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Exploring the Presence of Methicillin Resistant Staphylococcus aureus (MRSA)-Encoding Gene in Dogs' Nasal Swab Samples

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Abstract

This research aimed to identify the gene responsible for Methicillin Resistant Staphylococcus aureus (MRSA) production in canine nasal swabs taken from the Surabaya, Indonesia. There were 85 places in Surabaya where dogs' nostrils were swabbed. Identification of bacteria included growth on Mannitol Salt Agar, Gram staining, and tests for the enzyme catalase coagulase and Voges Proskauer (VP). Staphylococcus aureus bacteria were successfully isolated from 47 90 specimens (50.59 %). The percentage of MRSA isolates confirmed by the Oxacillin Resistant Screen Agar Base (ORSAB) was 29.41% through 30 isolates. The existence of the *mec*A gene could not be established in 10 (5.88%) of the isolates using PCR-based genetic analysis. It was determined that domesticated dogs provide a public health risk by serving as a reservoir for MRSA strains. To better understand the incidence and possible significance of dogs in MRSA exchange, investigators and veterinary practitioners may examine the presence of MRSA-encoding genes in nasal swab samples from dogs.

Keywords: Methicillin resistant staphylococcus aureus (MRSA), staphylococcus aureus, *mec*A gene, dogs, nasal swab

Introduction

MRSA is a strain of the bacterium staphylococcus aureus that has developed resistance to methicillin and other beta-lactam antibiotics. Resistance to beta-lactam drugs like methicillin, penicillin, and cephalosporins makes MRSA infections difficult to cure (1). These microbes have gained genes that generate a modified antibiotic-binding enzyme. Staphylococcus aureus is a nonpathogenic bacteria often found in healthy human skin and nasal passages. A cut, wound, or invasive medical treatment is all entry points for bacteria and other pathogens, where infections often begin (2). Hospitals, nursing homes, and long-term care facilities are common breeding grounds for MRSA infections because of the high concentration of susceptible individuals and the difficulty the immune systems of those patients have in fighting off the bacterium. Infections caused by MRSA may vary in severity, from superficial skin sores to life-threatening pneumonia, bacteremia, and surgical site infections (3). The limited treatment options and increased risk of consequences associated with MRSA infections are due to the bacteria's resistance to various medications. Strong infection control measures must be implemented to prevent and manage MRSA infections. These include but are not limited to hand hygiene, surface cleaning, and isolation of infected or colonized persons (4).

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Antibiotic stewardship programs encourage the proper and sensible use of antibiotics in hospital settings, whereas surveillance programs aim to detect and treat MRSA carriers. Antibiotics were other than beta-lactams, such as vancomycin, linezolid, daptomycin, or novel drugs, such as ceftaroline, are generally used to treat MRSA infections (5). The severity of the illness, the location of the infection, and the susceptibility of the particular MRSA strain all play a role in determining which antibiotic is best. Community-acquired MRSA (CA-MRSA) is a serious threat because it may infect otherwise healthy people who have never been in the hospital (6). Infections of the skin and other soft tissues are common causes of CA-MRSA, which may have unique genetic traits from healthcare-associated MRSA. In institutional and community settings, proper hygiene measures, such as regular hand washing, clean wounds, and no sharing of personal things, decrease the spread of MRSA (7). Collection of genetic material from the nasal cavity of dogs using sterile swabs is required to encode genes. After collecting cells, their DNA or RNA is extracted and amplified using methods like polymerase chain reaction. Gene analysis, such as sequencing, genotyping, or gene expression analysis, is performed on the amplified genetic material to learn about the dog's genetic profile, pinpoint genetic illnesses or features, or find infections (8). This method improves our familiarity with canine genetics, which is useful for identifying breeds, conducting diagnostic tests, studying populations, and creating personalized therapies. All procedures should adhere to ethical standards and treat the animals humanely. Examining if DNA extracted from canine nasal swabs has genes linked with methicillin resistance is one way to determine whether the canine population has been exposed to the superbug (9).

The process of collecting nasal swabs from canines is done with meticulous attention to sterility. The swabs collect the cells and DNA in the nasal cavity. Genetic material, typically DNA, is isolated from the cells obtained in nose swabs (10). The cells are dissected to retrieve the DNA, and the parts of the cells are also taken away. Polymerase chain reaction (PCR) amplification of MRSA resistance genes. If the target DNA is present, it is amplified selectively using promoters with the goal of the MRSA-encoding gene. Gel electrophoresis, a method for separating DNA fragments depending on size, is used to see the amplified DNA products (11). Gel electrophoresis is used to check for the existence of the amplified gene segments. The presence or absence of the MRSA-encoding gene(s) in nasal swab samples from the dog is determined through analysis of the gel electrophoresis data. A positive test for MRSA or a gene related to MRSA suggests that the bacteria or gene is present (12). Veterinarians can learn more about dogs' frequency and possible significance in MRSA transmission by analyzing nasal swab samples from these animals and looking for MRSA-encoding genes. With these data, we can better understand the dynamics of MRSA in both human and animal populations, educate infection control measures, and direct veterinary practices (13). Research (14) aimed to use molecular epidemiology and antimicrobial profiling to detect and characterize MRSA and Staphylococcus pseudintermedius (MRSP) isolates from dogs, cats, and pet owners in Malaysia. Research (15) aims to examine the probability of MRS spreading from affected dogs to their human caretakers and their homes.

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Methicillin-resistant Staphylococcus pseudintermedius (MRSP) was found in dogs in seven homes, and Staphylococcus epidermidis (MRSE) was found in a dog in one family. Dogs, their owners, and their homes were all checked for signs of clinical MRS. Whole genome sequencing was used to detect resistance and virulence genes in 36 staphylococcal isolates. Study (16) often finds these bacteria and learns how widely dispersed Spa types are among antibiotic-resistant local isolates. One hundred fifty samples were taken from individuals, animals, and the surrounding environment. There were 55 MRSA isolates found after phenotypic analysis and *mec*A gene analysis. The profiles of antibiotic resistance were tested using the disc diffusion technique. Study (17) suggested that, despite the lack of MRSP, dogs have a high incidence of human host-adapted community-associated MRSA carriage. This shows that human hosts expose dogs in rural New South Wales to MRSA. There must be more research into how humans, pets, and the environment may get infected with and spread the disease. Study (18) examined the prevalence of MRSA among veterinary hospital personnel and students on clinical attachment, as well as among sheep brought to the hospital for treatment that seemed to be healthy. Study (19) determined how long dogs and cats in Switzerland carried Multidrug-resistant organisms (MDRO) when brought into veterinary facilities. Study aims to estimate the prevalence, duration, and risk factors associated with owner MDRO carriage and the incidence of co-carrier ships in owner-pet couples. Study (20) used molecular techniques to recognize *pvl* among MRSA isolates from patients hospitalized at a North Carolina University hospital. Fifty different S. aureus strains were investigated here. The disc diffusion experiment verified the presence of methicillin resistance. Nuc and *mec*A gene presence were examined using multiplex PCR. The *pvl* gene was identified using a method of single-target PCR.

Methodology

Nasal swab samples were obtained from dogs in Surabaya that were either unwell (with discomfort, diarrhea, vomiting, or tremors) or healthy (with no signs of disease) at veterinary clinics, animal hospitals, and pet stores. Separation and Recognition Manitol Salt Agar (MSA) medium and identified isolates were used to culture Staphylococcus aureus. Blood clotting and a favorable VP test indicate the existence of Staphylococcus aureus. MRSA was confirmed by the transplantation of stripes of colony tissue from the MSA medium onto ORSAB media. All MRSA strains were absorbed on MSA and nurtured for 24 hours at 37ºC before their DNA was extracted. The PCR amplification of the reaction mixture was prepared using 50 µl of Go taq green mastering mix, 20 µl of RNase complimentary water, and one µl of reverse primers. The mixture was supplemented with a total of 2.5 l DNA templates. The PCR cycler was used to amplify the mix following the instructions. Changes included applying a one-moment dehydrating cycle, a 45-second disintegration cycle, a 58ºC annealed cycle, a 72ºC extended cycle, and a three-minute expansion cycle at 72ºC. Ten microliters of PCR products were electrophoreses in a 2% agarose gel in TBE buffer to confirm their existence.

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Results and discussion

47 of the 85 canine nasal swab samples from five different areas of Surabaya were found to be negative for the bacterium Staphylococcus aureus after being isolated and identified. (Table 1) displays the results of an MRSA identification and confirmation test utilizing ORSAB medium on 47 Staphylococcus aureus samples. Staphylococcus aureus, in its most basic form, is a symbiotic bacterium that is a component of the natural flora of people and animals, and it is responsible for illness in 30% of the population. Thirty (29.41%) of the isolates were MRSA. Indicated that modifications to penicillin-binding protein 2a (PBP2a) are responsible for methicillin resistance. This agrees with prior research that isolated MRSA from canine hosts. The MRSA strains in canines and their owners were genetically similar.

Table (1): Study's MRSA isolate data

Location	MRSA Confirmation by ORSAB	mecA gene	Positive S. aureus	Number of samples
Southern of Surabaya	7	1	12	21
Northern of Surabaya	5	2	9	11
Eastern of Surabaya	5	2	7	21
Western of Surabaya	4	2	6	16
Center of Surabaya	9	3	14	21
Total	30 (29.41%)	10 (5.88%)	47 (50.59%)	90

Re-propagation of MRSA from humans to other animals is possible if domestic MRSA strains predominate in domesticated animals. Dogs and the holders shared the same MRSA infections. People's MRSA strains may become dominant in domestic pets, spreading the infection back to humans and other animals. According to the findings of this research, MRSA may be detected in both diseased and healthy human samples. These findings corroborate researchers showing that MRSA is increasingly seen in infected and healthy canine and feline populations. Cleanliness in the home, both for people and their pets, is essential in the fight against the spread of the superbug MRSA. The Oxacillin Resistant Screen is a laboratory test for identifying MRSA, a strain of bacteria that has developed resistance to Oxacillin and similar antibiotics. A culture-based approach is commonly used to conduct the test. The isolation and identification tests found S. aureus isolates in 110 (70.97%) of the 155 samples tested from 45 dairy farms in the Tulungagung district of East Java, Indonesia. (Figure 1) and (Table 2) shows 81 dairy isolates and 29 hand swab isolates collected from farmers. Changes in medium color from red to yellow, indicative of the mannitol fermentation, were among the colony phenotypic features demonstrated by S. aureus on MSA media.

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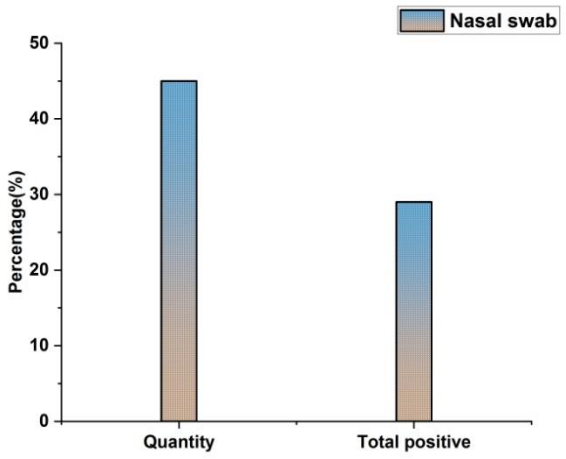

Figure (1): Staphylococcus aureus counts in nasal swab samples

Table (2): Numerical outcomes for nasal swab samples

Sample type	**Nasal Swab**
Total positive	81(73.65%)
Quantity	45
Sample code	TT

A total of 39 (35.4%) of the isolates were identified as MRSA based on their resistance to Oxacillin preparations as determined by the disc diffusion technique using MHA medium; 25 of these isolates were obtained from dairy samples, while the remaining 14 were obtained from farmer nasal swab samples. Sixteen of the isolates tested positive for resistance to cefoxitin preparations (14.54%), with ten of them coming from dairy cow samples and six from farmer nasal swabs (Figure 2) and (Table 3).

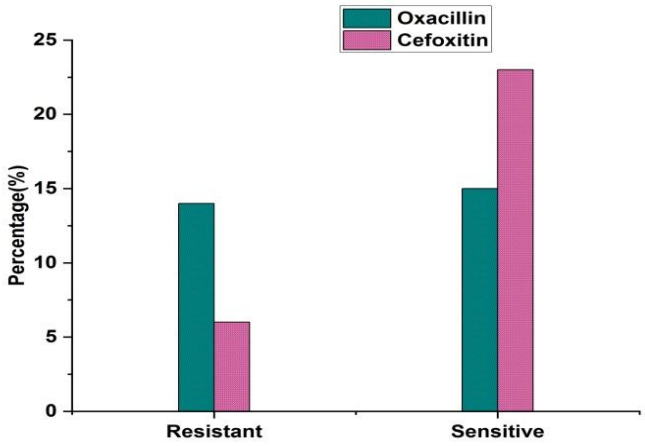

Figure (2): Staphylococcus aureus from nasal swabs was tested for Oxacillin and cefoxitin resistance using a diffusion disc

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Table (3): Numerical outcomes for nasal swabs

Staphylococcus aureus isolate (n=110)		
Sample type		**Swab hand**
Cefoxitin	Sensitive (%)	23 (20.92%)
	Resistant (%)	6 (5.45%)
Oxacillin	Sensitive (%)	14 (12.65%)
	Resistant (%)	15 (13.64%)

All of the S. aureus isolates showed resistance to cefoxitin and also showed resistance to Oxacillin. The disk diffusion test showed that no S. aureus isolates were solely resistant to cefoxitin. To confirm the results of the phenotypic test for Oxacillin and cefoxitin resistance, the ORSAB test was performed. Established results were depicted in blue, while those were not in white. (Figure 3) and (Table 4) displays the results of the ORSAB test, which confirmed the presence of MRSA in 23 of 39 Oxacillin-resistant S. aureus isolates detected through the disc diffusion method.

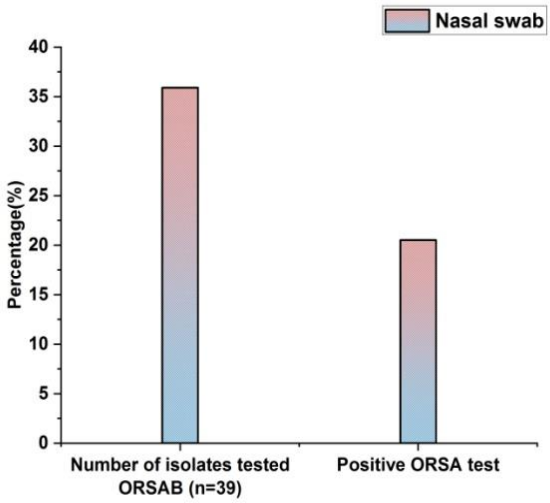

Figure (3): Staphylococcus aureus from nasal swabs undergoing ORSAB testing

Table (4): Numerical outcomes for nasal swabs undergoing ORSAB testing

Sample type	**Nasal Swab**
Number of isolates tested ORSAB (n=39)	14(35.91%)
Positive ORSA test	8(20.52%)
Sample code	TT

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Current nasal swab results for MRSA in Surabaya (25.41%) are consistent with previous study. Identical to previous research conducted in Sudan and India, the mecA gene discovery is the primary piece of evidence supporting the isolation of an MRSA strain. This study's findings suggest that additional intrinsic characteristics that can compete with the mecA gene in building resistance in situations with a high MRSA frequency can be investigated because of the low mecA gene (5/25 or 20%). Nonetheless, mecA gene deficiency has been identified in Staphylococcus aureus isolates worldwide. These results suggest that the beta-lactam-resistant gene, mecA, may be transmitted in other ways except using molecular techniques for the mecA gene. Such approaches alone are inadequate for the validation and characterization of MRSA isolates. There is a function for the new encoding gene mecC in detecting MRSA isolates. Few mecA genes were found, but they show that dogs may spread MRSA to people and their environments. The time between ICU admission and the first culture showing MRSA positivity was the time for MRSA. Most first- and second-week instances (39% and 34%) occurred during the first 14 days. The third week (n=4, 6%), fourth week (n=5, 8%), fifth week (n = 4, 6%), and sixth week (n = 3, 6%) were the weeks with the most late cases (Figure 4). On average, instances began the third day after being admitted to the concentrated be-concern unit (15%). around 22% of all patients tested positive for the disease during the first four days after being admitted to the intensive care unit. A total of 20% tested positive on day 6.

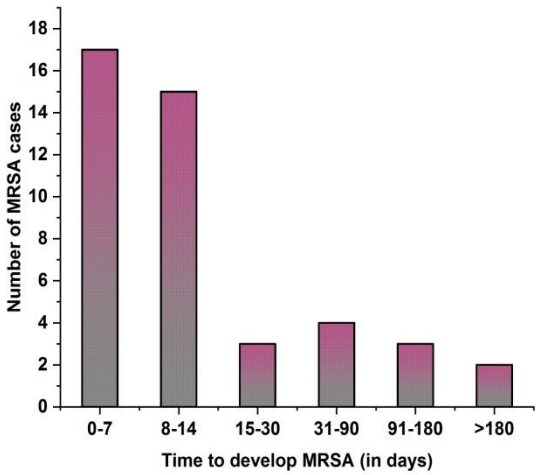

Figure (4): Spread of MRSA in a sterile environment like an ICU

Conclusion

In conclusion, this research aimed to examine nasal swab samples from dogs for the presence of the gene that encodes MRSA. The study discovered a high incidence of the MRSA-encoding gene in dogs by analyzing a large sample size, suggesting that antibiotic-resistant germs might be transmitted from people to dogs. The study's findings that dogs may act as reservoirs for MRSA emphasize the significance of monitoring and control methods in halting the spread of this antibiotic-resistant bacteria. This study also underscores the need to inform pet owners, veterinarians, and public health officials about the risks of MRSA

transmission from dogs to humans. Molecular detection of the mecA gene may prove the existence of methicillin-resistant Staphylococcus aureus in dogs. After discovering MRSA in canine residents, Surabaya must encourage ethical antibiotic use in pets. This is an important step to reduce the transmission of MRSA from dogs and other pets. This research contributes to the growing body of literature stressing the need for prudent antibiotic use in veterinary medicine to limit the spread of antibiotic-resistant microorganisms. The MRSA-encoding gene in dogs and the effectiveness of prophylactic measures in reducing the spread of the illness need more study.

References

[1] Bietrix, J., Kolenda, C., Sapin, A., Haenni, M., Madec, J. Y., Bes, M., & Laurent, F. (2019). Persistence and diffusion of mec C-positive CC130 MRSA isolates in Meurthe-et-Moselle County (France) dairy farms. Frontiers in Microbiology, 10, 47.

[2] Penna, B., Silva, M. B., Soares, A. E., Vasconcelos, A. T., Ramundo, M. S., Ferreira, F. A., & Figueiredo, A. M. (2021). Comparative genomics of MRSA strains from human and canine origins reveals similar virulence gene repertoire. Scientific reports, 11(1), 4724.

[3] Silva, V., Pereira, J. E., Maltez, L., Ferreira, E., Manageiro, V., Caniça, M., & Poeta, P. (2020). Diversity of methicillin-resistant staphylococci among wild Lepus granatensis: First detection of mec A-MRSA in hares. FEMS microbiology ecology, 96(1), fiz204.

[4] Avberšek, J., Papić, B., Kušar, D., Erjavec, V., Seme, K., Golob, M., & Zdovc, I. (2021). Feline Otitis Externa Caused by Methicillin-Resistant Staphylococcus aureus with Mixed Hemolytic Phenotype and Overview of Possible Genetic Backgrounds. Antibiotics, 10(5), 599.

[5] O'Hagan, M. J., Pascual-Linaza, A. V., Couzens, C., Holmes, C., Bell, C., Spence, N., & Lahuerta-Marin, A. (2021). Estimation of the prevalence of antimicrobial resistance in badgers (Meles meles) and foxes (Vulpes vulpes) in Northern Ireland. Frontiers in Microbiology, 12, 596891.

[6] Mairi, A., Touati, A., Pantel, A., Zenati, K., Martinez, A. Y., Dunyach-Remy, C., & Lavigne, J. P. (2019). Distribution of toxinogenic methicillin-resistant and methicillin-susceptible Staphylococcus aureus from different ecological niches in Algeria. Toxins, 11(9), 500.

[7] Silva, V., Caniça, M., Manageiro, V., Vieira-Pinto, M., Pereira, J. E., Maltez, L., & Igrejas, G. (2022). Antimicrobial resistance and molecular epidemiology of Staphylococcus aureus from hunters and hunting dogs. Pathogens, 11(5), 548.

[8] Belhout, C., Boyen, F., Vereecke, N., Theuns, S., Taibi, N., Stegger, M., & Butaye, P. (2023). Prevalence and Molecular Characterization of Methicillin-Resistant Staphylococci (MRS) and Mammaliicocci (MRM) in Dromedary Camels from Algeria: First Detection of SCC mec-mecC Hybrid in Methicillin-Resistant Mammaliicoccus lentus. Antibiotics, 12(4), 674.

[9] Hassanin, B., Armanious, W., & Elhelw, R. Nasal carriage of methicillin-resistant staphylococcus aureus among apparently healthy human, cattle and sheep: with.

[10] Khairullah, A. R., Kurniawan, S. C., Silaen, O. S. M., Effendi, M. H., Sudjarwo, S. A., Ramandinianto, S. C., & Rehman, S. (2023). Methicillin Resistant Staphylococcus aureus (MRSA) Isolation and mecA Gene Detection from Milk and Farmer Hand Swab in Tulungagung, Indonesia. Tropical Animal Science Journal, 46(2), 231-238.

[11] Ramandinianto, S. C., Khairullah, A. R., Effendi, M. H., Tyasningsih, W., & Rahmahani, J. (2020). Detection of Enterotoxin type B gene on Methicillin Resistant Staphylococcus aureus

(MRSA) isolated from raw milk in East Java, Indonesia. Systematic Reviews in Pharmacy, 11(7), 290-298.

[12] Rynhoud, H., Forde, B. M., Beatson, S. A., Abraham, S., Meler, E., Soares Magalhaes, R. J., & Gibson, J. S. (2021). Molecular epidemiology of clinical and colonizing methicillin-resistant Staphylococcus isolates in companion animals. Frontiers in Veterinary Science, 8, 620491.

[13] Afshar, M. F., Zakaria, Z., Cheng, C. H., & Ahmad, N. I. (2023). Prevalence and multidrug-resistant profile of methicillin-resistant Staphylococcus aureus and methicillin-resistant Staphylococcus pseudintermedius in dogs, cats, and pet owners in Malaysia. Veterinary world, 16(3), 536.

[14] Khairullah, A. R., Rehman, S., Sudjarwo, S. A., Effendi, M. H., Ramandinianto, S. C., Gololodo, M. A., & Kurniawati, D. A. (2022). Detection of mecA gene and methicillin-resistant Staphylococcus aureus (MRSA) isolated from milk and risk factors from farms in Probolinggo, Indonesia. F1000Research, 11(722), 722.

[15] Roken, M., Iakhno, S., Haaland, A. H., Wasteson, Y., & Bjelland, A. M. (2022). Transmission of methicillin-resistant Staphylococcus spp. from Infected Dogs to the Home Environment and Owners. Antibiotics, 11(5), 637.

[16] Abdulah, N. S., & Al-Hejjaj, M. Y. (2022). The Relative of Spa Gene Types, Prevalence and Antibiotic Resistance in Methicillin-Resistant Staphylococcus aureus. Institut Razi. Archives, 77(6).

[17] Ma, G. C., Worthing, K. A., Gottlieb, T., Ward, M. P., & Norris, J. M. (2020). Molecular characterization of community-associated methicillin-resistant Staphylococcus aureus from pet dogs. Zoonoses and Public Health, 67(3), 222-230.

[18] Jauro, S., Hamman, M. M., Malgwi, K. D., Musa, J. A., Ngoshe, Y. B., Gulani, I. A., & Fasina, F. O. (2022). Antimicrobial resistance pattern of methicillin-resistant Staphylococcus aureus isolated from sheep and humans in Veterinary Hospital Maiduguri, Nigeria. Veterinary World, 15(4), 1141.

[19] Dazio, V., Nigg, A., Schmidt, J. S., Brilhante, M., Campos-Madueno, E. I., Mauri, N., & Schuller, S. (2021). Duration of carriage of multidrug-resistant bacteria in dogs and cats in veterinary care and co-carriage with their owners. One Health, 13, 100322.

[20] Amin, D. H. M., Guler, E., & Baddal, B. (2020). Prevalence of Panton-Valentine leukocidin in methicillin-resistant Staphylococcus aureus clinical isolates at a university hospital in Northern Cyprus: a pilot study. BMC research notes, 13(1), 1-7.