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Course Code: 521



Phenotypic and genotypic resistance to antibiotics in *Staphylococcus aureus* strains isolated from cattle milk in Northern Kazakhstan

Section: 1

Introduction (Renad Binshonar: 446200355)

Mastitis in Kazakhstan:

- A significant infection in dairy cows caused by *Staphylococcus aureus*.

Challenge in Management:

- Difficulty in managing the disease due to a lack of clear information on antibiotic resistance patterns, making treatment challenging.

Impact of Antibiotic Use:

- The overuse of antibiotics in farming contributes to increased resistance.

Need for Understanding Resistance:

- To improve treatment and prevention strategies, a better understanding of antibiotic resistance mechanisms is essential.

Phenotypic Methods:

- These methods can detect whether bacteria are resistant but do not identify the specific enzymes responsible for resistance.

Genetic Methods:

- These methods can pinpoint the exact genes that cause resistance in bacteria.

Temporary Resistance Factors:

- Bacteria may exhibit temporary resistance due to environmental factors or the presence of biofilms.

Study Objective:

- The study aims to analyze both phenotypic and genetic resistance in *Staphylococcus aureus* from cow's milk to improve disease management practices.

Section: 2

Materials and Methods (Atiqa Riaz: 445206492)

Ethical Approval:

The study was approved by the Local Ethical Committee of the Research Institute of Applied Biotechnology of Kostanay Regional University (Protocol No. 1, May 19, 2020).

Study Period and Location:

Conducted from 2021 to 2022 across 16 dairy farms in Northern Kazakhstan. Analyses were performed at the Research Institute of Applied Biotechnology, Kostanay Regional University.

Sample Collection and Examination:

- Collected 675 milk samples from cows with clinical and subclinical mastitis.
- Isolated 87 *Staphylococcus aureus* strains. Identification utilized bacteriological methods and molecular genetic techniques.

Microbiological Methods:

- Used media such as egg yolk high salt agar, Baird–Parker agar, and CHROMagar Mastitis.
- Biochemical identification was performed with STAPHYtest systems. Final identification was done by real-time PCR (RT-PCR) and 16S RNA gene sequencing.

Antibiotic Susceptibility Testing:

- Tested antibiotic resistance using the disk diffusion method on Mueller-Hinton agar.
- Antibiotics tested included ampicillin, amoxicillin, cefoperazone, ceftiofur, and others.

Resistance Gene Identification:

- Extracted genomic DNA and used PCR to identify resistance genes: blaZ (β -lactam), ermC (macrolides), and tetK (tetracyclines).

- Amplification involved a 20- μ L reaction mixture with specific primers and probes.

Statistical Analysis:

- Calculated diagnostic sensitivity, specificity, and predictive values to assess the performance of disk diffusion compared to PCR.
- Kappa index was used to measure agreement between disk diffusion and PCR results.

This study aimed to analyze the phenotypic and genotypic characteristics of *Staphylococcus aureus* in mastitic milk and assess the effectiveness of antibiotic susceptibility testing methods.

Section: 3

Results (Noura Alshwieer: 446200069)

Antibiotic Resistance in *Staphylococcus aureus* Study Findings:

Through studying the genetic and phenotypic resistance of *Staphylococcus aureus*, it was revealed that all strains were resistant to multiple antibiotics, highlighting increasing challenges in infection control.

nuc Gene Detection:

- Universally found in all 87 *Staphylococcus aureus* isolates through RT-PCR.

blaZ Gene and Resistance:

- Found in 40 isolates, with 37 displaying benzylpenicillin resistance.
- The blaZ gene was not detected in nine *Staphylococcus aureus* isolates exhibiting resistance to benzylpenicillin.

Diagnostic Metrics for blaZ PCR:

- Sensitivity: 80.43%
- Specificity: 92.68%
- Positive Predictive Value (PPV): 92.50%
- Negative Predictive Value (NPV): 80.85%
- Kappa Value: 0.73 (discrepancies in classification)

ermC Gene and Resistance

There was a significant difference between PCR and the DD method in detecting the ermC gene. Out of 67 *Staphylococcus aureus* isolates, ermC was found. Macrolide resistance was detected in 53 isolates (32 to erythromycin, 28 to tylosin) using DD. 14 ermC-positive

isolates showed no phenotypic erythromycin resistance, while 12 tylosin-resistant isolates lacked *ermC* gene detection.

Diagnostic Metrics for *ermC* Gene:

- Sensitivity: 81.54%
- Specificity: 36.36%
- PPV: 79.1%
- NPV: 40%
- Kappa Value: 0.18 (significant discrepancy)

Prevalent Resistances:

β -lactam and Tetracycline Resistance:

- Varied patterns within tetracycline-resistant isolates.

***tetK* Gene and Tetracycline Resistance:**

- Absent in 6 tetracycline-resistant and 4 doxycycline-resistant isolates.

Diagnostic Metrics for *tetK* PCR:

- Sensitivity: 87.95%
- Specificity: 100%
- PPV: 100%
- NPV: 28.57%
- Kappa Value: 0.40 (significant discrepancy)

Section: 4

Conclusions (Fatimah Alfouzan: 446200107)

Global Spread of Antibiotic Resistance

- The resistance of infectious pathogens to antibiotics has rapidly spread globally, including in Kazakhstan.

Detection of *Staphylococcus aureus* Isolates

- 100% of *Staphylococcus aureus* isolates tested positive for the *nuc* gene, indicating high specificity in detecting *Staphylococcus aureus* using both microbiological and PCR methods.

Resistance to β -lactam Antibiotics

- High phenotypic resistance to β -lactams was observed, with 45.9% of isolates carrying the blaZ gene. Some discrepancies were found between phenotypic and genotypic results, indicating other resistance mechanisms might exist.

Cefoxitin Testing for MRSA

- Cefoxitin disk diffusion (DD) testing is highly sensitive for detecting MRSA. However, some isolates carried the blaZ gene without showing resistance, suggesting inconsistencies in detection

Resistance to Macrolides

- 60.9% of isolates were resistant to macrolides, and the ermC gene was detected in 77% of these. Some ermC-positive isolates were not erythromycin-resistant, indicating the potential presence of other resistance genes like ermA and ermB.

Resistance to Tetracycline

- 95.4% of isolates were resistant to tetracyclines, with the tetK gene detected in most of these isolates. Some tetracycline-resistant isolates did not carry tetK, suggesting other determinants like tetL, tetM, or tetO might be involved.

Comparison between DD and PCR Methods

- Resistance rates were generally higher using the DD method compared to PCR-based detection of genes. This suggests that other resistance mechanisms not investigated might be contributing to the observed resistance.

Presence of Resistance Genes without Phenotypic Resistance

- The study found cases where resistance genes were present without corresponding phenotypic resistance, implying that genetic resistance is only one of several mechanisms contributing to antibiotic resistance in *Staphylococcus aureus*.

Abbreviations	Explanation
PCR	Polymerase Chain Reaction
RT-PCR	Real Time Polymerase Chain Reaction
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
blaZ	A <i>Staphylococcus aureus</i> beta-lactamase gene
ermC	An inducible antibiotic resistance gene from <i>Staphylococcus aureus</i>
tetK	Tetracycline resistance gene
μ L	Microliter
β -lactam	Beta-lactam
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
DD	Disk Diffusion