Theses of doctoral (PhD) dissertation

THE CO-SELECTION OF RESISTANCE IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM POULTRY TO CRITICALLY IMPORTANT ANTIBIOTICS IN PUBLIC HEALTH

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1. Introduction and aims of research

The antimicrobial resistance has become one of the most significant global issues of the 21st century. A collaborative approach in the fields of animal and public health, applying the One Health principle, is necessary. The responsible use of antibiotics is in our common interest, and the impacts of resistance development and antibiotic use must be based on well-founded research results. Different active substances can exert stress-induced selective pressure on bacteria, leading to the development of various mutations. Our goal was to examine the cross-resistance relationship between different active substances using evolutionary and co-selection in vitro methods. Additionally, we assessed the sensitivity of Escherichia coli strains isolated from large-scale domestic chicken and turkey flocks to active substances of animal and public health significance, and we examined β-lactamase and extended-spectrum β-lactamase (ESBL) production and their public health implications.

The Microbial Evolution and Growth Arena (MEGA) plate is an in vitro system suitable for experimental evolutionary and selection studies, enabling the long-term examination of microorganisms exposed to increasing concentrations of active substances. This system allows for the study of evolutionary and co-selection processes in a closed environment without the need for repeated subculturing of bacteria. Resistant mutant strains stochastically emerge, allowing for a more balanced population dynamics. Following sampling from the developed resistant lines, their phenotypic and genotypic changes can be monitored.

We determined the phenotypic resistance expression in the context of the effects of the active substance itself and its effects on other active substances by determining the minimum inhibitory concentration (MIC) values for six different active substances. During our research, we examined the

phenotypic resistance expression to six different active substances by determining the minimum inhibitory concentration (MIC) values. Additionally, we conducted genomic studies to identify mutations in genes responsible for antimicrobial resistance. We also determined the sensitivity profile of poultry-derived E. coli strains collected nationwide, with particular attention to critically important active substances such as ceftriaxone, enrofloxacin, and colistin.

Our results underscore the importance of evolutionary and co-selection studies, as they provide deeper insights into the processes and correlations of resistance development. The results of phenotypic and genotypic studies also highlight the significance of regular surveys and monitoring studies.

2. Overview of new scientific results

We were the first in Hungary to implement the MEGA-plate methodology for evolutionary and co-selection studies of antimicrobial resistance. We applied this system for the first time to examine six active substances, authorized for use in poultry and significant from a public health perspective, using an ATCC Escherichia coli strain at 1×, 10×, 100×, and 1000× concentrations of its ¼ MIC value. This new methodology is suitable for studying the development of antimicrobial resistance and the cross-resistance correlations between individual active substances.

We were also the first in Hungary to conduct a comprehensive survey of the antimicrobial resistance status in large-scale domestic chicken and turkey flocks, comparing the results with public health sensitivity data, thereby establishing the "One Health" principle. This was further complemented by phenotypic and genotypic investigations focused on ESBL production.

Main findings of the study are as follows:

1. The adaptation and introduction of the MEGA-plate methodology into laboratory routine in Hungary.

2. Genetic mapping of the mutational selection pressure of various active substances used in veterinary and human medicine, observation of efflux pump activation, and SOS stress responses.

3. Assessment of the sensitivity of *Escherichia coli* strains to antibiotics of animal and public health significance in Hungary between 2022-2023.

4. Phenotypic examination of β-lactamase and ESBL production in *Escherichia coli* strains and the genetic mapping of these traits in strains isolated in Hungary between 2022-2023.

3. Own scientific publications related to the topic of the dissertation

Full text papers in peer-reviewed journals

Barnácz F., Kerek Á., Csirmaz B., Román I.L., Gál C., Hajduk E., Szabó Á., Jerzsele Á., Kovács L. (2024) **Antimikrobiális rezisztencia hazai nagylétszámú házityúkállományokban, hasznosítási irányok alapján 2022-2023 között**. *MAGYAR ÁLLATORVOSOK LAPJA* 146:339–356. https://doi.org/10.56385/magyallorv.2024.06.339-356

Kerek, Á., Török, B., Laczkó, L., Somogyi, Z., Kardos, G., Bányai, K., Kaszab, E., Bali, K., Jerzsele, Á. (2024). ***In Vitro* Microevolution and Co-selection Assessment of Amoxicillin and Cefotaxime Impact on *Escherichia coli* Resistance Development**. *ANTIBIOTICS*, 13 (3), 247. http://doi:10.3390/antibiotics13030247

Kerek, Á., Török, B., Laczkó, L., Kardos, G., Bányai, K., Somogyi, Z., Kaszab, E., Bali, K., Jerzsele, Á. (2023). ***In Vitro* Microevolution and Co-Selection Assessment of Florfenicol Impact on *Escherichia coli* Resistance Development**. *ANTIBIOTICS*, 12(12), 1728. http://doi.org/10.3390/antibiotics12121728

Kerek, Á., Török, B., & Jerzsele, Á. (2022). MEGA-plate – **Új evolúciós és koszelekciós mikrobiológiai vizsgálati módszer**. *MAGYAR ÁLLATORVOSOK LAPJA*, 144(7), 429–439.

Conference presentations

Kerek Á., Török B., Bányai K., Jerzsele Á.: ***In vitro* evolúciós és koszelekciós antimikrobiális rezisztencia vizsgálatok MEGA-plate segítségével *Escherichia coli* baktériumon: florfenikol antibiotikum hatása**. In *XXVII. Tavaszi Szél Konferencia 2024 - Absztraktkötet P. Hajdú, ed.,* Budapest: Doktoranduszok Országos Szövetsége (DOSZ). (pp. 689).

Kerek Á., Szabó K., Mag P., Jerzsele Á.: **Nagylétszámú baromfiállományokból izolált *Escherichia coli* törzsek kiterjedt spektrumú béta-laktamáz (ESBL) termelésének felmérése Magyarországon**. II. Magyar Agrártudományi Doktoranduszok Szimpóziuma, Doktoranduszok Országos Szövetsége (DOSZ), Budapest, Magyarország, 2024.

Kerek Á., Barnácz F., Csirmaz B., Somogyi Z., Jerzsele Á.: **Az antimikrobiális rezisztencia helyzetképe a nagylétszámú baromfiállományokban, kritikusan fontos antibiotikumok tükrében**. 39. Óvári Tudomány Nap Konferencia, Mosonmagyaróvár, Magyarország, 2023.

Kerek Á., Török B., Jerzsele Á.: **Microbial Evolution and Growth Arena (MEGA-plate), gyorsított evolúciós és koszelekciós vizsgálatok *Escherichia coli* törzzsel, kiterjedt spektrumú béta-laktamáz (ESBL) termelésre**. I. Magyar Agrártudományi Doktoranduszok Szimpóziuma, Doktoranduszok Országos Szövetsége (DOSZ), Debrecen, Magyarország, 2023.

Kerek Á., Szabó K., Bányai K, Jerzsele Á.: **Nagylétszámú baromfiállományokból izolált *Escherichia coli* törzsek ESBL termelésének felmérése Magyarországon**. MTA Akadémiai Beszámolók, Budapest, Magyarország, 2023.

Jerzsele Á., Kerek Á., Török B.: **MEGA-plate lemezen történő, gyorsított evolúciós antimikrobiális rezisztencia-koszelekciós vizsgálat**. MTA Akadémiai Beszámolók, Budapest, Magyarország, 2022.