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Antimicrobial drug resistance at the human-animal interface in Vietnam

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CHAPTER 8
DISCUSSION

Chapter 8: Discussion

Antimicrobial resistance (AMR) is increasing worldwide. The global trade and mobility of persons facilitate the spread of resistant microorganisms and/or AMR determinants. There is a longstanding debate on the attribution of the usage of antimicrobials in animals and the subsequent increase of resistance determinants in the animal reservoir, to AMR in humans. Given the increasing AMR burden in humans and the above debate, the project was initiated.

The majority of Vietnam's population is living in the rural areas and around 40% of the households engage in poultry raising. An approximately 94% of these households has a flock size of 50 chickens or less [1, 2]. However, limited data on AMR in the chicken microbiome and the association with AMR in the microbiome of the humans exposed to these animals are available.

Non-typhoidal *Salmonella* (NTS) and *E. coli* were the bacteria of interest in this study. We have chosen *Salmonella* because salmonellosis is a public health concern worldwide with poultry as a major source for humans [3]. In addition, we also included *E. coli* since commensal *E. coli* are considered as a reservoir for resistance genes and an indicator species for AMR surveillance [4, 5].

This is one of the first projects in Vietnam that used the One Health approach, with the simultaneous collection of samples from chickens and farmers, as well as from location-, age- and gender-matched humans without direct exposure to chickens.

High level of antimicrobial usage in both chicken farming and in the community

Data from Chapter 2 show that a wide range of antimicrobials from at least ten different antimicrobial classes was used in poultry, with many of them also used in human medicine such as quinolones and macrolides. In addition, antimicrobials were mainly used for prophylaxis (84%) rather than therapeutic purposes.

Our results also show that the amount of antimicrobials used to produce one 'meat' chicken in the Mekong delta was more than 6 times higher than in Europe [6]. Antimicrobial drug usage is

unacceptably high in small-scale farms in southern Vietnam. In contrast to what was hypothesized, back yard farming showed systematic usage of antimicrobial drugs and medicated feed, probably similar to intensive farming practices.

Data obtained from human study participants, presented in Chapter 6, show that amongst 510 households, 82 households (16.1%) reported the use of antimicrobials by at least one member during the month prior to the study visit. Antimicrobials were used in 33 (16.2%) of farmer households, 32 (15.7%) of rural households and 17 (16.7%) of urban households. The three most commonly used antimicrobials were cephalosporins (10.2%), penicillins (4.3%) and macrolides (2.1%). The data in our study are consistent with other studies that have published about the use of antimicrobials in humans in the community [7]. This is an obvious consequence of the lack of an effective enforcement of the antimicrobial prescribing and dispensing laws which regulated that antimicrobials should be prescribed by only clinicians or veterinarians.

High prevalence of colonization with antimicrobial resistant bacteria and antimicrobial resistance determinants

Almost half of chicken farms in our study tested positive for non-typhoid *Salmonella* (NTS). There was no statistically significant difference in the NTS farm-level prevalence between household farms (46/102, 45.1%) and small farms (47/102, 46.1%). The prevalence of asymptomatic NTS colonization in humans in our study is higher than the reported prevalence of asymptomatic NTS in developed countries (0.3% – 0.4%) [8-10]. However, our findings were comparable to other studies in the northern Vietnam (3.1%) [11] and Thailand (4.7%) [12].

Salmonella isolates in our study were commonly resistant to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole and ampicillin. The results were similar to the results of another survey also carried out in the Mekong Delta [13].

In general, the level of resistance was higher in *E. coli* compared to non-typhoidal *Salmonella* in our study. MDR *E. coli* isolates (resistant to at least 3 different classes of antimicrobials) were detected in 100% of the farms. The prevalence of antimicrobial resistant *E. coli* in these backyard chicken farms was higher than expected since we did not expect so much of antimicrobial use in backyard farms.

Enteroaggregative *E. coli* (EAEC), an important cause of diarrhea, was studied in Chapter 5. EAEC was only isolated from human faecal samples (3.3%, 15/458). These EAEC isolates exhibited a high level of resistance in which 88.2% and 50.0% of the isolates were multi-drug resistant and extended-spectrum beta-lactamase positive, respectively. The level of antimicrobial resistance among EAEC isolates was also higher compared with results from other countries [14].

As shown in Chapter 6, the prevalence of ESBL-Ec colonization was 20.0% in chicken farms, 31.1% in chicken farmers, 49.5% in rural individuals and 38.3% in urban individuals. This high prevalence of human colonization with ESBL-Ec is in agreement with the high and uncontrolled use of broad spectrum cephalosporins in the community [15]. However, the large difference in prevalence between chicken and humans was unexpected and was related to the comparatively low usage of cephalosporins in chicken.

In Chapter 7, we observed an extremely high prevalence of faecal colonization with *mcr-1* carrying bacteria in chicken faecal samples (59.4%) as well as in human samples (20.6%). We also noticed that the prevalence of faecal colonization with *mcr-1* carrying bacteria in urban individuals (9.1%) was much lower than in farmers and rural individuals (25.2 and 17.6%, respectively). Our isolation rate of *mcr-1*-positive *E. coli* in chickens (approximately 20.0%) was similar to previous published data from Vietnam and China [16, 17], but much higher than data from other countries such as Japan (0%), France (1.8%) and Brazil (3.0%) [18-20].

Association of antimicrobial usage and colonization with antimicrobial resistant bacteria and carriage of genomic antimicrobial resistance determinants

Data presented in Chapter 2 revealed the association of quinolones use and ciprofloxacin resistant *E. coli* in chicken farms. Our findings are in line with other field studies that demonstrated that the usage of quinolones selects for carriage of quinolone-resistant *E. coli* in poultry [4, 21]. In addition, data from chapter 7 showed that the detection of a plasmid-mediated colistin resistance encoding gene was associated with colistin use in chickens.

Results described in chapter 6 indicated that ESBL-Ec colonization was associated with the recent usage of antimicrobial drug in humans. The findings are in line with a previous study

where risk factors associated with faecal colonization with ESBL-producing Enterobacteriaceae in healthy individuals were reviewed [22].

The risk of human colonization with antimicrobial resistant bacteria and carriage of antimicrobial resistance determinants resulting from chicken farming

We initially hypothesized that exposure to chickens through farming results in higher risk of asymptomatic colonization with antimicrobial resistant bacteria. However, although having direct contact with chickens, farmers are not very likely to become a carrier of non-typhoidal *Salmonella* (Chapter 2). There is a difference between the prevalence of NTS colonization in farmers (4.4%) and general, unexposed individuals (2.6%), but the difference was not statistically significant. In addition, among 204 studied farms, there is only 1 farm in which the Weltevreden serovar is shared between chicken and farmer. Although in this study transmission was not specifically investigated for resistant *Salmonella*, it seems unlikely that transmission of resistant *Salmonella* would be different from susceptible *Salmonella*.

With regards to Enteroaggregative *E. coli*, our data in Chapter 5 suggest that there is little transmission of EAEC from humans to chickens or that transmission does not lead to subsequent colonization, since EAEC was detected only in human samples and not in chicken samples.

We did not find an association between non-intensive chicken farming and ESBL-Ec colonization in humans in Vietnam (chapter 6) indicating that non-intensive chicken farming is not a major source of ESBL-Ec colonization for humans in this setting. Data from chapters 4 and 6 suggest that resistance in *E. coli* in animals and humans is determined by the use of antimicrobials in each of the specific domains: a relatively high prevalence of resistance to fluoroquinolones in poultry due to the use of fluoroquinolones, and a higher resistance to 3rd generation cephalosporines in humans compared to poultry due to use of this type of antimicrobial in humans and hardly any use in poultry. Overall data suggest that the transfer between animals and humans has restrictions resulting in different reservoirs in poultry and humans.

However, in Chapter 7, we found that the risk of colonization with *mcr-1*-carrying bacteria was significantly higher in farmers who were exposed to *mcr-1* positive chickens than farmers with

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mcr-1 negative chickens or individuals not involved in chicken farming. The lack of human colistin usage in the community and the association of *mcr-1* presence with exposure to *mcr-1* positive chicken suggest that *mcr-1* transmission is predominantly zoonotic. This is, however, in contrast to the conclusions from the other chapters. An additional factor that may play a role is the exposure of farmers to colistin on farms when it is used for the animals. In that case, there is not transfer of bacteria but a common exposure to colistin.

In summary, in our study, we found that bacterial transfer (e.g. NTS, EAEC) maybe limited between humans and chickens. This observation could be the consequences of host adaptation. In contrast, transmission of AMR determinants is extremely complex. It is likely to occur in multiple directions, and may be restricted to certain AMR genes, AMR carrying mobile elements, or bacterial clones, a.o. depending on the selective pressure exerted through antimicrobial drug usage.

Limitations of the study

Our study is subject to several limitations. Firstly, the cross-sectional study design may preclude the demonstration of direct transmission of the antimicrobial resistant bacteria and/or resistance determinants between chickens and humans. Secondly, the number of non-typhoidal *Salmonella* isolates from humans and the number of ESBL *E. coli* isolates from chickens was relatively small, limiting the power to demonstrate any statistical difference between study cohorts. In addition, results of antimicrobial usage reported in our study are likely to underestimate total antimicrobial usage, since commercial feed commonly includes sub-therapeutic amounts of antimicrobials (chlortetracycline, bacitracin and colistin). However, data on antimicrobials added into animal feed were not precisely available.

The recommendations arising from the project

It is important to highlight that the epidemiological findings in this study might not applicable in countries with different healthcare/farming settings, different access to antimicrobial drugs for both humans and animals, as well as different level of antimicrobial resistance in the community.

Although there are large differences in antimicrobial drug legislation and regulation, policies for antimicrobial usage among countries, the usage of antimicrobials in small-scale farming and in

the community are unacceptable high in Vietnam. Given the potentially serious consequences of the spread of antimicrobial resistance determinants (e.g. ESBL genes and plasmid-mediated colistin resistance gene) between food-producing animals and humans as well as the association between usage of antimicrobials and the prevalence of antimicrobial resistance, prudent usage of antimicrobial drugs in the community and in agriculture should be enforced globally, especially in a developing country such as Vietnam. Clearly, antimicrobial prescribing and dispensing practices need to be changed in both human and veterinary medicine in the country. In order to implement that, enforcement needs to be improved for stricter regulation of over-the-counter sales of antimicrobial drugs.

Given the low levels of investment and ‘bio-security’ in backyard chicken farms in Vietnam and the fact that chickens and humans are living in almost the same environment, sharing of AMR bacteria and/or resistance determinants between different hosts are possible (e.g. *mcr-1* gene). As a result, the first priority is that the level of ‘bio-containment’ on farms should be improved to limit the conditions that release viable resistant bacteria and resistant determinants into the surrounding farming and living environments.

The complexity of antimicrobial resistance deserves joint focus of multiple disciplines. While collaboration between human and veterinary professions might be more common in other countries, it is certainly not the case in Vietnam. Strengthening the collaboration between human and animal health practitioners in southern Vietnam should be implemented in order to have overall and thoughtful action plans to combat AMR in Vietnam. This project illustrated a typical collaboration of the integrated “One-health” approach which involved both veterinarians as well as medical healthcare workers in the province, encouraged knowledge exchanges and understanding between different sectors.

Future research

This project assists us to identify important research aspects for the future. It is essential to investigate (1) the relatedness of *E. coli* and *Salmonella* isolates that share the same sequence types and the same phenotypic resistance patterns by using whole genome sequencing. This high-resolution analysis is essential to understand the phylogenetic relationship between chicken and human isolates; (2) the dynamics of antimicrobial resistance in chickens and humans based

on a longitudinal study. Since resistance development is a constant process a follow-up study should be performed in such a way that we could contemporaneously capture as much as possible the event (3) the role of farming environments in spreading antimicrobial resistant bacteria and/or antimicrobial resistance determinants in chickens and humans. This is necessary because antimicrobial resistant bacteria contamination and occurrence in the environment contribute to the spread of resistant bacteria and/or resistance determinants.

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