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Research Article

IN-VITRO INVESTIGATION OF DONKEY MILK EFFICACY AGAINST STANDARD STAPHYLOCOCCUS AUREUS STRAINS

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Abstract: *Donkey milk, which is very similar to breast milk in terms of protein profile and lactose content, has been used for therapeutic purposes since ancient times. Different studies report its antimicrobial activity against an important human pathogen such as S. aureus. Therefore, in our study, we aimed to examine the antimicrobial efficacy of donkey milk against standard MRSA and MSSA strains in-vitro environment. In our study, in-vitro antimicrobial efficacy of donkey milk obtained from 53 different donkeys against S. aureus ATCC 43300 (MRSA) and S. aureus ATCC 29213 (MSSA) was investigated by broth microdilution method. Donkey milk mixed with TSB medium at different serial dilution rates was studied in 96 microwell plates. When the effectiveness of different concentrations of donkey milk added to TSB medium in vitro against ATCC MRSA and MSSA strains was examined, no suppression was observed, and growth was found to be higher than the control results after 24 hours of incubation at high milk concentrations. In conclusion, according to the results of our study, no antimicrobial effect of donkey milk was observed in-vitro against standard MRSA and MSSA strains. This situation may be caused by the fact that S. aureus strains may have different virulence abilities, as well as the differences in protein structure in milk between species.*

Keywords: *MRSA, MSSA, Donkey milk*

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1. Introduction

Milk has also been an essential component of human nutrition since the domestication of animals, as it provides important nutrients to all mammals and plays a critical role in health. In recent years, the number of individuals suffering from intolerances and allergic reactions arising from cow's milk ingestion has increased, leading to the search for alternative foods [1, 2]. The donkey is a member of the Equidae family and has become widespread in Africa, Asia, South America, and Europe with the domestication process that started in 6000 BC. It has been used for lifetimes ranging from 30 to 50 years as a pack animal for transport or riding. The donkey is also an animal serving humanity in the fields of milk production (in the food and cosmetic industry), meat production, ecotourism, and onotherapy.

Donkey milk is known for its therapeutic properties and has been used since ancient times in the treatment of various diseases such as wound healing, bronchitis, asthma, joint pain, and gastritis. Today, it is also available as a commercial product for people allergic to cow's milk proteins, newborns, and the elderly [1]. The donkey milk used by Hippocrates and Avicenna as a remedy for different diseases or by the Egyptian Queen Cleopatra in her bath to keep her skin soft and moist has been found to be very

similar to breast milk, especially in terms of protein profile and lactose content. Thanks to this structure, it stimulates the absorption of calcium from the intestine and thus provides the development of intestinal flora in humans. In addition, it is thought that high lysozyme content selectively creates antibacterial properties against pathogenic microorganisms [2, 3].

Staphylococcus aureus (*S. aureus*) is an important zoonosis responsible for a wide range of diseases and is a commensal microorganism [4, 5]. Prior to the use of methicillin, methicillin-sensitive *S. aureus* (MSSA) strains were almost the only cause of both serious and uncomplicated *S. aureus* infections in healthy individuals [6]. But shortly after methicillin became available in clinics, strains of methicillin-resistant *S. aureus* (MRSA) were reported and, since then, MRSA outbreaks have been observed worldwide. Control of MRSA is still an important problem in hospitals [5]. In some studies, it has been reported that donkey milk have different levels of antimicrobial activity against *S. aureus* strains [7, 8]. Therefore, we aimed to examine the effectiveness of different dilution rates of donkey milk against *S. aureus* strains, which is an important problem all over the world, and to determine the antimicrobial effect of standard MRSA and MSSA strains in-vitro [8, 9].

2. Material Method

2.1. Bacterial origins

S. aureus ATCC 43300 (MRSA) and *S. aureus* ATCC 29213 (MSSA) strains were used in our study. *S. aureus* strains were incubated in 5% sheep blood agar (Oxoid, UK) at 37ºC in an environment with 5% CO2 for 24 hours. After incubation, 0.5 McFarland suspensions prepared in sterile saline were used from colonies of S. aureus ATCC 43300 (MRSA) taken from blood agar medium [9].

2.2. Donkey Milk samples

In our study, 53 different donkey milk offered for sale in different regions of Turkey were purchased raw and these milks were used for in-vitro treatment with MRSA and MSSA strains [10].

2.3. Determination of MIC and MBC values by examining the antimicrobial effect

The effect of donkey milk on *S. aureus* ATCC 43300 (MRSA) and *S. aureus* ATCC 29213 (MSSA) strains were examined and the liquid microdilution method was used to define the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) levels [11]. 5, 10, 20, 40, 80, 160, 320, 640 µL (0.5%, 1%, 2%, 4%, 8%) of donkey milk into a tube containing tryptic soy broth broth (Oxoid, UK) , 16%, 32%, 64%) were added and serial dilutions were made in a total volume of 1 mL. 180 µL of liquid TSB medium containing donkey milk was added to 96-well plates and 20 µL of 0.5 McFarland bacterial suspensions were added on top. Data obtained from 200 µL of milk-free TSB medium for negative control and 180 µL of non-milk-free TSB medium for positive control and 20 µL of bacterial suspensions were considered. After inoculations, 96-well plates were incubated at 37ºC for 24 hours. Bacterial growth in each well was measured at 600 nm using an Epoch spectrophotometer (Biotek, Germany) according to the manufacturer's instructions. The values at which growth was suppressed 100% were accepted as the MIC value. In order to determine the MBC value, the samples with MIC value were incubated in 5% sheep blood agar (Oxoid, UK) at 37ºC in an environment with 5% CO_2 for 48 hours and the values without growth were accepted as MBC [11, 12].

3. Results and Discussion

When the effectiveness of different concentrations of donkey milk added to TSB medium in-vitro against ATCC MRSA and MSSA strains was examined, it was found that there was no suppression

and that even at high milk concentrations, the growth was higher than the control group after 24 hours of incubation. The results are given in Table 1.

It was determined that the reproduction of MSSA strains was less affected by donkey milk than MRSA strains and MRSA strains were able to grow more in this medium at all dilution ratios. Since 100% suppression was not observed in all dilution ratios, MIC value and related MBC value were not determined.

Table 1. Growth percentages and standard deviation data of MRSA and MSSA strains in-vitro in donkey milk added to TSB medium

Donkey milk concentration in TSB medium	**0.5%**	**1%**	**2%**	**4%**
S. aureus ATCC 29213 (MSSA)%	97.22±1.5	95.78±2.4	98.43±1.4	105.72±1.8
S. aureus ATCC 43300 (MRSA)%	98.68±1.9	98.3±2.6	99.41±1.7	108.62±2.2
Donkey milk concentration in TSB medium	**%8**	**%16**	**%32**	**%64**
S. aureus ATCC 29213 (MSSA)%	112.41±1.1	114.74±1.7	121.56±2.7	128.3±3.2
S. aureus ATCC 43300 (MRSA)%	121.36±1.4	128.3±1.8	129.71±2.1	131.4±2.9

Donkey milk is traditionally consumed in Asia, Eastern Europe, and Africa, however, when the positive effect on human health became widespread, took its place in developed country markets with increasing consumer awareness [13]. The antimicrobial activity in donkey milk is mainly due to lactoferrin, lysozyme, immunoglobulins, and lactoperoxidase. It is thought to originate from some small whey protein structures such as ingredients. It can vary greatly between species, races, and individuals due to genetic or reproductive variants [14].

When the studies examining the antimicrobial activity of donkey milk against *S. aureus* were checked; In a study conducted by Yirmibeşoğlu and Öztürk in 2020 they examined the zone diameters of donkey milk-impregnated discs against *S. aureus*, they found this zone diameter to be 11,5 mm, and the ampicillin zone diameter they added for control purposes was 22 mm [7]. In Koutb et al.'s study in 2016, they found that donkey milk-impregnated discs formed a zone diameter of 18 mm against *S. aureus* ATCC 8095, and the minimum lethal concentration was 64 when they examined it [8]. In Saric et al.'s study in 2014, they reported that the antibacterial effect of donkey milk against *L. monocytogenes* and *S. aureus* increased especially during the bacterial lag phase, and inhibited the growth of bacteria in this period. In this study, *S. aureus* was inhibited after 8 hours. It was stated that there was not even a 1 log increase in reproduction compared to the control [15]. Contrary to the studies of Koutb et al. (8) and Yirmibeşoğlu and Öztürk [7], our results were similar to Saric's study, no value that would show a minimum inhibitory concentration was observed in the in-vitro environment. Since we did not examine the lag phase and different durations in our study as in Saric's study [15], we did not find the antimicrobial effect of donkey milk at different points. Similar to the data in our study, Pilla et al. reported that they found the presence of *S. aureus* in donkey milk samples when they examined the hygienic status of donkey milk in 2010 [16]. Their results also support the data in our study. Although different enzymes are mentioned in its content, milk does not show this effect despite *S. aureus* origins. We think that the reason for this may be due to the rich protein structure in its content.

4. Conclusion

In conclusion, according to the results of our study, no antimicrobial effect of donkey milk was observed against standard MRSA and MSSA strains in-vitro. The reason for this situation may be due

to the fact that the protein content of the milk obtained from different donkey species is different, as well as the ability of *S. aureus* to adapt to different environments and produce different virulence factors that can live there. We believe that comprehensive studies should be conducted on donkey milk, which is close to breast milk.

Conflict of interest

No conflict of interest or common interest has been declared by the author.

The Compliance to Research and Publication Ethics

This work was carried out by obeying research and ethics rules.

The Declaration of Ethics Committee Approval

The author declares that this document does not require an ethics committee approval or any special permission. Our study does not cause any harm to the environment.

Authors' Contributions

A.Y.: Conceptualization, Methodology, Formal analysis, Writing

M.D.: Conceptualization, Methodology, Resources, Investigation

S.K.A.: Methodology, Writing - Original draft preparation

B.S.K..: Methodology, Writing - Original draft preparation

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