

Summary of Ph.D. Thesis

**CHARACTERISATION OF LIVESTOCK-
ASSOCIATED METHICILLIN-RESISTANT
STAPHYLOCOCCUS AUREUS STRAINS
ISOLATED IN HUNGARY**

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1. Objectives of the study and premise

1.1 Introduction

Staphylococcus aureus is a worldwide facultative pathogenic bacterium, which may be carried asymptotically by 10–30% of the human and domesticated mammalian populations. The *S. aureus*-related syndromes range from benign skin and soft tissue infections to life-threatening necrotic pneumonia, endocarditis, and osteomyelitis. By the extensive use of antibiotics, the methicillin-resistant variants of *S. aureus* (MRSA) nowadays are among the most successful and best known multidrug-resistant pathogens. More recently, MRSA spreads rapidly in several species of domestic animals. Thus, the livestock-associated (LA-) MRSA belonging to the clonal complex (CC) 398 has become the most abundant lineage in Europe. This clonal lineage was found to be prevalent in pig herds in some European countries in the early 2000s and associated isolates were also recovered from diseased farm workers. Later, this lineage was proved to be of human origin, which retained its ability to successfully colonise and infect the human host.

1.2. Aims of the study

Since its first discovery in the early 2000s, studies assessing the prevalence and zoonotic potential of the LA-MRSA CC398 lineages were widely published from most part of Europe. While in Hungary, recent data on the occurrence and prevalence rates of LA-MRSA in different large animal species are still scarce. Hence, our **first goal was to investigate the occurrence and population genetics of MRSA in swine which is an important reservoir of LA-MRSA CC398** strains, and in other selected species in which staphylococci are considered as major pathogens (dairy cattle and horses).

Besides their veterinary importance, these strains pose potential human medical risk, and the re-adaptation of this lineage to the human host has already been suggested. Our **second aim was to assess the zoonotic potential of the recovered LA-MRSA CC398 isolates** by comparing animal and human originated strains, using an array of molecular epidemiologic tools.

The emergence of new resistance mechanisms poses a further risk in the case of pathogens which can colonise several host species, like *S. aureus*. The overlap between the antimicrobial usage of human and veterinary medicine

may give rise to the emergence of antimicrobial resistance genes between the different host species, through the interspecies colonisation with the pathogen, and horizontal gene transfer within the microbiota. Thus, **the third aim was to investigate the antimicrobial resistance profiles** of the isolates, focusing on resistances which are encoded on mobile genetic elements.

A special attention was dedicated to horses, in which species LA-MRSA CC398 emerged mainly as a nosocomial pathogen in Europe and means a severe challenge to veterinary medicine. Despite the relative long persistence of certain lineages in the European equine clinics, the occurrence and genetic relatedness of the Hungarian clinical and sub-clinically carried strains are yet not discovered. Our **fourth goal was to investigate the equine-related CC398 strains isolated in a 10-years period** in and outside the Department and Clinic of Equine Medicine, to have a better understanding of the clinic's role in the Hungarian equine-related MRSA epidemiology.

2. New scientific results of the study

2.1 Swine related MRSA

According to the European Food Safety Authority baseline study, in 2008, Hungary reported 3 positive farms out of the investigated 181 holdings. However, no such further official estimation of MRSA in pig settings has been made in the country since then. The sampling of 40 pig farms, in 2019, showed a dramatic increase in the occurrence of MRSA in the Hungarian swine industry, as 33 (83%) of them were tested positive. The key role of purchase networks – both domestic and transboundary ones– was reinforced in the spread of the pathogen, by the comparison of whole genome sequences, including other European isolates. Also, a high prevalence (70%) was observed in the investigated swine professionals (veterinarians and farm managers) working on these farms. The isolated LA-MRSA strains belonged exclusively to the livestock-associated clonal complex 398, and the WGS analyses revealed close relatedness between most farm isolates and isolates from swine professionals and diseased patients, pointing to the spill-over of the LA-MRSA to the human host. Besides their high zoonotic potential, the antibiotic resistance of these strains is also of concern. Half of the strains, isolated from pig farms,

showed resistance or a non-wild-type phenotype to at least six tested antibiotics (besides beta-lactams), and along with human isolates, they carried eight different types of multidrug-resistance genes, including *cf*, which encodes resistance to five classes of antimicrobials.

2.2 MRSA of bovine milk origin

In dairy cattle *S. aureus* is considered one of the most important bacteria causing contagious clinical or subclinical intramammary infection. However, the mastitis of MRSA origin, is an occasional observation and rarely causes a stock-level problem. The last domestic prevalence estimates on MRSA of dairy origin were made in the early 2000s and resulted in as low as 0-0.6%. In the frame of a passive surveillance, 626 individual *S. aureus* strains isolated from cow milk samples of 42 farms were collected and characterized between July 2017 and December 2018. The surveillance resulted in four (0.5%) MRSA CC398 strains from three (7.1%) farms, suggesting an unchanged occurrence and significance of MRSA of dairy origin in Hungary during the past decade. A retrospective investigation of the Veterinary Diagnostic Directorate's culture collection (Budapest, Hungary)

revealed 27 MRSA strains originating from ten dairy farms. The isolates belonged to various sequence types (STs) and clonal complexes (ST1, ST22, CC97, and ST398), of which all can colonize and infect the human host. Most investigated isolates were resistant to three or more antimicrobial classes, hence the low host specificity and multidrug-resistance of the strains calls for periodic revision on the role and distribution of the MRSA in the Hungarian dairy sector.

2.3 Equine MRSA

Equine MRSA was first recognized in the Department and Clinic of Equine Medicine (DCEM) of the University of Veterinary Medicine Budapest (Budapest, Hungary) in 2011 but related bacterial isolates have been collected and deposited in the culture collection of the Diagnostic Laboratory since 2009. By the molecular investigation of this collection series of outbreaks were identified between 2010 and 2018, caused by two distinct lineages of the equine hospital-associated (EHA-) MRSA CC398-t011-SCC_{mec}IV, dubbed as lineage 1 (L1) and lineage 2 (L2). Strains of L1 were totally replaced by that of L2 in 2011, and the latter gradually gained additional resistance and virulence genes while residing in the hospital environment,

most of them encoded on mobile genetic elements. Changes in the resistance pattern can be attributed to the introduction of the relevant agent into the hospital environment, and thus regarded as an evolutionary response to the selective pressure. The emergence of the equine specific leukocidin LukPQ within the clonal lineage, suggests a further step towards successful host adaptation. The same time-related pattern of microevolution was observed when MRSA strains from clinical specimens of other private veterinary practices, including a second equine clinic (Clinic 2), were analysed retrospectively from the timeframe of 2008-2018, suggesting a common origin of strains. Besides the genomic evidence, epidemiologic links could also be identified between the horses and equine clinics in some cases.

The observation of two apparently independent MRSA outbreaks in 2018, involving Clinic 2 and three related farms, and another in 2019 observed in a stud, resulted in the isolation of the same genetic lineage L2. Apart from these outbreaks, the pathogen is apparently rare in the horse population outside the clinic, as none of the 325 horses from 24 farms tested positive, and only 6% of 128 horses admitted to the clinic carried MRSA strains. Of

these, all but one belonged to L2, supporting the clonal uniformity of the Hungarian equine-derived MRSA strains further.

The caretakers and the environment of horses can act as a potent reservoir of EHA-MRSA. In both clinics and outbreak farms more than 20% of the staff carried the pathogen. The role of heavy contamination of the environment was underlined by the large proportion (47%) of MRSA positive horses leaving the DCEM.

The whole genome-based comparison of isolates to other European data reinforced the very close relatedness of the Hungarian L2 isolates. The results indicate a gradual adaptive microevolution of the clonal lineage taking place presumably in only a few epicentres, as well as its occasional spread from the epicentres into the horse population in Hungary. Such epicentres may include, besides the equine clinics, certain intensive horse-keeping establishments, e.g. studs, where the conditions required for the enrichment of the pathogen are present.

3. The publications by the author related to the topic of the thesis

Scientific publications in peer-reviewed journals that form the basis of the thesis

Albert, E., Sipos, R., Perreten, V., Tóth, Á., Ungvári, E., Papp, M., Dán, Á., Biksi, I.: High prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* in Hungarian pig farms and genomic evidence for the spillover of the pathogen to humans, *Transbound. Emerg. Dis.*, 2023. Article ID 5540019, 2023.

Albert E., Biksi, I.: Állati eredetű meticillin-rezisztens *Staphylococcus aureus* nagyállatokban és haszonállatokban – 1. rész Az MRSA előfordulása és jelentősége lovakban és a lovakkal kapcsolatban lévő emberekben (Irodalmi összefoglaló), *Magy. Állatorvosok Lapja*, 142. 503-512, 2020.

Albert, E., Sipos, R., Jánosi, S., Kovács, P., Kenéz, Á., Micsinai, A., Noszály, Z., Biksi, I.: Occurrence and characterisation of methicillin-resistant *Staphylococcus aureus* isolated from bovine milk in Hungary, *Acta Vet. Hung.*, 68. 236-241, 2020.

Albert, E., Biksi, I., Német, Z., Csuka, E., Kelemen, B., Morvay, F., Bakos, Z., Bodó, G., Tóth, B., Collaud, A., Rossano, A., Perreten, V.: Outbreaks of a methicillin-resistant *Staphylococcus aureus* clone ST398-t011 in a Hungarian equine clinic: emergence of rifampicin and chloramphenicol resistance after treatment with these antibiotics. *Microb. Drug Resist.*, 25. 1219-1226, 2019.

Conference oral presentations

Albert, E.: Experiences with methicillin-resistant *Staphylococcus aureus* derived from horses in Hungary, XXVII Annual Conference of the Hungarian Association of Equine Practitioners, Balatonkenese, Hungary, 2019.

Albert, E.: A multidrug-resistant bacterium in the Hungarian swine sector, XXVIII. Köves Napok, Egerszalók, Hungary, 2019.

Albert, E., Jánosi, S., Noszály, Z., Sipos., R., Erős, Á., Kelemenová., B., Biksi., I., Szenci, O.: Methicillin-resistant *Staphylococcus aureus* strains isolated from Hungarian dairy herds in the past 15 years, XVIII Middle-European Buiatrics Congress, Eger, Hungary, 2018.

Other publications related to the topic of the thesis

Albert, E., Sahin-Tóth, J., Horváth, A., Papp, M., Biksi, I., Dobay, O.: Genomic evidence for direct transmission of *mecC*-MRSA between a horse and its veterinarian, *Antibiotics*, 12. 408, 2023.

Sahin-Tóth, J., Albert, E., Juhász, A., Ghidán, Á., Juhász, J., Horváth, A., Steward, M. C., Dobay, O.: Prevalence of *Staphylococcus aureus* in wild hedgehogs (*Erinaceus europaeus*) and first report of *mecC*-MRSA in Hungary, *Sci. Total Environ.* 815. 152858, 2022.

Petróczki, F. M., Pásztor, Á., Szűcs, K. D., Pál, K., Kardos, G., Albert, E., Horváth, B., Ungvári, E., Béri, B., Peles, F.: Occurrence and characteristics of *Staphylococcus aureus* in a Hungarian dairy farm during a control program. *Pathogens*, 10. 104, 2021.

Német, Z., Albert, E., Dán, Á., Balka, G., Szenes, Á., Sipos, R., Bódizs, S., Biksi, I.: Genomic analysis of *Staphylococcus aureus* strains originating from Hungarian rabbit farms reinforce the clonal origin of various virulence types. *Animals*, 10. 1128, 2020.

Német, Z., Albert, E., Nagy, K., Csuka, E., Dán, Á., Szenci, O., Hermans, K., Balka, G., Biksi, I.: Virulence type and tissue tropism of *Staphylococcus* strains originating from Hungarian rabbit farms. *Vet Microbiol.*, 193. 1-6, 2016.