Salmonella in food products – a vector for distribution of antibiotic resistance




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Non-typhoidal *Salmonella* spp. are common food-associated pathogens, and *Salmonella* infections are one of the most common causes of death associated with food-associated illness, especially in developing countries. As in many other developing countries, raw food hygiene and antimicrobial resistance epidemiology are in their infancy in Vietnam. In addition, the lack of stringent controls on antimicrobial usage in human health and particularly in animal production systems increases the risk of food-borne pathogens harbouring and disseminating antibiotic resistance genes. For countries such as Vietnam, Thailand and other Asian countries, *Salmonella* vaccination is a more cost-effective way of controlling *Salmonella* in food production animals than the use of antibiotic therapy.

Salmonella pathogenicity

The ability of *Salmonella* spp. to cause infection is dependent upon the expression of virulence determinants and the complex activities these virulence determinants initiate upon contact and entry into the host cell [1-3]. Although a majority of *Salmonella* spp. are pathogenic to humans and animals, their virulence appears to vary from serovar to serovar and this diversity can be attributed to genetic variation or polymorphisms in virulence genes. Variations in surface structures such as lipopolysaccharides, fimbriae and products encoded by virulence plasmids have been shown to contribute towards the pathogenicity and host range of a *Salmonella* strain [3-7.] The virulence of a serovar is also largely affected by genetic modifications (insertion and deletion events and mutations) to genes clustered on *Salmonella* pathogenicity islands (SPI) [1,5,8,9]. Studies of SPI1 to SPI5 of various *Salmonella* spp. (e.g. Typhimurium and Sofia) have revealed a range of genetic changes in these islands which could contribute to the variation in virulence and host range of *Salmonella* spp. [5,8,10]. These genetic variations explain why some *Salmonella* serovars such as Typhimurium are virulent and a common cause of food-borne illness worldwide, while other serovars such as Sofia, which colonises avian species, is avirulent in people.

Distribution of *Salmonella* spp. in food products in Vietnam

In selected countries in Asia, *Salmonella* spp. is still a major cause of disease. In many cases, this is because meat is sold fresh in the market, there is no cold chain during transportation and the quality of slaughter conditions is poor. In a study on the contamination of raw food samples by *Salmonella* spp. isolated in Vietnam [11], there was a considerably high prevalence of *Salmonella* spp. in raw meat and poultry, in which 64% of pork samples, 62% of beef samples and 53.3% of chicken samples were contaminated with *Salmonella* spp [11]. Eighteen per cent of shellfish samples were also reported to contain *Salmonella* spp. in this study [11]. These samples were contaminated with *Salmonella* serovars associated with human salmonellosis in Asia and in Vietnam such as *S.* Typhimurium, *S.* Paratyphi B biovar java, *S.* Anatum, *S.* Panama, *S.* Rissen and *S.* Lexington. Serovar Typhimurium, the most predominant serovar in human clinical isolates [12], has been isolated from meat, poultry and shellfish sources, indicating a potential health risk if these raw food samples were not properly cooked. A high prevalence of *Salmonella* spp. in meat samples was also noted by Phan *et al.* [13], who reported that 21–70% of poultry and meat samples in the Mekong Delta, Vietnam, were contaminated with *Salmonella* spp.

The reported rates of *Salmonella* spp. contamination in retail meat and poultry were much lower in more developed countries such as in the United Kingdom (23–29%) [14,15], Ireland (2.8–26.4%) [16,17], The Netherlands (13.2%) [18], Spain (35.8%) [19], Belgium (36.5%) [20] and in Korea (36%) [21]. Advanced processing practices and equipment in slaughterhouses and more effective use of refrigeration in developed countries helps to reduce the level of bacterial contamination.

Antibiotic resistance characteristics of *Salmonella* spp. isolated from food samples in Vietnam and potential spread of antibiotic resistance

Information on the phenotypes and genotypes of antimicrobial resistance in food-borne microorganisms is largely restricted to first-world countries and there is a paucity of information on what is happening in developing countries. Where they are reported, rates of resistance to antibiotics of bacteria originating from meat were high in developing countries [22-25], possibly as a result of the inappropriate or uncontrolled use of antibiotics in farming practices.

In Vietnam, antibiotic resistance has been reported to occur in human bacterial isolates, including *Salmonella enterica* serovar Typhi and other diarrhoea-causing pathogens [26-30]. However, there has been very little published about the occurrence of antibiotic-resistant bacteria in raw food samples in Vietnam and even less about the molecular characteristics of these antibiotic-resistant bacteria. The extensive study on antibiotic resistance of food isolates by Van *et al.* [11] showed that antibiotic resistance in *Salmonella* spp. in raw food samples from Vietnam was significant. In this study, 91 *Salmonella* spp. isolates recovered from food samples were tested for antibiotic resistance against 15 antibiotics. The results showed that approximately half (50.5%) of the isolates were resistant to at least one antibiotic; the highest rate was in chicken samples (88.9%). Multiresistant *Salmonella* isolates, resistant to at least three different classes of antibiotics, were observed in all food types. The authors

demonstrated that *Salmonella* isolated from chicken and pork showed a greater degree of resistance than that from beef and shellfish (at a significance level of 0.05), revealing the higher use of antibiotics in poultry and pig farming in Vietnam. Antibiotic resistance patterns in *Salmonella* food isolates from Vietnam are similar to those of other studies in many countries, showing that *Salmonella* isolates in retail meats were commonly resistant to tetracycline, ampicillin, sulphonamides and streptomycin [31-36]. They also showed that resistance of *Salmonella* isolates to naladixic acid was particularly high in Vietnam (38.9% in chicken) and resistance to antibiotics such as ampicillin, chloramphenicol, and tetracycline was often observed. These antimicrobial agents are still used widely in human therapy in Vietnam due to their low cost and ready availability [37].

The authors also found that multiresistance occurred in potential human-pathogenic *Salmonella* serovars, including serovars Typhimurium, Albany, Anatum, Havana and London. Furthermore, one serovar Typhimurium, a pork isolate was resistant to eight antibiotics, and a serovar Albany isolate from chicken was resistant to seven antibiotics. These *Salmonella* serovars have also been isolated from clinical human isolates in Vietnam [12]. Therefore, resistance to these antibiotics in food-borne pathogens may create problems for disease treatment. A further study on molecular characterisation of these *Salmonella* isolates [38] demonstrated that they harbour a pool of mobile genetic elements such as plasmids and integrons, which contained various antibiotic resistance gene cassettes. Conjugation experiments showed the successful transfer of all or part of the antibiotic resistance phenotypes, demonstrating the role of raw food in the spreading of antibiotic resistance genes. *Salmonella* genomic island 1 (SGI1) is the first genomic island reported to contain an antibiotic resistance gene cluster in a 13-kb segment within a 43-kb genomic island and was identified in the multidrug-resistant *Salmonella enterica* serovar Typhimurium DT104. Until now, SGI1 and its variants have been identified in different *Salmonella* serovars and also in *Proteus*

mirabilis[39-46]. The variant SGI1-F antibiotic resistance gene cluster was detected in *Salmonella* serovar Albany isolated from chicken meat in Vietnam, in which *aadA2* gene, which confers resistance to streptomycin in serovar Typhimurium DT104, has been replaced by *dfrA1* (trimethoprim resistance) and *orfC* (of unknown function)[38]. Interestingly, this variant has previously been found in serovar Albany isolated from chicken in The Netherlands[47] and from fish meal in Thailand, which was imported into France[48]. Fish meal is commonly used as a protein supplement in animal feed for poultry and pigs, it is possible that this strain has been transferred to Vietnam from fish meal in Thailand.

Control of *Salmonella* in food products: the use of vaccines

Multiresistance is becoming more and more common worldwide[31,32,34,36,49,50] and resistance genes are often contained in mobile elements such as integrons, which are easy to transmit from one strain of bacteria to another[51]. Consequently, the use of antibiotics for controlling *Salmonella* is not recommended. The use of vaccines against *Salmonella* spp. is one of the control methods to protect livestock against *Salmonella* exposure and decrease bacterial shedding. Live *Salmonella* vaccines have the ability to replicate, colonise and invade intestinal and visceral organs of inoculated chickens, thus producing strong immunity in vaccinated chickens[52,53]. Vaccination with attenuated *Salmonella* strains can protect chickens from subsequent challenge with virulent *Salmonella*[53-56]. An *aroA* mutant of *S.* Typhimurium (strain STM1) is one of the attenuated *Salmonella* vaccine strains which have been used to protect livestock from *Salmonella* infection. For countries such as Vietnam, Thailand and other Asia countries, *Salmonella* vaccination is a more effective way of controlling *Salmonella* in food production animals than the use of antibiotic therapy; in this way *Salmonella* contamination in carcasses will be dramatically reduced and therefore the possibility of spreading antibiotic resistance should be minimised.

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