged to a single phage type, PT13a/7". It was found that there was interspecies sharing of the phages. Amongst the various antibiotics tested for sensitivity, "Norfloxacin, enrofloxacin, gentamycin and ciprofloxacin were most effective, whereas, doxycycline, ampicillin, amoxicillin and tetracycline were relatively less effective. The phenomenon of multidrug resistance documented in this study highlights the importance of control measures to curb this pathogen.

Das *et al*., 2012 [32] conducted a recent study for detection and molecular characterization of *Salmonella enterica*serovartyphi, isolated from humans with Typhoidial fever, using biochemical, phenotypical and virulence gene based polymerase

chain reaction (PCR) techniques. The isolated strains were also investigated for antibiotic susceptibility patterns. A total of 16 clinical samples were collected from Coimbatore, Erode and Salem districts of Tamil Nadu, India and were processed bybroth enrichment methods for isolation and identification of the causative agent *S. enterica*serovartyphi. Microbiological and biochemical studies confirmed the presence of *S.* typhi in16 samples. The biotyping of the isolates showed that all the isolates belonged to biotype IV. The PCR analysis confirmed the presence of invasion gene, tyveloseepimerase gene, phage-1 flagellin gene for d-antigen and Vi antigen gene in all 16 clinical samples. The antibiotic susceptibility test that was carried out showed 100 % resistance to only ampicillin and 100 % sensitivity to carbenicillin, chloramphenicol, clindamycin, gentamycin, kanamycin and tetracycline. This study confirmed the presence of virulent strains of *S. enteric* serovar typhi among human population, and also established that PCR-based diagnostic could be very useful for the rapid detection of *S.* typhi isolates.

Diarrheal disease is an important global problem that accounts for high rates of morbidity and mortality among young children around the world [33]. The prevalence of *Salmonella* serovars, and their distribution among diarrheal children, is not well reported, since *Salmonella* is identified up to genus level, and the serotyping procedures are not performed. Identification of *Salmonella* serovars and its pattern of antimicrobial susceptibility will be very useful in providing epidemiological data for effective antimicrobial therapy. A study was conducted by Bukitwetan *et al*., in 2007 [34] in Indonesia during January 2003 through August 2005, at two communityhealth centers in south Jakarta, Indonesia, to detect nontyphoidal *Salmonella* infections in children with diarrhea. Out of a total of 814 rectal swab samples collected, 56 (6.9%) were positive for *Salmonella*. Serotyping revealed that *Salmonella enterica*serovar typhimurium is the most frequent in 32.1% of all *Salmonella* isolates. Antimicrobial susceptibility showed that 5.6% to 66.7% of *Salmonella* serovars were resistant to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, and nalidixic acid. However, all serovars were susceptible to norfloxacin, ciprofloxacin, and ceftriaxone. The alarming increase in antimicrobial resistance of *Salmonella* serovars, and the impact which it has on public health and economy around the world, highlights the importance of initiating structured surveillance programs to help in control of Salmonellosis.

Seafood contamination with Salmonella is increasingly being reported in India [35]. *Salmonella* spp. associated with gastrointestinal tract of animals, including birdsreach the aquatic environments through faecal contamination [36]. A study by Bhowmick *et al*, 2012, [37] included fifty eight *Salmonella* isolates obtained from various seafood. All isolates were serotyped and epidemiological investigation was carried out using molecular fingerprinting methods, "Random Amplified Polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus sequence based-PCR (ERIC-PCR)" along with whole cell protein profiling using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in this study. Among the 58 *Salmonella* isolates, *S.* weltevreden was the most predominant serovar. Typing of *Salmonella* serovars using RAPD and ERIC-PCR suggested the existence of a genetic diversity. This study showed that use of protein profiling in combination with established typing methods such as RAPD and ERIC-PCR may provide useful information in typing of non-typhoidal *Salmonella* isolates recovered from seafood, and help in developing strategies to protect public from *Salmonella* infections.

Conclusions

Salmonella causes considerable burden globally with around an estimated 93.8 million illnesses, of which an estimated 80.3 million are food borne, leading to 155,000 deaths each year [38]. Worldwide, large scale production and distribution of food are major causes for rapid dissemination of pathogens; this furthermore, accompanied with multidrug resistance resulting from excessive and uncontrolled use of antimicrobial agents, poses major challenges for the control and prevention of the spread of *Salmonella* infections. Improving standards for food safety and reducing the incidence of *Salmonella* infection can only be achieved by promoting and implementing effective food safety measures on a global scale [39].

Considering the huge impact the pathogen has on overall disease dissemination in both animals and humans around the world, the need of the hour is to develop better and quicker methods of large scale identification of the pathogen from various sources as a first step towards control. Secondly it is very important to increase the awareness of the extent of damage that the pathogen can cause amongst people. Another important step towards curbing the pathogen is to initiate programs that would help in routine surveillances and reporting cases of illness caused by the pathogen within regions and also between different countries, leading to a unified and effective means of controlling the dissemination of this pathogen.

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