



A review and meta-analysis of non-typhoidal *Salmonella* in Vietnam: Challenges to the control and antimicrobial resistance traits of a neglected zoonotic pathogen

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ABSTRACT

Non-typhoidal *Salmonella* (NTS) is a food-borne zoonotic pathogen with important implications for human health. Despite its importance, the burden of NTS infections in Vietnam is unknown. We conducted a systematic review and a meta-analysis to describe the prevalence and phenotypic antimicrobial resistance (AMR) for NTS over time in Vietnam. Following PRISMA guidelines, we identified 72 studies from PubMed and Google Scholar containing data relevant to '*Salmonella*', '*Salmonellosis*', and '*Vietnam*', or '*Viet Nam*'. Of those, forty-two papers reporting prevalence of NTS, and twenty-six including data on phenotypic resistance were selected for meta-analysis. The prevalence of NTS ranged from 2% to 5% in humans and from 30% to 41% in samples from animals and the farm/slaughterhouse environment. Between 2000 and 2020 we observed a 27.3% ($p = 0.044$) increase in absolute terms in prevalence among individuals with enteric diseases and a 12.0% ($p = 0.047$) increase in aquaculture/seafood samples. The most commonly serovars identified across studies included *S. Weltevreden* (12.0%), followed by *S. Typhimurium* (10.1%), *S. Derby* (6.4%), *S. London* (5.8%), *S. Anatum* (4.4%), *S. Rissen* (3.9%), *S. Enteritidis* (3.7%), *S. Albany* (3.3%) and *S. 4,[5],12:i:-* (3.0%). Over the same period, there was an increasing trend in the pooled AMR prevalence for quinolones (15.6% relative increase), 3rd-, 4th-, and 5th-generation cephalosporins (23.7%), penicillins (16.1%), tetracyclines (12.9%), sulfonamides (8.8%), amphenicol (17.8%), and multidrug resistance (11.4%) (all $p \leq 0.014$). A broad range of AMR genes (ARGs) were detected in both human and animal populations. The observed prevalence and AMR trends in Vietnam underscore the need of adopting a One Health strategy encompassing surveillance systems, research initiatives, and awareness campaigns to effectively address the challenges posed by NTS.

1. Introduction

Salmonella enterica is a global pathogen responsible for enteric fever, diarrhea and invasive disease. >2500 *Salmonella* serovars have been identified worldwide [1]. Non-typhoidal *Salmonella* (NTS) are serovars other than Typhi. In developed countries the economic burden of NTS results from the large number of human gastroenteritis cases and serious sequelae in some cases [2]. Particularly in Africa, invasive NTS infections (iNTS) account for ~4100 annual deaths, mostly in children with malnutrition, malaria, and HIV [3].

NTS, being zoonotic, can be hosted by many animal species, and may further contaminate the environment, water and food. Given its

ubiquity, its impact on human health including implications for food safety in the farm-to-fork continuum and its capacity for acquisition of antimicrobial resistance (AMR) traits [4] control of NTS should follow a One Health approach. These recognize that the health of humans, animals, plants, and the wider environment (including ecosystems) are closely interdependent [5]. In many developed countries with well-established surveillance systems NTS is a notifiable disease and its epidemiological features are well characterized.

Until the early 1990's, Vietnam has been endemic for typhoid fever. However, improvements in sanitation and water quality alongside vaccination efforts have led to massive reductions over the past 25 years [6]. In parallel to this, more attention has been paid to NTS. In 2017

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Vietnam established active surveillance of AMR in NTS from pigs and chickens at slaughter points and markets [7].

Over the last two decades Vietnam has been experiencing rapid intensification in food animal production, and has now reached a leading global position especially in aquaculture [8], with some incipient exports of pig and poultry products. Intensification in animal production is associated with increased connectivity between different components in the value chain and may potentially contribute to increased dissemination of NTS strains.

The burden of human disease due to NTS in Vietnam is not well known. This is because of other pressing priorities in the health system as well as the technical challenges of diagnosis of diarrheal pathogens. Paradoxically, the Vietnamese authorities are aware of the importance of food safety in the country. Outbreaks of disease (often due to NTS) are detected and covered by the media. For example, in November 2022, an outbreak in Nha Trang province affected over 600 schoolchildren, resulting in one fatality [9]. There is also awareness of a potential higher risk of bacterial infections associated with seasonal flooding and may increase due to global climate change [10]. Still, Vietnam does not have a nationwide programme to monitor NTS and other foodborne infections in human populations.

A recent review and meta-analysis summarized AMR for NTS and other Enterobacteriales, but did not investigate differences between host animal species or changes over time [11]. The aim of this study is to describe the changes in prevalence and AMR of NTS in Vietnam over time. The goal is to provide a benchmark for future One Health interventions to control NTS in human and animal populations.

2. Methods

2.1. Study protocol and search strategy

We reviewed literature on NTS in Vietnam published in English from 1980 until 2023 following the PRISMA guideline, which includes a total of 27 checklist items [12].

2.2. Inclusion and exclusion criteria for article selection

Initially, the title and abstract of selected articles were screened for information on: (1) Prevalence of detection (per sample/individual); (2) Serovar; (3) Antimicrobial susceptibility; and (4) Antimicrobial resistance gene (ARGs). Articles were excluded if they did not contain any information on either the NTS prevalence or antimicrobial susceptibility or detection of ARGs. Moreover, review articles, book chapter, conference abstracts, letter, and articles written in other languages than English, and duplicated among records were also excluded. Article selection adhered to the Joanna Briggs Institute (JBI) Critical Appraisal Checklist, specifically designed for studies reporting prevalence data [13]. The two authors (N.T.N and D.H-P) independently conducted the selection process. In cases where discrepancies arose in study selection between the two reviewers, a third reviewer (J.C.M) was involved to resolve any disagreements regarding article inclusion.

2.3. Data collection

The date of sample collection was extracted from all studies. In the absence of an explicit date of sample collection, a date of two years prior to publication was assigned; for studies conducted over a time period, a mid-point was defined. From each publication, the following information was extracted: (1) Year of sample collection; (2) Species (for samples collected from humans and animals or their environment) (3) Sample type (faeces, floor swabs, farm waste, carcass, lymph node, caeca, meat, other samples from the abattoir environment); (4) Number of samples; (5) Prevalence of detection (per sample); (6) Number of NTS isolates; (7) Serovar; (8) Prevalence of resistance; (9) Methods used. Human isolates were further categorized based on whether they were

recovered from healthy subjects (i.e. enteric carriage), from cases of enteric disease or invasive infection.

2.4. Statistical analyses

Antimicrobial agents were grouped by class [14] and the average prevalence of resistance was calculated for each class. Only studies with prevalence and/or phenotypic resistance data were included in the meta-analyses. Logit-transformed proportions were analysed using a generalized linear mixed-effect model, including a random-effect model to identify the within- and between-study variances [15]. Heterogeneity across selected studies was assessed using the inverse variance index [16]. Studies were further categorized by the source of the isolates: (1) Human-enteric carriage; (2) Human-enteric disease; (3) Humans-invasive infection; (4) Poultry/poultry farm/abattoir environment/poultry meat; (5) Pig/pig farm/abattoir environment/pork; (6) Cattle/cattle farm/abattoir environment/beef; and (7) Aquaculture/seafood. Analyses were conducted on NTS prevalence and phenotypic resistance meta-analyses to identify significant differences among host species. Univariable meta-regression models were performed to investigate trends in NTS prevalence and phenotypic resistance over time. Results of the meta-analysis were presented using forest plots [17]. All statistical analyses and figure were performed using R programming language [18] with the 'meta', 'meta for', 'tidyverse' and 'ggplot2' packages.

3. Results

3.1. Article selection

A total of 6258 articles were initially identified. Of those, 95.7% were excluded since they were not research articles (e.g. books, reviews, letters to the editor, datasets, guidelines, etc.), were published in different language other than English, or their full text was unavailable. A further 2.6% were excluded since their abstracts did not contain any data. Of the remaining 103 articles, 31 lacked NTS data in the full-text. Therefore 72 articles were finally selected (Supplementary Table 1). For the meta-analyses, only articles that reported NTS prevalence ($n = 42$) and phenotypic resistance ($n = 26$) were selected (Fig. 1).

Of the 72 selected articles, 23 (31.9%) included NTS human data (including invasive disease, enteric disease and carriage). A total of 51 (70.8%) investigated NTS from animals; of those, 35 (68.6%) included data on NTS from pigs, 24 (47.1%) from poultry, 13 (25.5%) from aquaculture and 12 (23.5%) from cattle. A total of seven (9.7%) studies included NTS from environmental samples (i.e. outside animal farms) and wildlife/synanthropic fauna (i.e., geckos). Most (40.3%) studies were conducted in southern Vietnam, 30.5% in northern Vietnam and 4.2% in the central region; 8 (11.1%) covered more than one region, and 10 (13.9%) did not specify geographical area. The serovar identity was investigated in 39 (54.2%) studies, 29 by the agglutination test, 7 by Multi-Locus Sequence Typing (MLST) and 1 by Whole Genome Sequencing (WGS). In two studies the method was not specified. In 43 studies (59.7%) isolates were investigated for their antimicrobial susceptibility. The disc diffusion test was used in 32 studies and in 11 the minimum inhibitory concentration (MIC) was analysed using broth dilution (Sensititre), E-test, Vitek, and agar dilution. Full details are given in Supplementary Table 2.

3.2. Asymptomatic carriage in humans

A study in the Mekong Delta found a higher (albeit non-statistically significant) NTS prevalence among healthy adult chicken farmers (4.4%) compared with rural non-farming adult subjects (2.9%) and their urban matched controls (2.0%) [19]. Potentially limitations in sample size (102 individuals in each group) limits the statistical power of detection of a statistical difference.

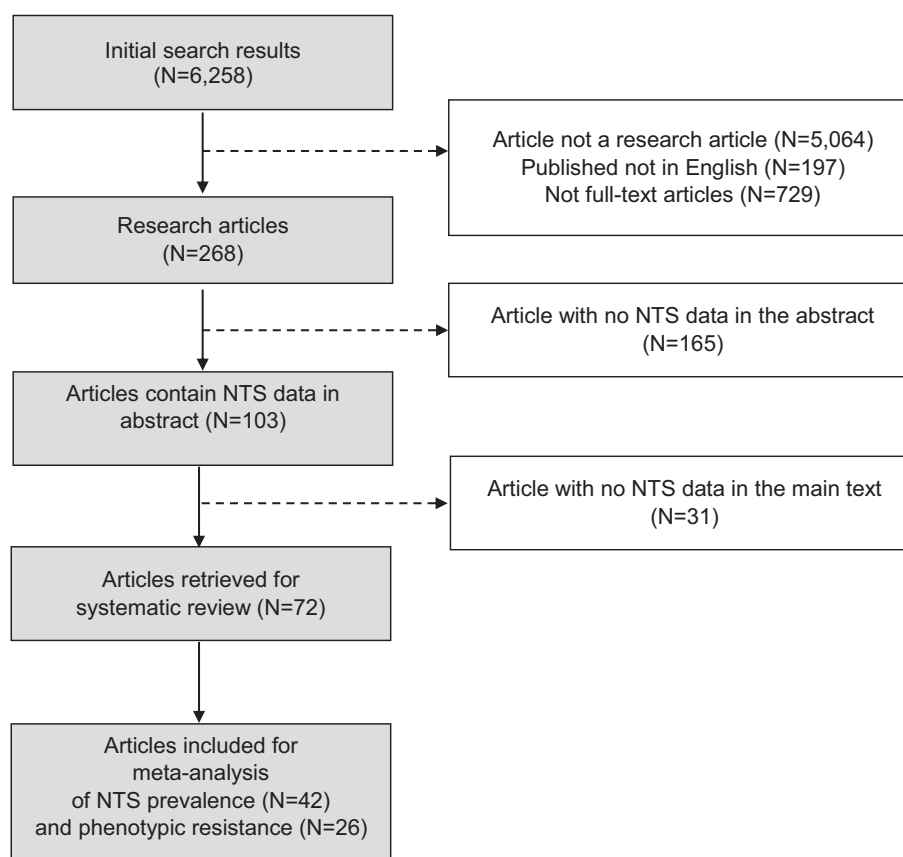


Fig. 1. The PRISMA flow diagram of the study selection process.

3.3. Enteric infection

In a 2001–2002 study, NTS was detected in 45/922 acute diarrheic children in the Mekong Delta region [20]. In contrast, NTS was not detected in 587 children with diarrhea in Hanoi (northern Vietnam) during the same period [21]. A subsequent case-control study (2009–2010) in Ho Chi Minh City (HCMC) on 2028 children (<60 months) failed to detect significant difference in prevalence of NTS between acute diarrhea cases and controls (4.0% and 5.6%, respectively) [22]. A prospective study of newborn children in the rural district of Dong Thap province and HCMC (2009–2013) revealed that in 57% (748/1309) diarrhea cases a pathogen was detected. *Salmonella* was detected by PCR in 18% positive samples after rotavirus (50%), norovirus (24%), and *Campylobacter* spp. (20%). Interestingly, the percent of children colonized with NTS in the Mekong Delta was three times higher than in a large urban center (HCMC) [23]. Among 16 patients with gastroenteritis symptoms in Ben Tre province (Mekong Delta), 7 (43.8%) were *Salmonella*-positive [24]. A 2014–2016 study of 3166 children (<16 years) with dysentery admitted in southern (HCMC) hospitals identified NTS by stool culture in 15%. The peak of cases was between May and September (mid-rainy season) [25].

3.4. Invasive infection

A hospital in Hue (central Vietnam) reported four cases of *Salmonella* meningitis from 2003 to 2008 in <7 month-old children (two *S. Claibornei*, *S. enterica* subsp. *arizonae*, one to *S. Paratyphi* B and one non-specified) [26]. A 2008–2013 study of 147 individuals with an invasive NTS (iNTS) in HCMC identified 43% as *S. Enteritidis* and 30% as *S. Typhimurium*. A total of 71% patients were HIV-positive adults [27]. NTS accounted for 10.9% of 1070 invasive bacterial isolates recorded in HCMC hospitals (2011–2013) [28].

3.5. NTS in animals and food

A total of 32 studies reported 23 prevalence estimates for pigs, 18 for poultry, 7 for cattle and 6 for aquaculture (Table 1). The sample prevalence by year is displayed in Fig. 2.

The meta-analysis of prevalence is presented in Supplementary Figs. 1 and 2 and summarized in Table 1. Detection in samples ranged from 2% to 5% in humans, and from 30% to 41% for animal samples. High levels of heterogeneity were found among the selected studies within each type (all $I^2 > 88\%$, $p < 0.01$), except for humans with enteric carriage ($I^2 = 38\%$, $p < 0.20$). Humans with enteric disease (+27.3%; $p = 0.044$), and aquaculture/seafood (+12.0%; $p = 0.047$) had significant increases over time.

3.6. NTS in animal feeds and other foods at retail

Five of 123 pig commercial feeds (4.1%) were contaminated with NTS (all *S. Weltevreden*) [52]. The investigation of 9 vegetable samples in canteens in Hanoi (2003–2004), failed to detect NTS [54]. A 2014–2015 study on raw pepper detected NTS in 5/84 samples [61]. A survey of 420 cooked and fermented, ready to eat foods from 21 hospital and university canteens in Hanoi detected *Salmonella* in 5% of samples [62]. A survey in the Mekong Delta identified NTS in 12.8% fresh vegetable samples [63]. Of 160 vegetable samples collected from markets in HCMC, 17.5% were contaminated with NTS [64]. No studies have been conducted on table or embryonated eggs.

3.7. NTS in wildlife and the environment

A study on wastewater used for aquaculture and crops in Hanoi found *Salmonella* at a concentration of 108 MPN/100 mL and coliform contamination over 110 times the legal limits, indicating considerable

Table 1
Meta-regression analyses of NST prevalence by different host species and time of study. *P*-values reflects the significance level of the coefficient being different from 0 obtained generalized linear mixed-effect models with the logit-transformed prevalence.

Host categories	Meta-analyses					Univariable meta-regression analyses (Year of sampling)		
	No. prevalence estimates*	I^2	Pooled prevalence (%)	95% CI	p-value	β	95% CI	p-value
Individual analyses by separate host species								
Human (enteric carriage) (a)	3	38%	2.0	0.0- 59.0	0.200	0.334	-2.31-2.98	0.355
Human (diarrhea) (b)	7	97%	5.0	1.0-24.0	<0.01	0.273	0.01-0.54	0.044
Human (invasive) (c)	3	99%	4.0	1.0-25.0	<0.01	0.227	-1.90-2.36	0.404
Poultry and poultry meat (d)	18	99%	30.0	19.0-43.0	<0.01	0.061	-0.04-0.16	0.220
Pig and pork products (e)	23	98%	35.0	25.0-46.0	<0.01	-0.006	-0.09-0.08	0.884
Aquaculture/seafood (f)	6	88%	30.0	12.0-56.0	<0.01	0.120	0.00-0.24	0.047
Cattle and beef products (g)	7	97%	41.0	22.0-63.0	<0.01	-0.032	-0.19-0.12	0.620
Sub-group analyses								
Human studies (a,b,c)	13	NC	4.0	1.0-25.0	0.610	0.273	0.09-0.45	0.006
Animal studies (d,e,f,g)	54	NC	33.0	27.0-40.0	0.720	0.030	-0.02-0.08	0.244
Human and animal studies (a,b,c,d,e,f,g)	67	NC	24.0	18.0-31.0	<0.01	0.071	0.01-0.14	0.035

I^2 : inverse variance index; NC: not calculated. *The number of prevalence estimates ($n = 67$) was from 42 studies selected for meta-analyses of prevalence of NTS. Of 67 estimates, seven for human with diarrhea [20–25,29], 3 for invasive infection [26,28,30], 3 for enteric carriage [19,21,22], 23 for pig [7,31–52], 18 for poultry [7,19,31,34–40,42,48,50,53–57], 7 for cattle [31,34,35,40,42,50,58], and 6 for aquaculture [31,35,40,54,59,60].

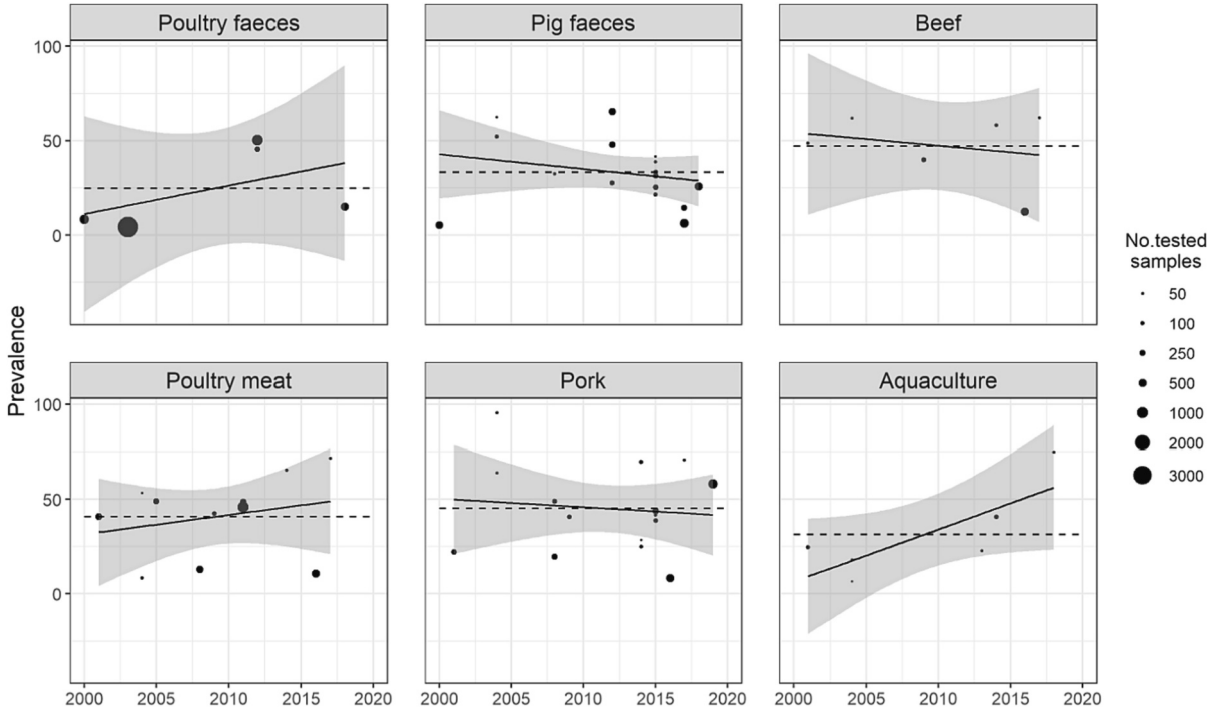


Fig. 2. Prevalence of NTS detection in animal sources over time. The dashed lines indicate the average prevalence across studies.

faecal contamination resulting from inadequate waste treatment. A city drainage canal was most contaminated (>455 MPN/100 mL) [65]. A study of 72 household waste and agriculture field samples reported a NTS prevalence of 46-79% (concentration 164-1441 MPN/100 mL) [46]. A study on river and irrigation water detected NTS in 43/141 (30.5%) samples, where river water had highest contamination (75%) [64]. A study of faecal samples from 101 geckos in the Mekong Delta identified NTS in 23.8%. In positive faecal samples, NTS were present at a concentration of 4.5 log CFU/g [66]. In another study, 156 of 959 (16.3%) of geckos collected from Hue and Mekong Delta provinces were NTS positive [67].

3.8. Differences by type of farm and retail type

No significant differences in prevalence were detected between small-scale and household chicken flocks in the Mekong Delta [19]. However, medium-scale pig herds had higher prevalence than small-scale ones [39]. Similarly, a study in northern pig farms reported significant higher prevalence of NTS in medium-scale compared with small-scale farms [43]. A study on chicken farms using European Union (EU) sampling methods (pooled faeces and dust) [68] showed that the prevalence of infection was highest in layers, followed by broilers and breeder flocks. In pig farms, fattening pigs displayed the highest prevalence (both per sample and at farm-level) [38]. A study in HCMC found NTS in 90.0% chicken meat samples from wet markets (52.6% from supermarkets) [42]. The prevalence of NTS in shrimp from wet markets

(80%) was also higher than those from supermarkets (60%) [60]. A study on shrimp farms in the Mekong Delta (data from 2015 or earlier) identified a 22.3% prevalence of NTS, but did not detect any differences between farm sizes [59].

3.9. Serovar distribution

In total, 6235 isolates were tested for their serovar identify. A total of 8.4% were untypable. A total of 109 different serovars were identified (Supplementary Table 3). The most common serovars identified were, in descending order, *S. Weltevreden* (12.0%), followed by *S. typhimurium* (10.1%), *S. Derby* (6.4%), *S. London* (5.8%), *S. Anatum* (4.4%), *S. Rissen* (3.9%), *S. enteritidis* (3.7%), *S. Albany* (3.3%) and *S. 4,[5],12:i:-* (3.0%). Each of the remaining serovars accounted for <3% of the total. The serovar distribution of the five host categories are displayed in Fig. 3.

By animal host type, the highest correlation with the human serovar distribution corresponded to pork ($r = 0.501$; $p = 0.002$), followed by cattle ($r = 0.321$; $p = 0.060$), poultry ($r = 0.308$; $p = 0.072$), and aquaculture ($r = 0.239$; $p = 0.167$). The fraction of NTS isolates identified as *S. Weltevreden* decreased from 2006 to 2010 to 2016–2020 in aquaculture (from 21.6% to 1.6% of all serovars in individual studies), cattle (28.6% to 4.2%), pigs (18.8% to 6.3%). Over the same time period, *S. Derby* in pigs decreased from 32.3% to 5%, and *S. Anatum* in cattle from 28.6% to 3.1%.

In a 2014–2016 study monophasic *S. Typhimurium* (mSTM) (*S. 4,[5],12:i:-* ST34) accounted for 120/450 (26.7%) hospitalized children with dysentery [25]. In a study conducted in the Mekong Delta (2012–2013), mSTM was found in ducks (15% of farms) and pigs (6%) [39]. High prevalence of mSTM (11%) were also reported in poultry and

pig farms in central Vietnam [38] and in 13.9% pig farms in northern Vietnam [47]. This serovar was also detected in raw meat samples of chicken, pork and beef (1.3–1.7% of isolates) in HCMC [40].

3.10. Phenotypic resistance

Twenty-six (36.1%) studies had data on phenotypic resistance suitable for meta-analyses (Fig. 4, Table 2). Among highest priority critically important antimicrobials (CIAs), macrolides and quinolones had the highest pooled resistance prevalence (12.0% and 10.0% respectively). The pooled prevalence was for 3rd-, 4th-, and 5th-generation cephalosporins and polymyxins was 1.0%. From 2000 to 2020 there was an increase in absolute terms in resistance to quinolones (15.6%, $p = 0.001$) and to 3rd-, 4th-, and 5th-generation cephalosporins (23.7%; $p = 0.005$). Regarding high priority CIAs, the highest resistance was seen for penicillins (adjusted pooled prevalence 21.0%), followed by aminoglycosides (8%). No resistance against carbapenems was detected. An increase in AMR levels over time was only found for penicillin antimicrobials (16.1% increase from 2000 to 2020, $p < 0.001$). Regarding highly important antimicrobials, the highest resistance corresponded to tetracyclines (39.0%), followed by sulfonamides (32.0%) and amphenicols (21.0%).

NTS from poultry and pigs displayed the highest MDR prevalence (54–55%) compared to other host species ($\leq 39\%$). Between 2000 and 2020 there was an overall 11.4% increase in MDR ($p = 0.014$).

Resistance was highly variable across serovars. Particularly high levels of MDR have been reported in *S. Typhimurium* (80%), mSTM (78.9%), *S. Kentucky* ST198 (70%) and *S. Newport* (59.1%) isolates from invasive infections in HCMC [25]. A study in Mekong Delta

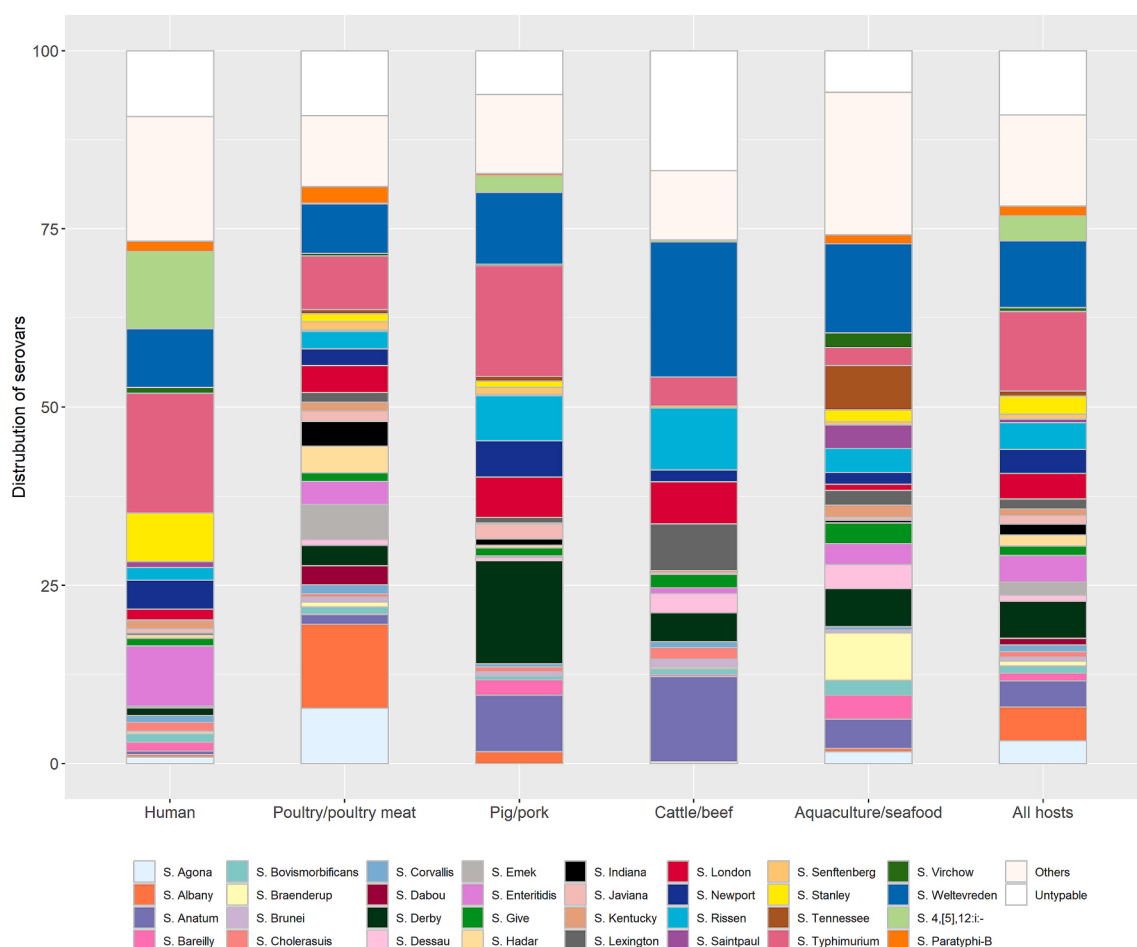


Fig. 3. Serovar distribution in five host categories.

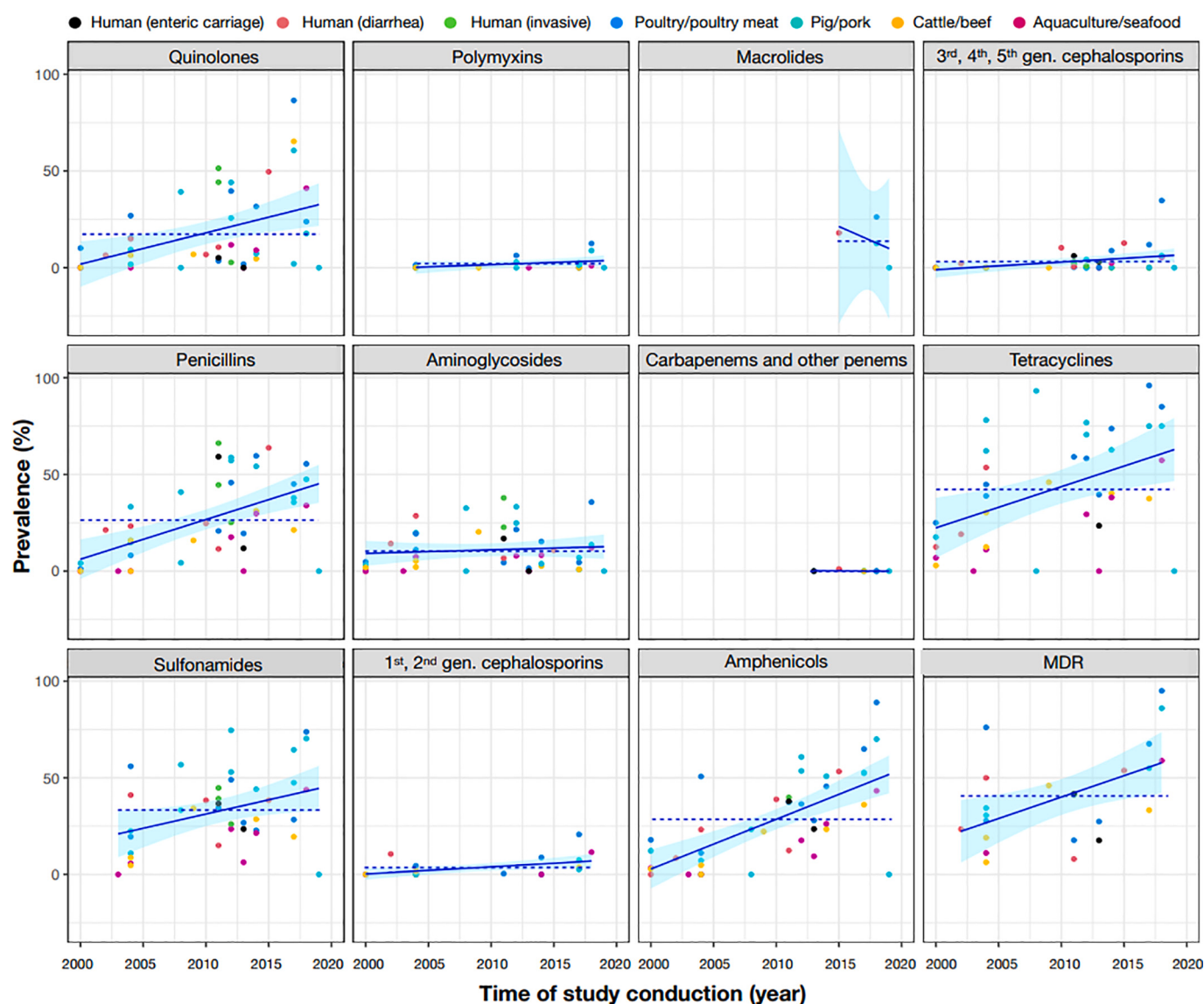


Fig. 4. (Crude) prevalence of AMR in NTS over time. The plotted lines correspond to linear regression lines. The dashed lines indicate the mean prevalence among studies. MDR = multi-drug resistance.

revealed >37% MDR among *S. Typhimurium* and mSTM from pigs and poultry [39]. In other studies, 100% of mSTM isolates from pig and poultry were MDR [38,47]. In study on NTS from retail meat, MDR was most common in *S. Kentucky* (91.7% isolates), *S. Derby* (87.5%), and *S. London* (71.4%); in contrast MDR was absent in *S. Weltevreden* and *S. Lexington* isolates [42].

A 1995–2001 study on *S. Weltevreden* from several countries (19 from Vietnam) reported <6% of resistance against all tested antimicrobials [75]. The analysis of 12 Vietnamese *S. Weltevreden* isolates (2001–2005) from fish and snails imported to the USA revealed full susceptibility to a range of antimicrobials [73]. Similarly, studies in the Mekong Delta reported that all 26 *S. Weltevreden* isolates from vegetables (2017–2019) and 58 isolates from wild geckos (2012–2015) were fully susceptible against all examined antimicrobials [63,67].

3.11. Genotypic antimicrobial resistance

A total of 17 publications reported ARGs in NTS isolates from humans ($N = 5$, [25,28,71,76,77]), poultry ($N = 7$, [37,40,71,76–79]), pigs ($N = 9$, [37,71,76–82]), cattle ($N = 2$, [58,79]), aquaculture ($N = 5$, [40,74,78,83,84]), vegetables ($N = 2$, [64,74]) and river water ($N = 1$,

[64]). A total of 79 ARGs conferring resistance against 12 antimicrobial classes were identified (Table 3).

Of the ten different β -lactamase encoding genes described, *bla*_{CTX-M} and *bla*_{TEM} were most frequently detected in both human and animal isolates. A study investigating organisms associated with bacteremia in HCMC (2011–2013) revealed that *bla*_{CTX-M} and *bla*_{TEM} were the only genes detected in ESBL producing *S. Choleraesuis* strain [28]. Recent study of children in HCMC (2014–2016) reported that 58.7% of NTS strains harbored *bla*_{TEM} gene [25]. In studies conducted in retail markets (2008–2009) in northern Vietnam, *bla*_{TEM} was found in 90–92.3% of NTS strains resistant against ampicillin [37,58]. Co-occurrence of *bla*_{ampC} and *bla*_{CMY-2} was reported in four *S. Braenderup* and one *S. Typhimurium* isolates (all MDR and ESBL producers) from poultry samples collected in markets (2012–2015) in HCMC [40]. Mutations of the *gyrA* gene was the most detected mechanism conferred to quinolone resistance. Other plasmid-mediated resistant genes such as *acc(6')-Ib-cr*, *oqxAB* and *qnrS* were also detected. Mutation at codon Ser83 of *gyrA* gene was detected in 18/23 quinolone resistant NTS from meat samples (2008–2009) while no other mechanisms observed [37]. In a study of NTS from human, *qnrS1* was present in around half of collection (47.8%) whereas mutations of *gyrA* and *parC* genes were only detected in 8.4% of

Table 2

Sub-group analyses of resistance prevalence and trends over time. *P*-values reflects the significance level of the coefficient being different from 0 obtained generalized linear mixed-effect models with the logit-transformed prevalence.

Antimicrobials (no. of studies)	Host species								Meta-regression analyses (year of study conduction)		
	Human (Enteric carriage)	Human (Diarrhea)	Human (Invasive)	Poultry/poultry meat	Pig/pork	Aquaculture/seafood	Cattle/beef	Total*	p-value	β	p-value
Quinolones ¹ (n)	2	6	3	9	12	6	6	44			
Pooled prevalence (%)	4.0	12.0	23.0	19.0	8.0	5.0	6.0	10.0	0.230	0.156	0.001
95% CI	(0.0-94.0)	(4.0-33.0)	(0.0-95.0)	(6.0-45.0)	(2.0-25.0)	(0.0-40.0)	(1.0-34.0)	(6.0-16.0)		(0.06-0.25)	
Polymyxins ¹ (n)	–	–	–	4	7	2	3	16			
Pooled prevalence (%)	–	–	–	4.0	2.0	1.0	0.0	1.0	0.630	0.164	0.063
95% CI	–	–	–	(0.0-25.0)	(0.0-8.0)	(0.0-100.0)	(0.0-100)	(0.0-4.0)		(-0.01-0.34)	
Macrolides ¹ (n)	–	–	–	–	2	–	–	2			
Pooled prevalence (%)	–	–	–	–	12.0	–	–	12.0	1.0	nc	nc
95% CI	–	–	–	–	(1.0-79.0)	–	–	(1.0-79.0)			
3,4,5 gens of Cephalosporins ¹ (n)	2	6	3	8	9	4	5	37			
Pooled prevalence (%)	6.0	2.0	2.0	1.0	0.0	3.0	0.0	1.0	0.280	0.237	0.005
95% CI	(0.0-91.0)	(0.0-17.0)	(0.0-9.0)	(0.0-14.0)	(0.0-13.0)	(1.0-10.0)	(0.0-100)	(0.0-2.0)		(0.07-0.40)	
Penicillins ² (n)	2	6	3	9	12	7	6	45			
Pooled prevalence (%)	33.0	23.0	45.0	24.0	29.0	4.0	10.0	21.0	0.10	0.161	<0.001
95% CI	(0.0-100.0)	(9.0-48.0)	(12.0-83.0)	(10.0-47.0)	(16.0-47.0)	(0.0-47.0)	(2.0-37.0)	(14.0-29.0)		(0.10-0.22)	
Aminoglycosides ² (n)	2	5	2	9	12	7	6	43			
Pooled prevalence (%)	9.0	12.0	30.0	9.0	8.0	7.0	4.0	8.0	<0.01	0.024	0.061
95% CI	(0.0-100.0)	(6.0-25.0)	(2.0-92.0)	(4.0-20.0)	(3.0-19.0)	(2.0-19.0)	(1.0-14.0)	(6.0-12.0)		(-0.00-0.05)	
Carbapenems ² (n)	–	–	–	3	3	–	–	6			
Pooled prevalence (%)	–	–	–	0.0	0.0	–	–	0.0	1.0	nc	nc
95% CI	–	–	–	(0.0-100)	(0.0-100.0)	–	–	(0.0-100.0)			
Tetracyclines ³ (n)	–	3	–	9	11	7	6	36			
Pooled prevalence (%)	–	29.0	–	61.0	51.0	14.0	25.0	39.0	0.010	0.129	0.003
95% CI	–	(4.0-81.0)	–	(39.0-79.0)	(20.0-82.0)	(3.0-47.0)	(11.0-49.0)	(26.0-53.0)		(0.05-0.21)	
Sulfonamides ³ (n)	2	4	3	8	11	6	5	39			
Pooled prevalence (%)	35.0	32.0	37.0	39.0	45.0	16.0	17.0	32.0	0.050	0.088	0.007
95% CI	(4.0-87.0)	(16.0-53.0)	(20.0-57.0)	(25.0-55.0)	(29.0-61.0)	(4.0-42.0)	(7.0-37.0)	(26.0-39.0)		(0.03-0.15)	
1,2 gens of Cephalosporins ³ (n)	–	3	–	6	6	4	5	24			
Pooled prevalence (%)	–	6.0	–	2.0	0.0	0.0	1.0	1.0	0.060	0.140	0.050
95% CI	–	(1.0-30.0)	–	(0.0-16.0)	(0.0-16.0)	(0.0-97.0)	(0.0-6.0)	(0.0-3.0)		(0.00-0.28)	
Amphenicols ³ (n)	2	6	–	9	12	7	6	42			
Pooled prevalence (%)	36.0	21.0	–	42.0	21.0	11.0	10.0	21.0	0.010	0.178	<0.001
95% CI	(4.0-87.0)	(8.0-46.0)	–	(24.0-63.0)	(6.0-50.0)	(3.0-36.0)	(2.0-34.0)	(14.0-31.0)		(0.12-0.24)	
Multi-drug resistance (n)	2	4	–	6	4	2	4	22			
Pooled prevalence (%)	38.0	30.0	–	55.0	54.0	36.0	24.0	39.0	0.380	0.114	0.014
95% CI	(5.0-88.0)	(7.0-70.0)	–	(19.0-87.0)	(17.0-86.0)	(0.0-100)	(7.0-56.0)	(27.0-54.0)		(0.03-0.20)	

¹Highest priority CIAs, ²high priority CIAs, ³highly important antimicrobials. *The total number of prevalence estimates was from 26 studies selected for meta-analyses of phenotypic AMR prevalence of NTS. Six studies conducted on humans with NTS diarrhea [20,22,25,69–71], 3 on invasive NTS infections [27,28,72], 2 studies on human with NTS enteric carriage [19,70], 12 studies in pigs [7,33,35,36,38,40,42,47,51,52,69,71], 9 studies in poultry [7,19,35,38,40,42,53,69,71], 6 studies on aquaculture [35,40,59,60,69,73,74] and 6 studies on cattle [35,40,42,58,69,71].

isolates [25]. Similarly, the presence of *qnrS1* was detected in 32/69 (46.4%) NTS isolates from pigs in slaughterhouse and markets in the North (2014–2019) [81].

A study of 450 NTS isolates from children with diarrhea in HCMC hospitals reported high concordance between AMR phenotype and genotype [25]. This was confirmed by a further study on isolates from human and animals [77]. The study revealed that 84% of ARGs were located on plasmids and 95% of ARGs were within 10 kb of insert sequence IS6/IS26, which allows exchange of ARGs between plasmids

and other parts of the genome.

Three studies reported Class 1 integrons in between 13%–28% isolates [71,74,78]. Gene cassettes found in class 1 integrons included *dhfrXII*, *dfrA1*, *dfrA12*, *dfrA17* (conferring resistance against trimethoprim), *aadA1*, *aadA2*, *aadA5*, *sat* (aminoglycosides), and *bla_{PSSE}*, *bla_{OXA-30}* (β -lactams). The transfer of integrons and AMR determinants from *Salmonella* to *E. coli* through conjugation experiment was possible for 17/83 (20.5%) integron-positive isolates [71].

Table 3

Number of studies where ARGs were detected in NTS isolates from different sources in Vietnam.

Gene	No. studies					Gene	No. studies				
	Human	Poultry/ poultry meat	Pig/ pork	Cattle/ beef	Aqua- culture/ seafood		Human	Poultry/ poultry meat	Pig/ pork	Cattle/ beef	Aqua- culture/ seafood
Aminoglycosides						Polymyxins					
<i>aac(3)-IV</i>	2	2	2	1		<i>mcr-1</i>	1	1	3		
<i>aac(3)-IIa</i>	2	1	3	1		<i>mcr-3</i>			1		
<i>aac(3)-IId</i>	1	1	2			Macrolides					
<i>aac(6')-Iaa</i>			1		1	<i>mefB</i>	1	1	3		
<i>aac(6')-Iy</i>			1			<i>mphA</i>	1	1	2		
<i>aadA1</i>	1	4	6	2	1	<i>mphB</i>	1	1	1		
<i>aadA2</i>	2	2	5	2	1	<i>ermB</i>	1				
<i>aadA5</i>	1	1	1			<i>ermF</i>	1				
<i>aadA12</i>	1	1	1			Tetracyclines					
<i>aphA1</i>		1	1			<i>tetA</i>	1	3	5	2	1
<i>aphA1-1AB</i>		1	1	1		<i>tetB</i>	1	2	4	2	1
<i>aph(6')-Id</i>			1			<i>tetC</i>			1	1	
<i>aph(4)-Ia</i>	2	2	2			<i>tetG</i>	1	2	2	1	
<i>aph(3')-Ia</i>	2	2	2			<i>tetM</i>	1	1	3		
<i>aph(3')-IIa</i>	2	3	3	1		Sulfonamides					
<i>aph(3')-Ib</i>			1			<i>sodC1</i>			1	1	
<i>sat</i>	1	1	1			<i>sul1</i>	2	3	4	1	
<i>strA</i>	2	2	3			<i>sul2</i>	2	2	4	1	
β-lactams						<i>sul3</i>	2	1	4	1	
<i>bla_{ampC}</i>		1				Trimethoprim					
<i>bla_{CMY-2}</i>	3	3	2			<i>dhfr1</i>					1
<i>bla_{CARB}</i>	3	2	2			<i>dhfrXII</i>			1		1
<i>bla_{CTX-M-9}</i>					1	<i>dfrA1</i>	3	3	3	1	
<i>bla_{CTX-M-14}</i>	1					<i>dfrA12</i>	3	4	6	1	
<i>bla_{CTX-M-15}</i>	1					<i>dfrA14</i>	2	2	3		
<i>bla_{CTX-M-55}</i>	3	2	3		1	<i>dfrA17</i>	2	2	2		
<i>bla_{LAP-2}</i>			2			<i>dfrA23</i>	1	1	1		
<i>bla_{NDM-1}</i>	1					<i>dfrA27</i>	1	1	1		
<i>bla_{OXA-1}</i>	3	3	3	1		Phenicol					
<i>bla_{OXA-30}</i>	1	1	1			<i>avrA</i>		1	1	1	
<i>bla_{PSE-1}</i>	1	3	2			<i>cmIA1</i>	2	4	7	2	
<i>bla_{TEM}</i>	4	4	6	2	2	<i>catA1</i>	2	1	1		
<i>bla_{SHV}</i>	1					<i>catA2</i>	2	2	3		
Quinolones						<i>catB</i>	3	1	1		
<i>gyrA*</i>	4	3	3			<i>cat2</i>			1	1	
<i>gyrB</i>		1	1	1		<i>floR</i>	3	4	6	2	
<i>parC*</i>	1				2	<i>mgtC</i>		1	1	1	
<i>aac(6')-Ib-cr</i>	3	2	2			<i>ssaQ</i>		1	1	1	
<i>oqxAB</i>	3	2	3			Rifampicin					
<i>qacH</i>			1			<i>arr-2</i>	1	1	2		
<i>patA</i>	1					Lincosamides					
<i>qnrB</i>	1					<i>linG</i>			1		
<i>qnrD</i>	1					<i>lnu(F)</i>	1	1	3		
<i>qnrS</i>	2	1	3		2	Fosfomycin					
						<i>fosA7</i>			2		

287 *Quinolone resistance determinant region mutations consist of *gyrA*-83, *gyrA*-87 and *parC*-80.*Quinolone resistance determinant region mutations consist of *gyrA*-83, *gyrA*-87 and *parC*-80.

3.12. Colistin resistance

Colistin resistance was detected in 1/67 (*S. Albany*) isolate from poultry in 2004 while none of 63 NTS from beef at retail in Hanoi (2009) [58,71]. A 2011–2012 study of NTS ($N = 138$) from pigs in northern Vietnam identified phenotypic colistin resistance in 2.9% isolates (*S. Typhimurium* and *mSTM*) [47]. A 2012–2013 study reported colistin resistance in 3/48 (6.2%) isolates (all *S. enteritidis*) from chickens, but none from 51 pig isolates [38]. A further study (2013) described high ($\text{MIC} \geq 16$ mg/L) colistin resistance in a *S. Rissen* isolate from a Vietnamese pig, mediated by a *mcr-1* gene identical (100%) to one identified in a pig in Hong Kong on the same year and harbored by an almost identical (96.86%) plasmid [80]. A study on 69 NTS isolates from pork

(2014–2019) revealed that 21.7% were fully resistant (4.3% intermediate); both the *mcr-1* and *mcr-3* genes were identified [81]. In a further study (2016–2017) of 40 NTS isolates from pork purchased in HCMC markets, colistin resistance was detected in 1 isolate (*S. London*; $\text{MIC} = 8$ $\mu\text{g/mL}$), but not in chicken meat ($n = 37$) and beef ($n = 36$) [42]. Surveillance data from pigs and chickens sampled at markets from 2017 to 2019 revealed an overall prevalence of colistin resistance of 9% in pigs and 13% in chickens [7]. In a study (2018) of 90 NTS from shrimps purchased at retail in HCMC, colistin resistance was identified in 1 (1.1%) isolate (*S. enteritidis*; $\text{MIC} > 2$ $\mu\text{g/mL}$) [60].

4. Discussion

Our study demonstrates an increase of NTS in Vietnam over the 2000–2020 period, mostly in human diarrhea cases and aquaculture. Over this period, the aquaculture output in Vietnam has increased ten-fold (from 0.51 to 4.68 million of metric tons) [85]. The Vietnamese per capita meat consumption has increased 154% (from 24 kg in 2000 to 61 kg in 2020) [86]. The observed rises in human NTS diarrhea may also reflect increased exposure due to changes in consumption.

Furthermore, the increase in urbanization experienced over recent decades (i.e. a total of 20.3% of Vietnamese lived in urban settings in 1990 versus 38.8% in 2022) [87] may have contributed to reduction of levels of herd immunity over time.

The data suggests that human challenge with NTS is of higher magnitude in rural environments potentially due to higher challenge associated with higher exposure to farm animals and their excreta. Farmers in the Mekong Delta exhibited a higher prevalence of NTS carriage compared with rural non-farming and urban adults [19] and detection of NTS in children with diarrhea living rural areas was 3.5 higher than those in urban areas [23]. Furthermore, there is evidence of strong protective serological immunity which is transferred from mother to offspring [88].

The data confirms widespread distribution of NTS in farms, slaughterhouse environment and food products. However, contamination in many relevant food sources (i.e. table eggs) remains unknown in Vietnam. Eggs have been incriminated as a primary source of NTS worldwide, particularly due to *S. Enteritidis* [89,90].

Three studies reported data on NTS carriage in healthy humans in Vietnam, with an overall 2.0% pooled prevalence. Studies from North Africa, Mexico and the Middle East have shown variable prevalence of NTS in healthy individuals globally (range from 3.4 to 11.8%) [91–93].

The data conclusively confirms overall increases in AMR and MDR over time. An early study (1996–1999) of (human) community acquired NTS infection Vietnamese isolates had a comparatively low AMR (<1–13% by antimicrobial). No resistance to nalidixic acid was detected [94]. This suggests that the development of AMR has accelerated over the 2000–2020 period, and this is likely to be a result of the high levels of antimicrobial consumption reported in humans and animals in the country [95].

The prevalence AMR in Vietnamese NTS isolates was generally higher than in the EU. For example, in 2021 the EU average resistance of NTS against tetracycline, ampicillin, gentamicin, and colistin for broilers was, respectively 34.0%, 21.7%, 1.6% and 2.5% [96], compared with 61.0% (tetracyclines), 24.0% (penicillins), 19.0% (aminoglycosides) and 4.0% (polymyxins) in Vietnam. However, the Vietnamese data displayed an upward trend and therefore it is likely that the differences would be even more marked if only recent data were used. It is not known whether these differences reflect a different distribution of serovars or a genuine higher prevalence of AMR given the higher levels of AMU in Vietnam compared with the EU [95].

The relative contribution of exposure via contaminated food or direct contact with animals to human NTS infection in Vietnam is not clear. A study characterizing sequence type and AMR patterns of NTS isolates from humans and animals indicated overlapping between sources. Accordingly, NTS in human blood were likely to have greater similarity (54%) to chicken isolates whereas NTS in human stools could be largely (40%) attributed to pig isolates [72]. In contrast, another study showed that *S. typhimurium* isolates from humans and animals (duck, pig and chicken) clustered into distinct clades in the phylogeny indicating limited evidence for the transmission of this pathogen [76]. Conducting attribution studies of different animal sources using serovar characterization would be desirable under a One Health approach, but are technically challenging given the vast diversity of NTS in animals and the environment (including geographical diversity) and the difficulties in obtaining representative data. A quantitative microbial risk assessment indicated that the annual probability of acquiring salmonellosis from

consumption of boiled pork was 17.7% [41].

S. Enteritidis and *S. Typhimurium* accounted for 73% of invasive infections in hospital settings in southern Vietnam [27]. This probably reflects a higher pathogenic potential of these serovars which represent 31.5% of isolates recovered from humans across the country (14.9% for all species combined). mSTM is an emerging serovar associated with numerous outbreaks in humans worldwide in association with livestock, particularly pigs [97]. Over the past decade MDR mSTM has become more prevalent in Vietnam, and has been associated with iNTS disease in HIV-patients [76]. In contrast, serovar *S. Weltevreden* is widespread but has considerably reduced its prevalence over time, and is associated with low levels AMR and low virulence in humans [77,98].

In Vietnam, meat products are often purchased unrefrigerated in informal street markets, which allow for any bacteria present to quickly multiply after slaughter. Chicken meat from wet markets had higher level of contamination with NTS bacteria (median 3.2 log₁₀ MPN/g) than beef or pork [42]. It has been proposed that restricting sales of meat to the cooler morning hours may reduce bacterial contamination [43]. Given the high prevalence of NTS in vegetables [63,64], and the practice of consuming raw leaf vegetables, we suggest that this could be an additional source of NTS to humans.

5. Conclusions

This review demonstrates the magnitude of circulation of NTS in humans, animals and the environment. Levels of human infection appear to be higher in rural settings, probably reflecting environmental exposure and closer animal contact. The observed increases in NTS in aquaculture and poultry over the 2000–2020 period probably reflects intensification of these sectors. The increase in AMR and MDR of NTS isolates over the same period are likely to have repercussions for the treatment of invasive NTS infections. Since most laboratories do not conduct routine investigation of NTS in outpatients the burden of infection in the community is now well known. The prevalence and increased AMR trends of NTS infections underscore the need of adopting a One Health strategy for surveillance, research, and awareness campaigns to address the challenges posed by this pathogen. Unchecked, the rise in NTS infections and AMR could strain healthcare resources, hinder economic progress in the agriculture sector, and jeopardize food safety in Vietnam.

Ethics statement

No ethical approval was required for this review work.

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CRediT authorship contribution statement

Nguyen Thi Nhung: Conceptualization, Formal analysis, Methodology, Writing – original draft. **Doan Hoang Phu:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Juan J. Carrique-Mas:** Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Pawin Padungtod:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2024.100698>.

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