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ANTIBIOTIC RESISTANCE OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM ANIMALS AND BIRDS IN THE TERRITORY OF KOSTANAY REGION

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Abstract

This article reveals the features of the resistance of *Staphylococcus aureus* strains isolated from animals, birds and animal products in the territory of Kostanay region. *Staphylococci* can infect any tissue or organ of an animal, causing more than 100 different diseases. According to literary sources, more than a dozen species of non-pathogenic or conditionally pathogenic *staphylococci* are isolated from many agricultural and domestic animals and birds. For the first time in the Kostanay region, the analysis of antibiotic resistance of *staphylococcus* strains isolated in livestock farms and animal products was carried out.

The analysis of antibiotic resistance of *staphylococcus* strains isolated in livestock farms of Kostanay region and animal products was carried out. The study showed that the largest number of isolates of *Staphylococcus aureus* showed high resistance to beta-lactam antibiotics by an average of 55.3%, fluoroquinolones by 47.1% and tetracyclines by 45.6%.

Of the 69 studied isolates of *Staphylococcus aureus*, antibiotic resistance was shown by 43 (62.3%) isolates, 26 (37.7%) isolates were sensitive to all groups of AMD.

It was found that the largest number of isolates is 62.3% in the group of beta-lactams resistant to various antibiotics, the least resistant strains are 7.9% in the group of aminoglycosides and 20.3% in the group of sulfonamides.

Key words: antibiotics; bacteria; microbiology; sensitive; *staphylococci*; strains; resistance.

Introduction

Resistance is primarily referred as the ability of germs to tolerate therapeutic doses of antibiotics, sulfonamides, and nitrofurans, which would normally be fatal to other microbes.

The bacterial genome undergoes spontaneous changes, which promote the creation of resistant strains of microorganisms. The most recent research indicates that selective agents also have a role in the development of antibiotic-resistant bacteria, and that their connection to the DNA is not the only cause. Chemotherapeutic medications cause the death of susceptible bacteria during the selection process, whereas resistant germs survive, proliferate, and spread. Future bacterial generations encounter a barrier when acquired resistance becomes established. The kind and strain of the microbe determine the speed and stability of its development. development of the most *Staphylococci*, *Escherichia coli*, *mycoplasma*, *proteus*, and the blue pus bacillus are among the organisms that exhibit rapid and considerable antibiotic resistance [1, 3, 8].

Staphylococcus methicillin-resistant (MRS) is a marker for resistance to all lactam antibiotics, with vancomycin having the highest therapeutic importance. There are various subgroups of β -lactam antibiotics, a significant class of antibiotics used in veterinary medicine. Based on their methods of resistance, β -lactam antibiotics can be divided into four major categories: penicillin, cephalosporins, monobactams, and

carbapenems. The susceptibility of lactam antibiotics is closely related to their susceptibility, and their efficiency is negatively correlated with their propensity to produce resistance [2, 4, 5].

It is determined which antibiotic classes, such as aminoglycosides, tetracyclines, macrolides, and fluoroquinolones, isolates are susceptible to. Most isolates have been found to be susceptible to rifampicin (6.0%) and trimethoprim-sulfamethoxazole (20–40%).

Methicillin-resistant *Staphylococci* were mostly investigated as pathogens in hospital acquired illnesses in recent years; however, this scenario has changed as these pathogens are spreading more and more in the population. They contribute to serious diseases arising from animals and items generated from animals by infecting both people and animals. This community is currently a major source of infections, acting as reservoirs in two instances. Since their initial discovery in the early 1980s, the incidence of MRSA infections has increased over time [7, 9].

Objectives: To isolate *Staphylococcus aureus* strains from animals, birds, and products derived from them in the Kostanay region and conduct phenotypic characterization, as well as assess susceptibility to antibiotics and the prevalence of resistant and multidrug-resistant *Staphylococci*.

Materials and Methods

The Microbiology Laboratory of the Institute of Biotechnology at Kostanay A. Baitursynov State University conducted microbiological research from 2021 to 2023. Samples comprised milk and milk-derived

products, animal and avian biological components, and animal-derived goods.

A 3% solution of erythritol salt, plasma from JSC "NPO Mikrogen," a combination of stains for Gram staining, and control strains (*S. aureus* ATCC 25923, *S. aureus subsp. aureus* ATSS 6538) were used to adapt the isolated strains. The following selective media were used in tests to identify staphylococci: salt agar, mannitol salt agar, milk-salt agar, mannitol-mannitol agar, Baird-Parker agar, CHROMagar Mastitis, CHROMagar, France, and blood agar, HiMedia, India. The study of antibiotic resistance by the Disc-diffusion method was conducted in the Muller-Hinton environment (Research Center of Pharmacotherapy, St. Petersburg).

Utilizing the "Staphy-test" test systems, biochemical validation of isolates was done (ERBA Lachema, Czech Republic). Traditional microbiological techniques were used to determine the biological properties of staphylococci.

- Disk diffusion was used to test the antibiotic susceptibility (Pasteur Epidemiology and Microbiology Research Institute, St. Petersburg). The following antibiotics were tested: ampicillin (10 µg), amoxicillin (25 µg), benzylpenicillin (10 IU), streptomycin (10 µg), cefoperazone (75 µg), cefoxitin (30 µg), kanamycin (30 µg), neomycin (30 µg), gentamicin (120 µg), tetracycline (30 µg), doxycycline (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), erythromycin (15 µg), tylosin (15 µg), trimethoprim-sulfamethoxazole (1.25/23.75).

Equipment includes a drying cabinet (VK-75-01), an incubator, a thermostat (TC-1/80SPU), analytical balances (Precisa), adjustable volume

pipettes (1 - 1000 l), and an OPTIKA B510BF binocular microscope.

Direct staining and gram staining were done on colonies made from cultured material. The smears, which were created as a thin layer, were used to identify gram-positive cocci. Onto salt agar were first streaked all of the samples. For 24-48 hours, the tubes were incubated at 37°C. The colonies were then harvested from the incubator and placed on specialized diagnostic media, including milk-salt agar, egg yolk mannitol salt agar, Baird-Parker agar, Chromagar Mastitis, and blood agar. The potential for plasma to coagulate allowed researchers to identify the coagulase activity of bacteria. It was confirmed that staphylococci grew on salt agar. The colonies were then transferred to blood agar and streaked with mannitol agar or milk-salt agar. Staphylococci colonies began to form after 24-48 hours of incubation at 37°C with samples in petri plates. Convex, 2.0–2.5 mm in diameter, and colored yellow, golden, lemon yellow, light green, white, or translucent, *Staphylococcus* colonies on mannitol agar. A method called disk diffusion methods (DDM) was employed to test the antibiotic susceptibility.

The cultured microorganism's suspension (also known as the inoculum) was made. Determining the suspension standard of the bacterial growth is one of the crucial steps in all test techniques. The bacterial suspension standard should have a concentration of 1.5–10 CFU/mL. The Biosan DEN-1 densitometer was used to calculate the optical density. The basis for the instrument's operation is the measurement of optical density, and the

output is then displayed in McFarland units.

Colony-forming units (CFU) of sterile isolates were modified using sterile isotonic saline to match the McFarland turbidity criterion of 0.5 for the creation of the inoculum. Direct suspension of colonies in sterile bacteriological saline was the technique employed. A number of the colonies that multiplied on the agar after 24 hours after being suspended in sterile bacteriological saline were chosen. Based on their morphological traits, similar colonies were grouped together. Within 15 to 20 minutes, the collected material was used after being suspended in sterile isotonic saline.

DDM, or disk diffusion method. Mueller-Hinton agar was made in accordance with the directions. The thickness of the agar layer in the Petri

dish is one of the crucial details in figuring out the sensitivity of DDM. The agar layer should evenly cover the bottom of the Petri dish and be about 4 mm thick (plus or minus 0.5 mm). Before setting the plates down, the prepared agar needs time to firm. There shouldn't be any apparent condensation on the inside of the lid or the agar surface; it should be smooth and even.

The plates were slanted prior to incubation to prevent the disks from coming away from the agar surface. The plates were incubated at +37°C for 24 hours after the antibiotic disks were placed and left in place for 15 minutes. After incubation, the locations where the antibiotics had prevented microbial growth had been seen. This demonstrates the microorganism's susceptibility to the evaluated antibiotic (see Figure 1 in the source).



Figure 1 - Identification of susceptibility to antibacterial agents with disk diffusion method.

This report outlines the outcomes obtained through the disk diffusion method, a means of identifying the responsiveness of bacteria to various antibiotics. Following the completion of the incubation period, Petri dishes were positioned upside down and examined against a dark backdrop to count bacterial colonies. A uniform and continuous layer of bacterial growth was observed on the agar surfaces. The area around the antibiotic disks displayed a clearly defined inhibition zone. The size of these zones, indicative of bacterial growth inhibition, was measured in millimeters using a caliper (see Figure 2).

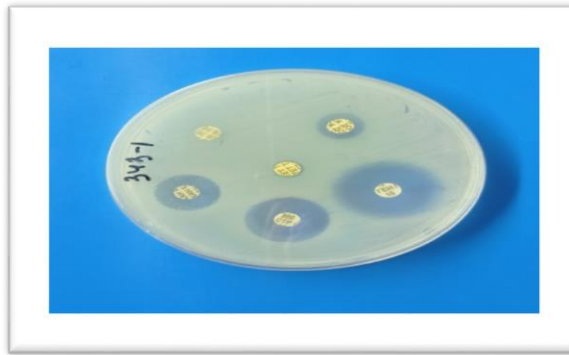


Figure 2 - Inhibition Zones of *Staphylococcus* Growth by Antibiotics

Results

In accordance with the criteria set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 11.0 [10, 12], the Clinical and Laboratory Standards Institute (CLSI) [11, 13], and the "results of susceptibility testing to antimicrobial agents using a collection of discs," the following observations were made [14].

Research Results: A total of 69 isolated strains of *Staphylococcus aureus* underwent testing for their susceptibility to various categories of pharmaceutical agents, which included:

- β -lactam antibiotics (ampicillin, amoxicillin, benzylpenicillin, cefoperazone, cefoxitin).
- Aminoglycosides (streptomycin, kanamycin, neomycin, gentamicin).
- Tetracyclines (tetracycline, doxycycline).
- Macrolides (erythromycin, tilosin).

- Fluoroquinolones (ciprofloxacin, norfloxacin).
- Sulfonamides (trimethoprim/sulfamethoxazole).

Previous studies have consistently indicated that *S. aureus* is among the microorganisms with a notably high resistance to antibiotics [2, 3]. If *S. aureus* continues to prevail in medical settings, it signifies that the issue of drug resistance in *Staphylococcus* reservoirs is increasingly pressing, and current infection control measures are insufficient to curb the proliferation of these bacteria. Our conducted research provides further evidence of this reality.

Based on the results of testing the isolated strains of *Staphylococcus aureus*, the highest proportion of isolates exhibited resistance to amoxicillin - 41 (59.4%), ampicillin - 39 (56.2%), cefoxitin - 38 (55.1%), cefoperazone - 37 (53.6%), benzylpenicillin - 36 (52.2%), ciprofloxacin and tetracycline - 34 (49.2%), norfloxacin and tilosin - 31 (44.9%) (see Table 1).

Table 1 – Antibiotic Susceptibility of *S.aureus* Strain

| Name | ABD drugs | Name of the ABD | Number of | Number of | Group average % |
|------|-----------|-----------------|-----------|-----------|-----------------|
|------|-----------|-----------------|-----------|-----------|-----------------|

| | | | resistant strains | resistant strains% | |
|----------------|------------------|--------------------|-------------------|--------------------|----------|
| S. aureus n=69 | β-lactams | ampicillin | 39 | 56,2±3,64 | 55,3±3,7 |
| | | amoxicillin | 41 | 59,4±3,4 | |
| | | benzylpenicillin | 36 | 52,2±4,0 | |
| | | cefoperazone | 37 | 53,6±3,88 | |
| | | cefoxitin | 38 | 55,1±3,76 | |
| | amino-glycosides | streptomycin | 6 | 8,7±7,64 | 7,9±5,5 |
| | | kanamycin | 5 | 7,2±7,76 | |
| | | neomycin | 0 | 0,0 | |
| | | gentamicin | 11 | 15,9±7,03 | |
| | tetracyclines | tetracycline | 34 | 49,2±4,24 | 45,6±4,5 |
| | | doxycycline | 29 | 42,0±4,85 | |
| | macrolides | erythromycin | 27 | 39,1±5,09 | 42,0±4,8 |
| | | tylosin | 31 | 44,9±4,61 | |
| | sulfonamides | sulfamethoxazole / | 14 | 20,3±6,67 | 20,3±6,6 |
| | fluoroquinols | trimethoprim | 34 | 49,2±4,24 | 47,1±4,4 |
| | | ciprofloxacin | 31 | 44,9±4,61 | |

Looking at the table, it's evident that most *Staphylococcus aureus* isolates displayed the highest resistance to β-lactam antibiotics, with 55.3% resistance, followed by fluoroquinolones at 47.1%, and tetracyclines at 45.6%.

Out of the 69 *Staphylococcus aureus* isolates subjected to testing, 43 (62.3%) exhibited resistance to antibiotics, while 26 (37.7%) isolates remained susceptible to all categories of antibiotics.

Among the different antibiotic classes, the lowest level of resistance was observed in the aminoglycosides group at 7.9%, and sulfonamides at 20.3%.

Among the 43 isolates showing resistance, 41 (95.3%) *Staphylococcus aureus* isolates displayed resistance to the β-lactam antibiotic class. Within this

category, 5 (12.2%) isolates were resistant to a single antibiotic, 13 (36.1%) were resistant to two antibiotics, and 14 (34.1%) were resistant to three antibiotics. Additionally, 5 (12.2%) isolates were resistant to four antibiotics, and 4 (9.8%) isolates were resistant to all five antibiotics within the β-lactam class.

Furthermore, among the identified resistance patterns, 7 (16.3%) isolates out of the 43 *Staphylococcus aureus* isolates exhibited resistance to a single β-lactam class, 12 (27.9%) isolates were resistant to two β-lactam classes, 9 (20.9%) isolates were resistant to three β-lactam classes, 7 (16.3%) isolates were resistant to four β-lactam classes, and 5 (11.6%) isolates showed resistance to all six groups (see Figure 3).

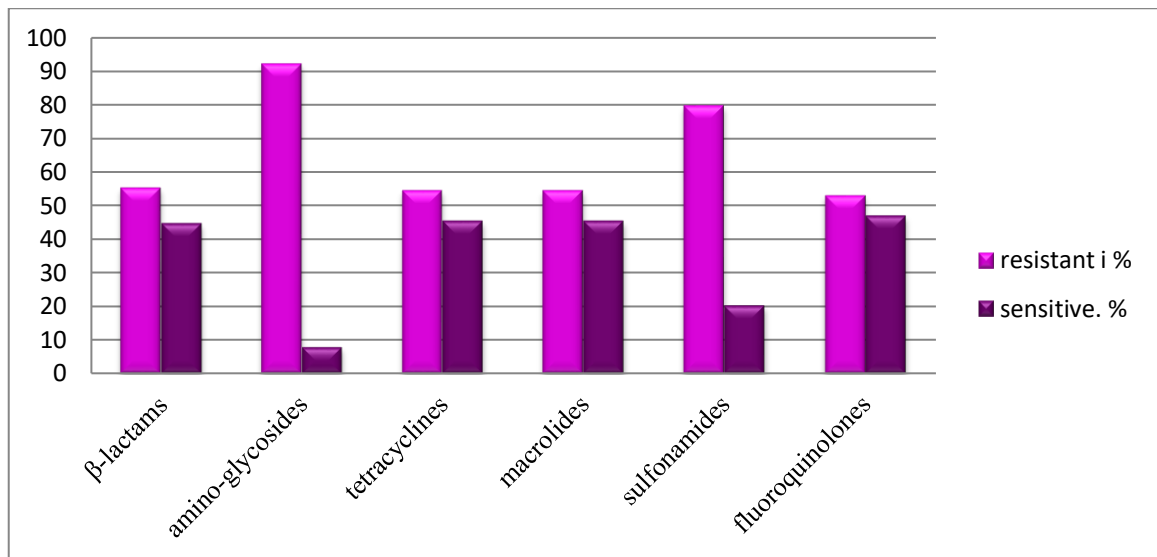


Figure 1- Correlation between the Susceptibility and Resistance of *S. aureus* Strains to Different Antibiotic Categories (%)

Discussion

Coagulase-negative staphylococci do not have the same pathogenicity as *Staphylococcus aureus*, but, according to researchers, they are highly resistant to antibacterial drugs. Coagulase-negative staphylococci act as an important reservoir of mobile genetic elements associated with resistance, which contribute to the rapid horizontal transfer of antimicrobial resistance genes and resistance genes between staphylococcus species.

Conclusion

A research investigation was carried out to evaluate how *Staphylococcus aureus* strains, collected from animals, birds, and agricultural products in the Kostanay region, respond to antibiotics. The study showed that the largest number of isolates of *Staphylococcus aureus* showed high resistance to beta-lactam antibiotics by an average of 55.3%, fluoroquinolones by 47.1% and tetracyclines by 45.6%.

Of the 69 studied isolates of *Staphylococcus aureus*, antibiotic resistance was shown by 43 (62.3%) isolates, 26 (37.7%) isolates were sensitive to all groups of AMD.

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Information on financing

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