DATA BASIS FOR ALGORITHMIC MANAGEMENT OF ANTIMICROBIAL RESISTANCE IN BEEF PRODUCTION WITHIN AN INTEGRATED FARM SYSTEM

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Abstract: The aim of the study was to detect and characterize antimicrobial-resistant bacterial isolates from the beef production environment and to establish a data basis for an antimicrobial resistance algorithmic module integrated into farm management systems. Samples were collected from the caecum, cattle carcasses, and equipment used during slaughterhouse processing. Microorganisms were isolated according to relevant ISO standards, identified by 16S rRNA gene sequencing, and tested for antimicrobial susceptibility using the broth microdilution method in accordance with EUCAST guidelines. A total of 8 bacterial species were identified, with the highest occurrence of resistance observed in *Escherichia coli* from caeca. Two strains exhibited concurrent resistance to two antibiotics (AMP+TET; COL+ATM). Among staphylococci, resistance to clindamycin and erythromycin was detected, while *Enterococcus faecium* showed resistance to penicillin and ampicillin. The results confirm the importance of the gastrointestinal tract as a reservoir of resistance genes and highlight the role of equipment as a potential vector for transmission. The obtained data will be retrospectively integrated into an algorithmic module to support the rational use of antibiotics within integrated farm systems.

Keywords: antimicrobial resistance, bacteria, beef production, integrated farm system, slaughterhouse

MATERIAL AND METODOLOGY

1. Samples: four caecal samples were collected after evisceration, four swab samples from cattle carcasses, and four swab samples from equipment at the slaughterhouse

2. Culture-based microbiological examination according to ISO standards: **STN EN ISO 6887-1 (2024), STN EN ISO 6887-6 (2014), STN EN ISO 6888-1 (2022), STN EN ISO 7899-1 (2001), STN ISO 15214 (2002) and STN ISO 16649-2 (2001).**

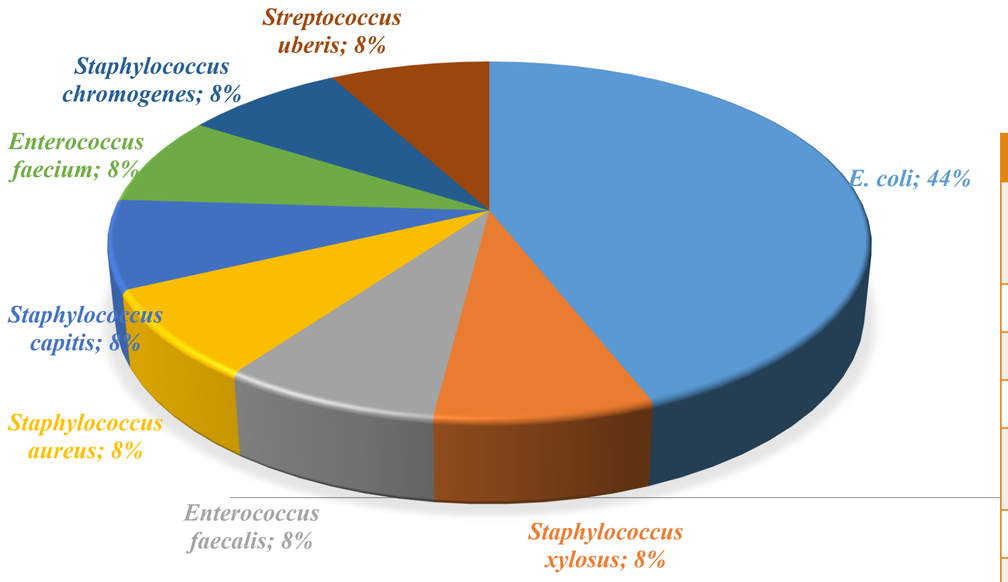
3. Bacterial species identification:

- DNA extraction using a modified method according to **De Bruin and Birnboim (2016).**
- Amplification**:** using a **CFX Opus 96 Real-Time PCR System** (Bio-Rad, USA) with **SYBR®Green Master Mix** (Bio-Rad, USA), primers **27F and 1492R** (the 16S rRNA gene) according to Frank et al. (2008).
- Sequencing analysis: The amplified PCR fragments were sequenced by a commercial company (SEQme s.r.o., Dobříš, Czech Republic). The obtained sequences were compared with those available in the GenBank—EMBL database using the National Center for Biotechnology Information (NCBI) BLAST database.

4. Antimicrobial susceptibility testing: microdilution method with using **the ErbaScan system** (Erba Mannheim, Mannheim, Germany) in combination with **ErbaExpert software** (Erba Mannheim, Germany).

RESULTS

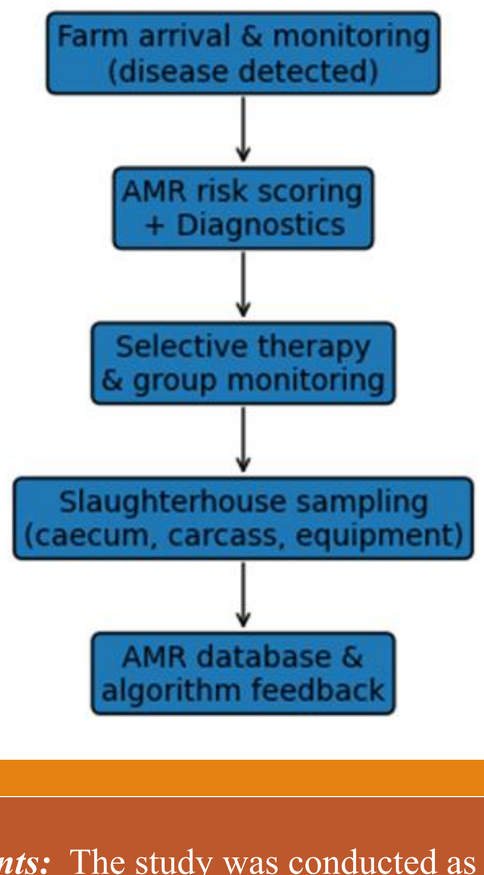
Species identification of bacterial isolates



Occurrence of antimicrobial-resistant bacterial isolates in beef production

Species	Sample	Antimicrobial resistance	MDR profile
E. coli	caecum	AMP (n=2), TET (n=1), COL (n=1), ATM (n=1)	AMP+TET (n=1), COL+ATM (n=1)
E. coli	equipment	no resistance detected	–
Staphylococcus aureus	equipment	CLI (n=1)	–
Staphylococcus capitis	equipment	ERY (n=1)	–
Staphylococcus chromogenes	carcass	no resistance detected	–
Staphylococcus xylosus	caecum	no resistance detected	–
Streptococcus uberis	carcass	no resistance detected	–
Enterococcus faecalis	caecum	no acquired resistance*	–
Enterococcus faecium	equipment	PEN, AMP (n=1)	PEN+AMP

Workflow for antimicrobial resistance monitoring from farm to slaughterhouse



CONCLUSION

The results of the study confirmed the presence of antimicrobial-resistant bacteria in various parts of the beef production chain, with the gastrointestinal tract identified as an important reservoir of resistance genes. The detection of multidrug-resistant ***Escherichia coli*** strains and a resistant ***Enterococcus faecium*** highlights the need for systematic AMR monitoring already at the level of primary production, while slaughterhouse equipment may represent an important factor in the transmission of resistant bacteria during carcass processing. The integration of the obtained microbiological data into an algorithmic module will enable comprehensive monitoring of the relationships between animal health, antibiotic use and the occurrence of resistant bacteria, thereby supporting the rational use of antimicrobials and contributing to improved beef production safety in accordance with the One Health concept.

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