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Differential antimicrobial susceptibility profiles between symptomatic and asymptomatic non-typhoidal *Salmonella* infections in Vietnamese children

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Short Paper

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Abstract

Non-typhoidal *Salmonella* (NTS) serovars, sequences types and antimicrobial susceptibility profiles have specific associations with animal and human infections in Vietnam. Antimicrobial resistance may have an effect on the manifestation of human NTS infections, with isolates from asymptomatic individuals being more susceptible to antimicrobials than those associated with animals and human diarrhoea.

There are an estimated 93.8 million cases of *Salmonella* gastroenteritis globally each year resulting in 155 000 deaths [1]. Consequently, non-typhoidal *Salmonella* (NTS) infections represent an important public health issue and a major contributor to global morbidity and mortality. The resistance of *Salmonella* to most first-line antimicrobials has been increasing in recent decades, prompting the World Health Organization (WHO) to include them on the list of priority pathogens for which new antimicrobials are needed [2].

NTS can persist in infected individuals for several months or even years without inducing clinical symptoms [3]. Although asymptomatic carriers are thought to be a key reservoir for NTS organisms in human populations, the epidemiology of asymptomatic infections is not well described. In this study, we aimed to describe the population structure of non-invasive symptomatic (diarrhoeal) and asymptomatic NTS infections in humans and animals and identify potential sources of antimicrobial-resistant NTS organisms in humans.

We combined data from three cross-sectional studies conducted in Vietnam: (i) The Vietnam Initiative on Zoonotic Infections (VIZIONs): community-based studies of zoonotic disease transmission; (ii) the aetiology of diarrhoea in provincial hospitals; and (iii) Vietnam Bacterial Resistance (VIBRE) [4–8]. Of 524 NTS stool isolates analysed, 113 (21.56%) came from asymptomatic individuals, 98 from individuals with diarrhoea and 313 (59.73%) from animals (chickens, ducks, pigs and rodents). Ethical approval for these studies was obtained from the Oxford Tropical Research Ethics Committee (OxTREC) and/or the Scientific and Ethics Committee of corresponding hospitals and health departments: VIZIONs (protocol 157/12); the aetiology of diarrhoea in provincial hospitals (protocol 0109); VIBRE (protocol 48/11) and. the Australian National University Human Research Ethics Committee (protocol 2018/229).

Bacterial culture and multi-locus sequence typing (MLST) was performed as previously described [4, 6–8]. Antimicrobial susceptibility of isolates was determined according to the 2014 Clinical and Laboratory Standards Institute (CLSI) guidelines against ampicillin (AMP), amikacin (AK), ceftazidime (CAZ), ceftriaxone (CTX), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN) and trimethoprim-sulfamethoxazole (SXT). Resistance to clinically important agents for NTS was defined as resistance to one or more of the following: AMP, CTX, CIP, GEN, or SXT. Multi-drug resistance (MDR) was defined as non-susceptibility to ≥1 agent in ≥3 antimicrobial classes.

Chi-square tests were used to test for heterogeneity of STs, AMR profiles and ST-AMR profiles between human asymptomatic/diarrhoeal and animal isolates. To evaluate an association

Table 1. Prevalence of antimicrobial susceptibility profiles among NTS isolates in Vietnam

Antimicrobial susceptibility profile	Human stool isolates – asymptomatic ($n = 113$), No. (%)	Human stool isolates – diarrheal ($n = 98$), No. (%)	Animal isolates ($n = 313$), No. (%)
Fully susceptible	73 (64.6)	38 (38.78)	167 (53.35)
R-Ampicillin	13 (11.5)	59 (59.18)	72 (23)
R-Amikacin	7 (6.19)	14 (14.29)	21 (6.71)
R-Ceftazidime	1 (0.88)	6 (6.12)	3 (0.96)
R-Ceftriaxone	0 (0)	6 (6.12)	3 (0.96)
R-Chloramphenicol	14 (12.39)	37 (37.76)	71 (22.68)
R-Ciprofloxacin	12 (10.62)	5 (5.1)	61 (19.49)
R-Gentamicin	8 (7.08)	19 (19.39)	18 (5.75)
R-Trimethoprim-sulfamethoxazole	17 (15.04)	36 (36.73)	72 (23)
R-Clinically important agent[a]	35 (30.97)	39 (39.8)	136 (43.45)
MDR[b]	9 (7.96)	41 (41.84)	47 (15.02)

[a]Resistant to ampicillin, ceftriaxone, ciprofloxacin, gentamicin and/or trimethoprim-sulfamethoxazole.
[b]Resistant to ≥1 agent in ≥3 antimicrobial categories including penicillins (ampicillin), aminoglycosides (gentamicin, amikacin), sulphonamides (trimethoprim-sulfamethoxazole), quinolones (ciprofloxacin), cephalosporins (ceftriaxone, ceftazidime) and phenicols (chloramphenicol).

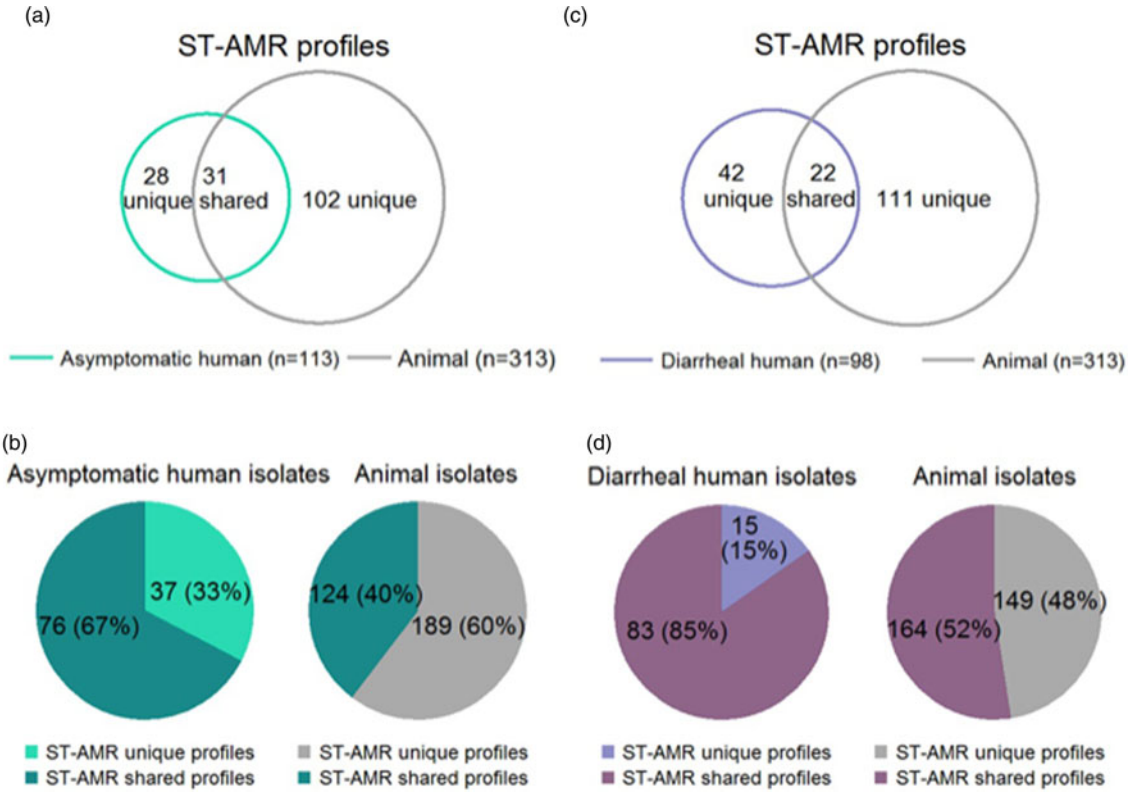

Fig. 1. Distribution of Salmonella sequence types and AMR profiles in animal and human *Salmonella* in Vietnam. The number and proportion of unique and shared ST-AMR profiles among asymptomatic human and animal NTS isolates (a,b) and diarrhoeal human and animal NTS isolates (c,d) in Vietnam.

between antimicrobial resistance and the source of isolate, we estimated odds ratios (ORs) with associated 95% confidence intervals (95% CI) using logistic regression; P values ≤0.05 were considered significant. To determine the distribution of unique and shared STs, AMR profiles and ST-AMR profiles, Venn diagrams and pie charts were constructed; analyses were performed using Stata software version 15 (Stata Corporation, College Station, TX, USA).

We identified 74 STs comprising 40 serovars. Serovar Weltevreden (ST365 and 1500) was the most common serovar both in human asymptomatic and animal isolates, accounting for 28.3% (32/113) and 13.7% (43/313) isolates, respectively.

The most common isolate from human diarrhoeal specimens was serovar Typhimurium (ST34, 19, 99, 313 and 1544) accounting for 38.8% (38/98) isolates. Of 63 STs identified in asymptomatic humans and animals, 12 were unique to humans, 24 were unique to animals and 27 were shared by both. Likewise of the 63 STs identified in human diarrhoeal samples and animals, 12 were unique to humans, 29 to animals and 22 were shared by both species.

The source of the isolate was a significant predictor of antimicrobial susceptibility. Organisms associated with diarrhoea in humans demonstrated the highest level of antimicrobial resistance, whereas NTS associated with asymptomatic infections were susceptible to the majority of tested agents (Table 1). Human diarrhoeal isolates were resistant to a mean of 1.85 antimicrobials (s.d. 1.93; range 0–8); human asymptomatic isolates to a mean of 0.64 agents (s.d. 1.11; range 0–5); and animal infections to a mean of 1.03 agents (s.d. 1.47, range 0–6). Organisms from asymptomatic infections were significantly less resistant to antimicrobials than those from animal infections (OR 0.63; 95% CI 0.4–0.98; $P = 0.04$). By contrast, isolates from human diarrhoea were significantly more resistant to antimicrobials than animal isolates (OR 1.22; 95% CI 1.04–1.42; $P = 0.012$) and isolates from asymptomatic infections (OR 1.70; 95% CI 1.28–2.25; $P < 0.001$). Similarly, the source of the isolate was an important predictor of MDR with NTS diarrhoeal isolates being more likely to be MDR when compared to human asymptomatic (OR 2.88; 95% CI 1.94–4.28; $P < 0.001$) and animal isolates (OR 1.6; 95% CI 1.35–1.89; $P < 0.001$). Correspondingly, human asymptomatic isolates were less likely to be MDR when compared to animal isolates; however, this association was not statistically significant (OR 0.49; 95% CI 0.23–1.04; $P = 0.062$). Overall, we identified 45 unique antimicrobial susceptibility profiles. In comparing human asymptomatic isolates and animal isolates, three were unique to humans, 18 to animals and 16 were shared by both. In comparing human diarrheal isolates and animals, eight were unique to humans, 22 were unique to animals and 12 were shared.

Lastly, to investigate the potential role of animal reservoirs in AMR NTS infections in humans, we compared the 200 ST-AMR profiles identified across all NTS isolates. Among the 161 ST-AMR profiles associated with human asymptomatic infection and animals, 28 (17.4%) were unique to humans, 102 (63.4%) to animals and 31 (19.3%) were shared by both. Concerning the 175 profiles identified in organisms associated with human diarrhoea and animals, 42 (24.0%) were unique to humans, 111 (64.4%) to animals, but only 22 (12.6%) were shared by both (Fig. 1).

Our data show that specific NTS serovars, STs and antimicrobial susceptibility phenotypes appear to have associations with animal and human infection. We identified a comparable population structure between NTS isolated from animals and asymptomatic human in Vietnam, highlighting the role of animal reservoirs in the disease transmission. However, a high prevalence of human asymptomatic NTS infections suggests that carriers may play a role in disease transmission. Furthermore, asymptomatic NTS organisms were less resistant to antimicrobials in comparison with human diarrhoeal isolates. This observation suggests that AMR may have a significant effect on disease manifestation and outcome [9]. Lastly, due to the high infectious disease burden and inappropriate use of antimicrobials in humans and animal production in Vietnam [10], it is necessary to adopt principles of antimicrobial stewardship and conduct more studies examining the antimicrobial resistance in a One Health context.

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Conflict of interest. The authors declare no competing interests.

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