Zábolyová, N., Lauková, A., Troscianczyk, A., Pogány Simonová, M.

Detegovanie génov rezistencie na antibiotiká u kmeňov stafylokokov zo surového kozieho mlieka

Kozie mlieko

- https://pikbest.com/png-images/drink-goat-milk_9102390.html

Bielkoviny

- podobné množstvo ako kravské; zloženie odlišné
- menej α-S1-kazeínu (príčina alergií)

Tuky

- menšie tukové častice - lepšia stráviteľnosť
- vyšší obsah stredne dlhých MK (zdroj energie)

Sacharidy

- podobný obsah ako kravské mlieko
- ľudia s miernou neznášanlivosťou laktózy znášajú lepšie kozie

Vitamíny a minerály

- Ca - vysoký obsah
- K, Mg, Se - vyšší obsah ako kravské
- vit. A, B - viac A + niektoré zo skupiny B

Benefity kozieho mlieka

Lepšia stráviteľnosť:
menšie tukové častice

Menej alergénne:
nižší obsah alfa-S1-kazeínu

Nižší obsah laktózy:
niektorí ľudia s laktózovou intoleranciou ho znášajú lepšie

Nižšia kyslosť:
vyhovujúce najmä pre ľudí s tráviacimi ťažkosťami / prekyslením

Imunitný systém:
vyšší obsah selénu; podporuje imunitu

Kontaminácia mlieka

Hygiena zvierat:

- nečistoty na vemene, koži zvierat
- ochorenie - mastitídy

Prostredie a vybavenie:

- dojárenské zariadenia
- kvalita vody

Spracovanie a skladovanie:

- teplota (4°C a menej)
- tepelné spracovanie (pasterizácia)

Kontaminácia z prostredia

- prach, nečistoty, chemické kontaminanty

Krmivo, voda ...

Staphylococcus spp.

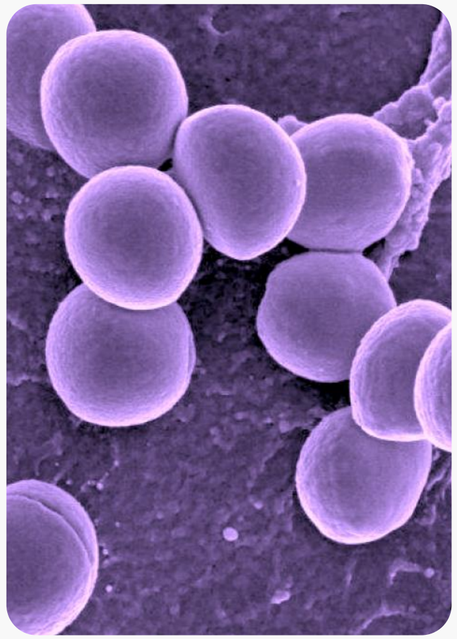
Ríša: Bacteria

Kmeň: Firmicutes

Trieda: Bacilli

Rad: Bacillales

Čeľaď: Staphylo-coccaceae



https://ianrnews.unl.edu/research-aims-prevent-resistance-staph-infection-treatment

Hindawi
International Journal of Food Science
Volume 2022, Article ID 4595473, 8 pages
https://doi.org/10.1155/2022/4595473

Research Article

Microbiome Associated with Slovak Raw Goat Milk, Trace Minerals, and Vitamin E Content

Andrea Lauková,[1] Lenka Micenková,[2] Ľubomíra Grešáková,[1] Michaela Maďarová,[1] Monika Pogány Simonová,[1] Valentína Focková,[1] and Jana Ščerbová[1]

[1]Centre of Biosciences of the Slovak Academy of Sciences, Institute of Animal Physiology, Šoltésovej 4-6, 040 01 Košice, Slovakia
[2]Recetox, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Correspondence should be addressed to Andrea Lauková; laukova@saske.sk

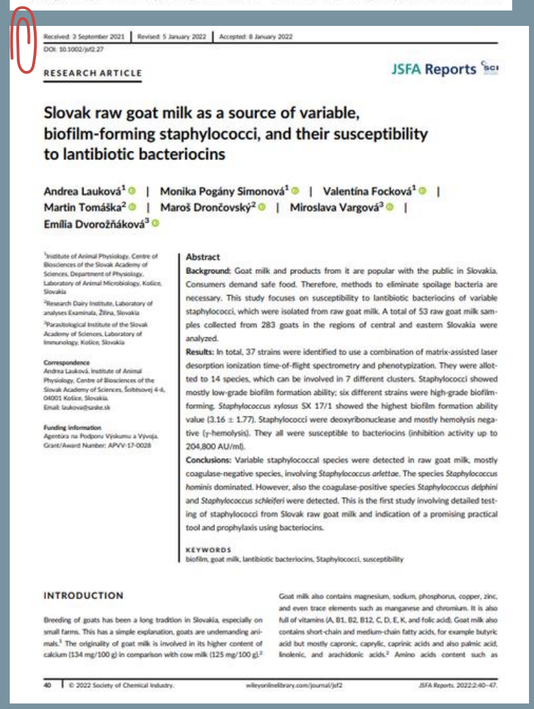
Received 25 April 2022; Revised 27 July 2022; Accepted 9 August 2022; Published 30 August 2022

Academic Editor: Parise Adadi

In Slovakia, goat milk production for direct consumption and cheese processing has attracted growing interest. However, there is a lack of information regarding the microbial consortium in Slovak raw goat milk analyzed by next-generation sequencing and trace elements and vitamin E as well. A randomly selected samples (G24-G50) of raw goat milk from different animals at farms in Slovakia were analyzed. The phylum Actinobacteria dominated (62.8%), followed by the phyla Firmicutes (20.5%), Proteobacteria (7.4%), and Bacteroidetes (6.4%). The family *Microbacteriaceae* was detected in the highest percentage (60.2%) followed by *Staphylococcaceae*, *Bacteroidaceae*, *Streptococcaceae*, *Lactobacillaceae*, *Enterobacteriaceae*, and others. Regarding the genera, the most prevalent was genus *Curtobacterium* (47.4%) followed by the genera such as *Staphylococcus* (8.3%) and *Bifidobacterium* (4%). The genera *Streptococcus*, *Lactococcus*, *Enterococcus*, *Lactobacillus*, and *Lacticaseibacillus* were evaluated in abundance percentage in range 1%-3.2%. The genus *Veillonella* reached abundance 3.2%. The genera *Enterobacter*, *Pseudomonas* (1.3% and 0.5%), and *Bacteroides* (6.4%) were evaluated in small percentage abundance too. Zinc was detected with the highest mean value (2.561 ± 0.6823 mg/L) in raw goat milk, followed by iron (1.383 ± 0.5087 mg/L). The mean value of copper and manganese was 0.1746 ± 0.0463 mg/L and 0.051 ± 0.0238 mg/L. The vitamin E reached the mean value 0.3783 ± 0.1976 mg/L. This study is an original contribution showing microbial consortium in raw goat milk from Slovak farms. It also contributes to trace elements and vitamin E status in raw goat milk showing it as a nutritionally healthy food.

1. Introduction

The breeding of goats has had a long tradition in Slovakia, especially on small farms. Moreover, goat breeding is often associated with agrotourism. Nowadays, goat milk production for direct consumption and cheese processing has attracted growing interest [1]. Goat milk is a highly nutritive product that has advantages over other milk. The immunoglobulin is more abundant (up to 80 μg/mL) than for example in breast milk [2]. In addition, the contents of protein (3.43%), fat (3.21%), minerals (up to 150 mg in case of Cl), vitamins (vitamin E, 0.4-0.11 mg/kg), and other nutrients of goat milk are much higher than other milk [3, 4]. Goat milk is, for example, more valuable in iron (Fe, 0.07-1.025 mg/L), copper (Cu, 0.05 mg and higher), zinc (Zn, 0.56 mg), and manganese (Mn, 0.032 mg) than, e.g., cow milk [5, 6]. However, mineral content can vary during the lactation period, which closely relates to the production season [7]. The content of vitamin E usually increases during the lactation period, e.g., up to 0.11 mg/kg [7]. Besides many general benefits, raw milk is also a beneficial medium for the growth of food-borne pathogens and spoilage psychrotrophic bacteria [8]. Although strict hygienic and zoohygienic conditions are kept during milk processing, spoilage microbiota, e.g., staphylococci, can contaminate goat milk because of farming production [9]. There exist studies mapping the occurrence of beneficial and/or spoilage bacteria in raw milks using standard microbiological analyses [9, 10, 11, 12, 13, 14] and also next-generation sequencing techniques [10, 14, 15]. However, regarding the microbial consortium



Received: 3 September 2021 | Revised: 5 January 2022 | Accepted: 8 January 2022
DOI: 10.1002/jsf2.27

RESEARCH ARTICLE

JSFA Reports

Slovak raw goat milk as a source of variable, biofilm-forming staphylococci, and their susceptibility to lantibiotic bacteriocins

Andrea Lauková[1] | Monika Pogány Simonová[1] | Valentína Focková[1] | Martin Tomáška[2] | Maroš Drončovský[2] | Miroslava Vargová[3] | Emília Dvorožňáková[3]

[1]Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Department of Physiology, Laboratory of Animal Microbiology, Košice, Slovakia
[2]Research Dairy Institute, Laboratory of analyses Examinala, Žilina, Slovakia
[3]Parasitological Institute of the Slovak Academy of Sciences, Laboratory of Immunology, Košice, Slovakia

Correspondence
Andrea Lauková, Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Šoltésovej 4-6, 04001 Košice, Slovakia.
Email: laukova@saske.sk

Funding information
Agentúra na Podporu Výskumu a Vývoja, Grant/Award Number: APVV-17-0028

Abstract

Background: Goat milk and products from it are popular with the public in Slovakia. Consumers demand safe food. Therefore, methods to eliminate spoilage bacteria are necessary. This study focuses on susceptibility to lantibiotic bacteriocins of variable staphylococci, which were isolated from raw goat milk. A total of 53 raw goat milk samples collected from 283 goats in the regions of central and eastern Slovakia were analyzed.

Results: In total, 37 strains were identified to use a combination of matrix-assisted laser desorption ionization time-of-flight spectrometry and phenotypization. They were allotted to 14 species, which can be involved in 7 different clusters. Staphylococci showed mostly low-grade biofilm formation ability; six different strains were high-grade biofilm-forming. *Staphylococcus xylosus* SX 17/1 showed the highest biofilm formation ability value (3.16 ± 1.77). Staphylococci were deoxyribonuclease and mostly hemolysis negative (γ-hemolysis). They all were susceptible to bacteriocins (inhibition activity up to 204,800 AU/ml).

Conclusions: Variable staphylococcal species were detected in raw goat milk, mostly coagulase-negative species, involving *Staphylococcus arlettae*. The species *Staphylococcus hominis* dominated. However, also the coagulase-positive species *Staphylococcus delphini* and *Staphylococcus schleiferi* were detected. This is the first study involving detailed testing of staphylococci from Slovak raw goat milk and indication of a promising practical tool and prophylaxis using bacteriocins.

KEYWORDS
biofilm, goat milk, lantibiotic bacteriocins, Staphylococci, susceptibility

INTRODUCTION

Breeding of goats has been a long tradition in Slovakia, especially on small farms. This has a simple explanation, goats are undemanding animals.[1] The originality of goat milk is involved in its higher content of calcium (134 mg/100 g) in comparison with cow milk (125 mg/100 g).[2] Goat milk also contains magnesium, sodium, phosphorus, copper, zinc, and even trace elements such as manganese and chromium. It is also full of vitamins (A, B1, B2, B12, C, D, E, K, and folic acid). Goat milk also contains short-chain and medium-chain fatty acids, for example butyric acid but mostly caproinic, caprylic, caprinic acids and also palmic acid, linolenic, and arachidonic acids.[3] Amino acids content such as

Lauková a kol. (2022) detegovali v surovom kozom mlieku 14 druhov stafylokokov vrámci 37 identifikovaných kmeňov (rod Staphylococcus bol detegovaný v úzkom percente abudancie)

Antibiotická rezistencia?

Neustále vzrastajúca antibiotická rezistencia bola v roku 2019 Svetovou zdravotníckou organizáciou zaradená medzi desať najväčších hrozieb pre globálne zdravie

IZOLÁCIA VZORIEK

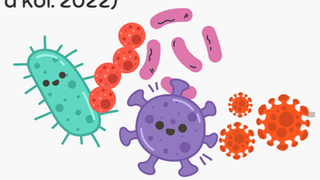
Vzorky boli izolované zo surového kozieho mlieka.

SPRACOVANIE

Spracovanie vzoriek prebehlo štandartnou mikrobiologickou metódou podľa ISO noriem

IDENTIFIKÁCIA A TESTOVANIE

Boli identifikované a testované na produkciu biofilmu a ďalšie vlasnosti (Lauková a kol. 2022)

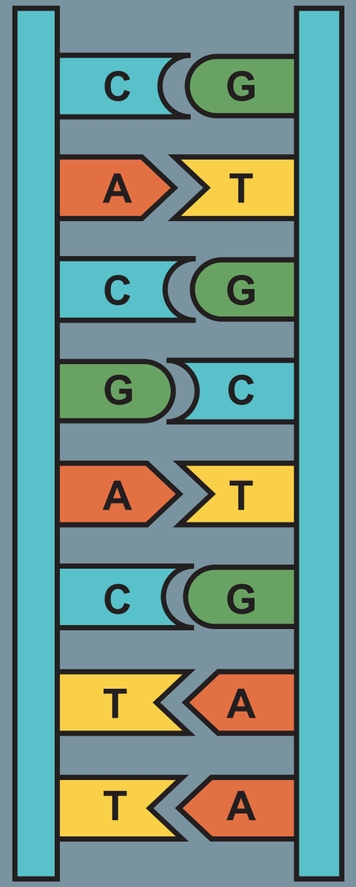


PRÍTOMNOSŤ GÉNOV?

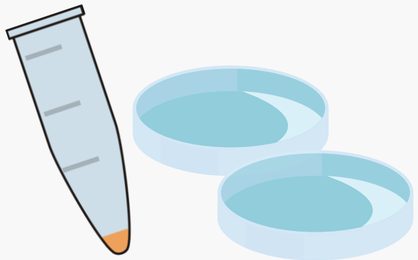
27 kmeňov stafylokokov bolo vybraných na testovanie prítomnosti génov pre antibiotickú rezistenciu

Testované gény

	Sekvencia primerov (5´-3´)	PB	Zdroj
*mec*A	F:TCCAGATTACAACTTCACCAGG R: CCACTTCATATCTTGTAACG	162	Oliveira a de Lencastre (2000)
*mec*C	F: TCACCAGGTTCAACYCAAAA R: CCTGAATCWGCTAATAATATTTC	356	Garcia-Alvarez a kol. (2011)
*bla*Z	F: ACTTCAACACCTGCTGCTTTC R: TGACCACTTTTATCAGCAACC	173	Martineau a kol. (2000)
*erm*A	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190	Strommenger a kol. (2003)
*erm*B	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	210	Strommenger a kol. (2003)
*erm*C	F: AATCGTCAATTCCTGCATGT R: TAATCGTGGAATACGGGTTTG	299	Strommenger a kol. (2003)
*tet*K	F: TTAGGTGAAGGGTTAGGTCC R: GCAAACTCATTCCAGAAGCA	647	Aarestrup a kol. (2000)
*tet*L	F: GTTGCGCGCTATATTCCAAA R: TTAAGCAAACTCATTCCAGC	456	Aarestrup a kol. (2000)



Materiál a metodika



Do skúmaviek Eppendorf bolo pridaných:

- 100 µL TE pufra (pH=8)
- 1,5 log baktérie

Mixovanie (vortex)

- 2 min

Zahrievanie (termoblok)

- 95°C/15 min.

Mixovanie (vortex)

- 2 min

Centrifuga

- 12 tis. otáčok /2 min

Supernatant

Materiál a metodika

Obsah vzorky

- 12,5 µL PCR Mix Plus (A&A Biotechnology, Poland)
- 1,0 µL priméru
- 6,5 µL vody
- 2,5 µL DNA (lyzát)

PCR reakcia

Amplifikačný program prebehol podľa programu zahŕňajúceho cykly:

- denaturácie,
- annealovania a
- predlžovania

Vizualizácia

PCR produkty sme analyzovali:

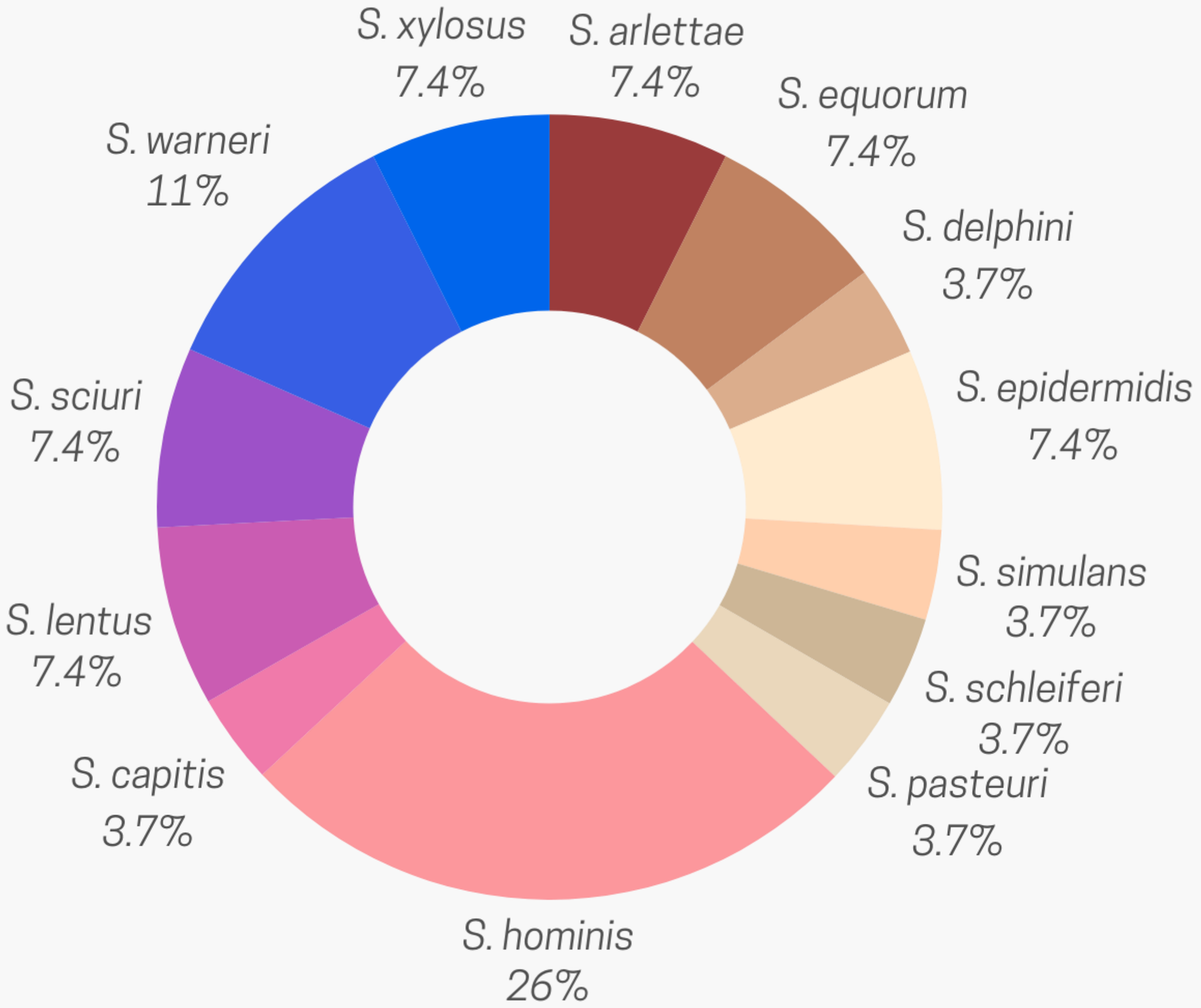
- elektroforézou na 1% agarózovom géli

Vizualizácia prebehla pomocou:

- GelDoc Go Imaging System (Bio-Rad Laboratories, USA).

Výsledky

- Pôvodný súbor stafylokokov (27)
- Najviac zastúpený - *S. hominis* (7)



Detegované gény

mecC

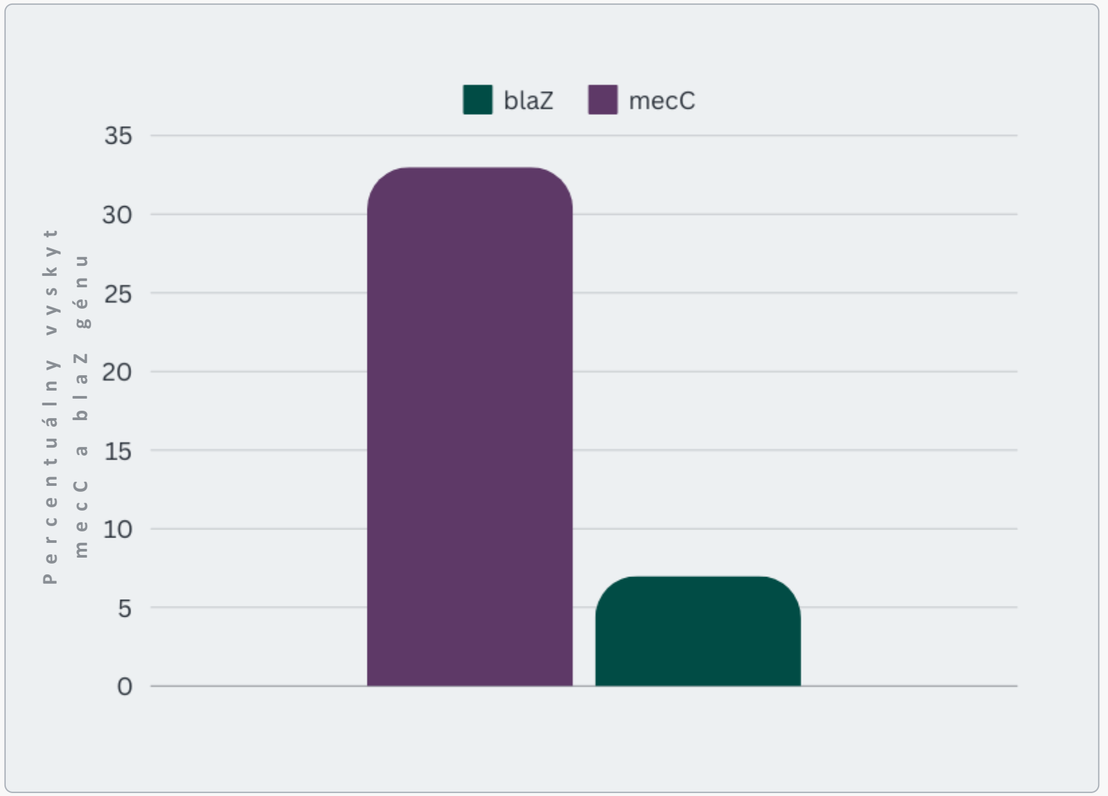
- kóduje meticilín-rezistentný proteín PBP2a
- znižuje afinitu stafylokokov k β-laktámovým antibiotikám
- alternatívny mechanizmus k *mec*A génu
- bežnejší v zvieracej ríši (kozie mlieko)

blaZ

- kóduje enzým β-laktamázu,
- penicilínová rezistencia
- na plazmidoch - horizontálny prenos
- rýchle šírenie v bakteriálnych populáciách

Výskyt génov rezsistencie u kmeňov stafylokokov izolovaných zo surového kozieho mlieka

kmene	*mecC* gén	*blaZ* gén
S. equorum Sq 32/2	+	-
S. hominis SHo 12/1	-	+
S. hominis SHo 31/1	+	-
S. capitis Sca 32/1	+	-
S. sciuri Sci 50/1	+	-
S. sciuri Sci 52/1	+	-
S. lentus SL 37/1	+	-
S. warneri SW 39	+	-
S. xylosus SX 1/2	-	+
S. xylosus SX 1/2	+	-



Záver

2 kmene koaguláza-pozitívnych stafylokokov

- SD30 a Ssch6/2
- bez výskytu génov

Koaguláza-negatívne stafylokoky

- prevaha *mec*C génu (meticilínová rezistencia)

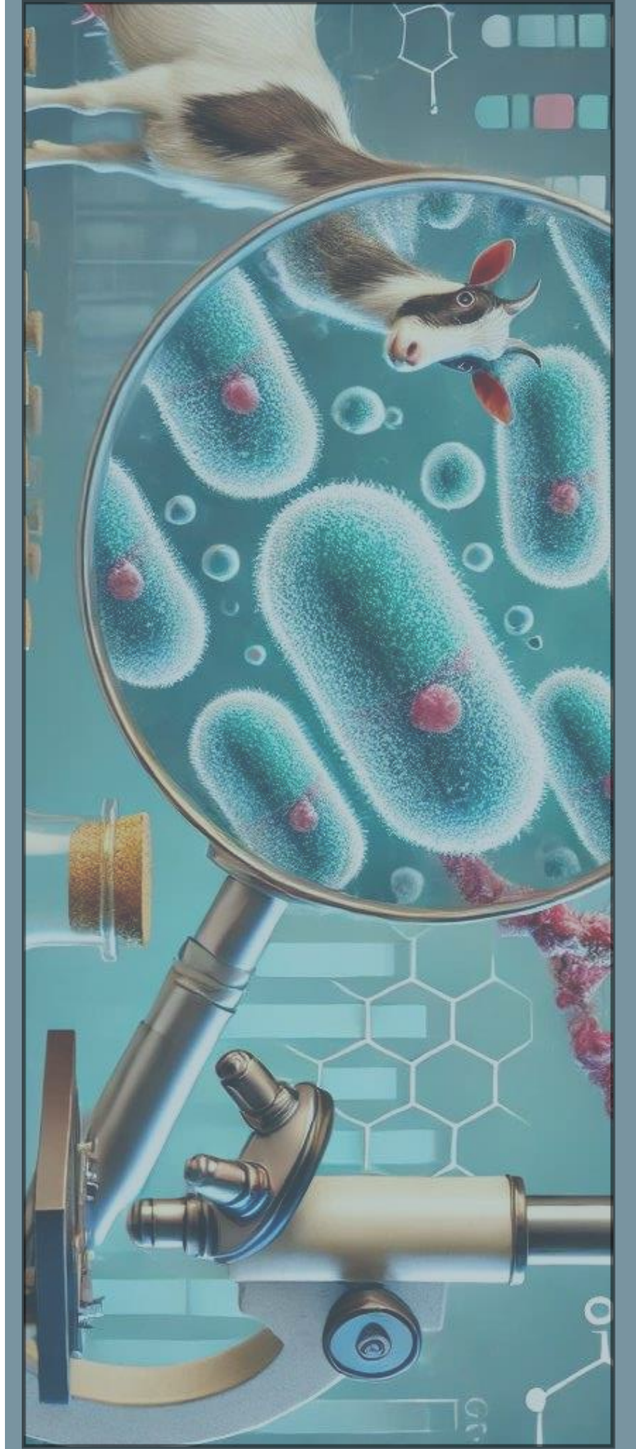
Prítomnosť génov druhovo nezávislá

Celková prítomnosť génov (<50%)

- detegované stafylokoky nepredstavujú striktné nebezpečenstvo

Používanie ATB v poľnohospodárstve

- rezistencia na ATB
- ohrozenie zdravia konzumenta
- riešenie = POSTBIOTIKÁ (BAKTERIOCÍNY)??



Ďakujem

za pozornosť