University of Veterinary Medicine Budapest

Doctoral School of Veterinary Science

A One Health approach study on sources in the process of animal-to-human antimicrobial resistance gene transfer

PhD thesis

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1 Background and aims of the doctoral thesis

The spread of antimicrobial resistance (AMR) is one of the most significant medical challenges of the 21th century, ranked as one of the top 10 global health issues by many international organisations, including WHO (World Health Organization). Although, the constant use of antibiotics inevitably contributes to the growing detection rates of AMR, antimicrobial resistance genes (ARGs) that are primarily responsible for the appearance of AMR and of the negative healthcare effects are the natural elements of the bacterial genomes. These genes can be transferred between bacteria, either pathogens or non-pathogens, with horizontal gene transfer. For the execution of horizontal gene transfer, some requirements must be fulfilled, out of which the presence of mobile genetic elements is one of the most crucial. Such mobile genetic elements facilitate the spread of various genes among bacteria. Therefore, in case of ARGs, being accompanied by such elements is a key factor of the spread of AMR. If ARGs, facilitated by mobile genetic elements, enter a pathogenic bacterium, a clinical consequence may be the decrease of the efficacy of antibiotics.

It is essential to know in which media the requirements of horizontal gene transfer are fulfilled and which ARGs can enter the human body from the possible sources. According to recent publications, 70% of the global antibiotic-use can be related to the animal husbandry sector. Such use of antibiotics puts a constant selective pressure on bacteria in and around domesticated animals, increasing bacterial ARG assets. In the recent past, great scientific attention has been focused on the static examination of ARGs, namely if their presence or lack was detectable. Nevertheless, this aspect cannot really reflect on the actual public health risk that certain ARGs potentially have.

More wide-spectrum studies, covering the dynamic aspects related to ARGs, can help deepening our understanding of ARG spreading potential. Such studies have gained a wider popularity in the past few years, owing to the development of various reliable, widely accessible high-throughput sequencing technologies.

Throughout our research, we aimed to identify sample sources of animal origin that may be involved in the animal-to-human ARG spread routes.
Our methods encompassed bioinformatic analyses based on shotgun next-generation sequencing. ARGs can be transferred from animals to human in either direct or indirect ways, and we examined the broadest spectrum possible of ARG transfer media. A priority was put on screening samples of animal origin with a great live bacterial content that may be contacted by a wide range of people on a daily basis. Several foods with animal origins are consumed with high viable bacterial counts (raw and probiotic products), while others animal-deriving products may contact the surface of food to be prepared and cooked, thus contribute to the indirect spread of the farm-borne bacterial gene content. Furthermore, close physical contact and common, regular veterinary interventions related to companion animals may also take place in the animal borne spread of AMR (e.g. saliva of dogs).

2 New scientific findings of the thesis

THESIS 1: Foods consumed without heat treatment, such as raw milk, can transfer antimicrobial resistance genes and contribute to the spread of antimicrobial resistance.

Several ARGs were identified in publicly available raw milk samples including the phage integrase-associated *blaZ* that was predicted to derive from a plasmid. Some of these ARGs were detected in contigs from *Acinetobacter spp.*. In conclusion, the consumption of raw milk may have significant implications for public health.

THESIS 2: Milk deriving from animal farms contains bacteria with antimicrobial resistance genes that can encounter potentially proliferating probiotic bacterial strains included in the fermentation process of dairy products. This can lead to the spread of antimicrobial resistance.

In bacteria associated with the fermentation process of yoghurt and kefir, 23 ARG types were identified, including ones that were mobile such as (*lmrD*) or act against antibiotics that are critically important for human medicine. Considering that dairy products often derive from environments where antibiotics are applied and the ARG content of fermented foods appeared to be able to grow due to bacterial multiplication, the starting culture strains of fermented foods should be monitored and selected carefully in order to decrease the intake of ARGs via foods.

THESIS 3: Probiotic bacterial strains used for food fermentation and in probiotic dietary supplements contain a broad set of potentially mobile antimicrobial resistance genes.

Based on the large-scale study *Bifidobacterium animalis* and *Lactococcus lactis* appeared to be highly rich in ARGs. In contrast, no *Lactobacillus casei* or *Lactobacillus paracasei* strains contained any ARGs, and in *Lactobacillus delbrueckii, Lactobacillus helveticus* and *Lactobacillus brevis*, ARGs were relatively less frequent. A high proportion of the identified ARGs appeared to be mobile. While acquiring mobile ARGs does not always confer AMR, extending current recommendations to detect potential functional traits of concern, including the selection of less ARG-rich bacterial species and strains used for food fermentation could be considered, with screening for mobile ARGs in probiotic bacteria.

THESIS 4: Based on the study of canine saliva samples, close physical bond with pet dogs can contribute to the spread of antimicrobial resistance.

In the genome of potentially pathogenic bacterial species, which are some of the most relevant bacteria in dog bite infections, 69 ARGs were detected. Several ARGs, including ones against amoxicillin–clavulanate, the most commonly applied antimicrobial agent for dog bites, were predicted to be potentially transferable. According to our findings, canine saliva may be a source of transfer for ARG-rich bacteria that can either colonize the human body or transport ARGs to the host bacteriota, and thus can be considered significant in the interspecies spread of AMR.

THESIS 5: Farm animal-associated samples, such as swine feces contain antimicrobial resistance genes that can be mobile.

Throughout the metagenomic analysis of swine fecal samples, 54 ARG types, including potentially mobile ones were detected. Similar surveillance studies at large-scale farms may lead to valuable discoveries in connection with the appearance and spread of AMR.

3 List of publications related to the PhD

1. Tóth A. G., Csabai I., Krikó E., Tőzsér D., Maróti G., Patai Á. V., Makrai L., Szita G., Solymosi N. (2020a): Antimicrobial resistance genes in raw milk for human consumption. *Scientific Reports*, 10(1). 7464.

2. Tóth A. G., Csabai I., Maróti G., Jerzsele Á., Dubecz A., Patai Á. V., Judge M. F., Nagy S. Á., Makrai L., Bányai K., G. Szita, N., Solymosi (2020b): A glimpse of antimicrobial resistance gene diversity in kefir and yoghurt. *Scientific Reports*, 10(1). 22458.

3. Tóth A. G., Csabai I., Judge M. F., Maróti G., Becsei Á., Spisák S., Solymosi N. (2021a): Mobile antimicrobial resistance genes in probiotics. *Antibiotics (Basel, Switzerland)*, 10(11). 1287.

4. Tóth A. G., Papp M., Jerzsele Á., Borbély F., Reibling T., Makrai L., Solymosi N. (2021b): Szoptató kocák bélsárrezisztomja egy hazai nagy létszámú sertésállományban. *Magyar Állatorvosok Lapja*, 143(4). 203-214.

5. Tóth A. G., Tóth I., Rózsa B., Dubecz A., Patai Á. V., Németh T., Kaplan S., Kovács E. G., Makrai L., Solymosi

N. (2022a): Canine saliva as a possible source of antimicrobial resistance genes. *Antibiotics (Basel, Switzerland)*, 11(11). 1490.

6. Tóth A. G., Judge M. F., Nagy S. Á., Papp M., Solymosi N. (2023a): A survey on antimicrobial resistance genes of frequently used probiotic bacteria, 1901 to 2022. *Eurosurveillance*, 28(14). 2200272.

7. Tóth I., Tóth A. G., Solymosi N. (2024a): Az új generációs genomszekvenálás lehetséges helye a kutyaharapások kezelésében. *Magyar Sebészet*, 77(4). 89–93.

8. Tóth A. G. (2021): A One Health approach study on alimentary products, sources in the process of animal-to-human antimicrobial resistance gene transfer. One Health Antimicrobial Stewardship Conference. Conference presentation. Alberta, Canada.

9. Tóth A. G., Tóth I., Rózsa B., Dubecz A., Patai Á. V., Németh T., Makrai L., Solymosi N. (2022b): Canine saliva: a possible interspecies medium for mobile antimicrobial resistance genes. 47th World Small Animal Veterinary Association Congress and XVIII FIAVAC Congress. Conference poster. Lima, Peru.

10. Tóth A. G., Tóth D. L., Remport L., Tóth I., Németh T., Dubecz A., Patai Á. V., Makrai L., Solymosi N. (2024b): A One Health approach metagenomic study on the antimicrobial resistance traits of canine saliva. Antimicrobial Resistance – Genomes, Big Data and Emerging Technologies Conference. Conference poster. Wellcome Genome Campus, Hinxton, UK.