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Antimicrobial resistance patterns of *Staphylococcus Aureus* isolates in a veterinary laboratory in Nyeri, Kenya

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Abstract
Staphlococcus aureus is a bacteria of veterinary and public health importance where it causes a vast array of pathogenic infections. In human, they cause skin and soft tissue infections, bloodstream infections, pneumonia and bone or joint infections. In animals, they can cause many infections one of the most common being mastitis which is a major concern in dairy farming. It causes swelling of the mammary tissues and changes in consistency of milk. Infections of the udder with various pathogens have led to the use of intra-mammary antibiotics which are mostly sold over-the-counter without prescriptions. Farmers and animal health service providers usually treat animals without antimicrobial sensitivity testing and this has over time contributed to resistance of bacteria to antibiotics. In addition to antimicrobial resistance, interaction between humans and animals and animal products can lead to transfer of resistance genes across species. The over-use of this antibiotics is leading to the silent antimicrobial Tsunami. The aim of the study was to determine the proportion of *Staphylococcus aureus* isolates in samples submitted at the Regional Veterinary Investigation laboratory, Karatina and also to determine Antimicrobial susceptibility patterns of the isolates through a retrospective study.
From 452 samples submitted. 96% were milk samples and 4% were organs sampled during postmortem examination. From the samples 239 (53%) were *Staphylococcus* isolates with 108 (24%) being *Staphylococcus aureus* isolates. Antimicrobial susceptibility patterns were observed for 8 antibiotics, highest resistance being for Ampicillin 50%, followed by Cotrimoxazole 44.4 %, Sulphamethoxazole 44.4%, Streptomycin 26.9%, Chloramphenicol 15.7%, Kanamycin 13.9%, with the least resistance being for Gentamycin 2.8% and Tetracycline 1.99%. Isolates showed increasing resistance towards common antibiotics used to treat animals. A multi-disciplinary approach by the farmers, animal health and human health providers, government agencies and all relevant stakeholders would help to control this silent Pandemic. Use of laboratory facilities would help animal health service providers and farmers to identify suitable drugs to treat animals. Regulating and monitoring use and access of antibiotics would help avert over-use and misuse of antibiotics.

Keywords: *Staphylococcus aureus*, mastitis, antimicrobial resistance, Nyeri, Kenya

1. Introduction
Staphylococcus aureus is a gram-positive cocci that is a commensal on healthy animals but can also be a pathogenic bacteria. It causes diseases in humans which range from mild organ infections to severe infections like bacteremia [1]. In animals it causes many diseases with the major disease in dairy cows being mastitis which occurs once the bacteria penetrate the skin barrier due to cracks in the teats or open teat canals. Mastitis can occur either in sub-clinical or clinical forms. High case rates of mastitis are reported in high producing areas and in high yielding cows. A study done by Kalayu., *et al.* on Burden and antimicrobial resistance of *Staphylococcus aureus* found that Mastitis causes huge economic losses to the farmer due to decline in milk production and also financial constrains in treating mastitis [2]. During cases of sub-clinical mastitis, farmers are not aware of any disease leading to delays in detecting mastitis and delays in implementing treatment as also found by Hussein *et al.* in Ethiopia [3]. When there is a drop in milk production or changes in milk consistency, farmers who notice these changes revert to use of intra-mammary drugs and this has led to the increased use of antibiotics leading to antimicrobial resistance. Milk and livestock products from these affected animals could pose a health risk to young suckling animals and also to human beings consuming them.

There is also a risk of transfer of antibiotic resistant bacteria to humans directly from animals or through consumption of animal products like milk. The objectives of this study were to determine proportion of *Staphylococcus aureus* isolated from milk and other samples and to identify their antimicrobial susceptibility patterns.

2. Methodology

2.1 Study Area and Population

This study was carried out at Regional Veterinary investigation Laboratory, Karatina in Nyeri County, Kenya. From samples submitted to the laboratory and those collected during post-mortem examination, we determined proportion of *Staphylococcus aureus* and its antimicrobial susceptibility patterns.

2.2 Study Design

The study design was a retrospective records review where data collected for diagnostic purposes was analyzed. Upon reception of milk samples in the laboratory, California Mastitis Test (CMT) is carried out in milk as stated by Esther Kamau., *et al.*, 2020 [4] Milk and other samples were cultured on Blood agar and MacConkey and incubated for 24hrs at 37.5 °c in an incubator. After the 24hr hours, pure bacteria cultures were isolated, sub-cultured on Blood agar and incubated. After incubation for 24hrs, Gram staining was done and the positive cocci were identified using biochemical methods (catalase and coagulase tests). Pure culture strains of *Staphylococcus aureus* NCTC 6571 were used as controls. Antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion method with Muller Hilton Agar as the media. A loop full of *Staphylococcus aureus* was incubated in nutrient broth for 3hrs to increase the number of colonies. Normal saline was added into the solution and standardized using the MacFarland standard. The standardized suspension was then streaked using a swab on the Muller Hilton Agar. The antibiotic disc containing 8 antibiotics was placed in the middle of the media and incubated for 24 hours at 37°c.The antibiotics used were: Ampicillin 25mcg, Tetracycline 25mcg, Cotrimoxazole 25mcg, Streptomycin 10mcg, Kanamycin 30mcg, Gentamycin 10mcg, Sulphamethoxazole 200mcg and Chloramphenicol 30mcg. After incubation, the zone of inhibition were measured using a ruler in mm as sensitive or resistant using the CSLI standard [5].

2.3 Study Period

The study period was June 2021 to August 2021

2.4 Data Collection and Analysis

Data that had been collected in an excel file for the 3 months was cleaned and analyzed. Proportions (percentages) for *Staphylococcus* isolates and resistance/susceptibility patterns against common antibiotics were calculated.

3. Results

From a sample size of 452 isolates, 239 (53%) *Staphylococcus* strains were isolated: 108 (23.9 %) being *Staphylococcus* aureus and 131(29%) being other *Staphylococcus* species (*Staphylococcus epidermidis*). Out of these, antimicrobial sensitivity testing was carried out. Isolates showed resistance to the drugs as follows:-with the highest being for Ampicillin 50%, followed by Cotrimoxazole 44.4 %, Sulphamethoxazole 44.4%, Streptomycin 26.9%, Chloramphenicol 15.7% and Kanamycin 13.9% with the least resistance from Gentamycin 2.8% and Tetracycline 1.99% as shown in figure 1 below.

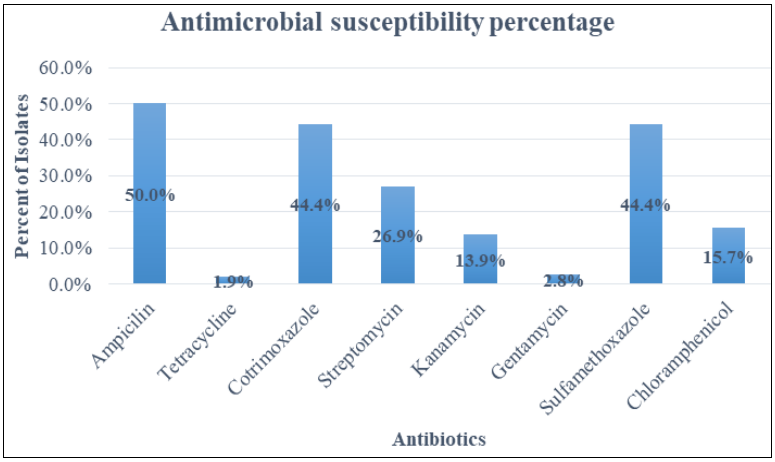

Fig 1: Drug susceptibility patterns of *Staphylococcus aureus* from specimens isolated at RVIL Karatina, 2021.

4. Discussion

From the findings, the results concurred with a study done by Esther Kamau., *et al.* in 2020 that mastitis either clinical or sub clinical is a common occurrence in dairy farms although prevalence of *Staphylococcus* species was found to be much lower in our study compared to the study by Kamau *et al.* [4]. Occurrence of mastitis in dairy herds has in turn prompted the over-use and misuse of antibiotics as noted by World Health Organization [6].

Staphylococcus species are common causes of mastitis and other conditions as shown by their high prevalence, 53% for *Staphylococcus* species, 24% for *Staphylococcus aureus* and 29% specifically was *Staphylococcus epidermidis* which was also noted to be an increasing mastitis causative agent in a study done by Mbindyo C.M [7]. The *Staphylococcus aureus* bacteria tends to show a high level of resistance to all the 8 antibiotics tested for, this being a critical health concern.

5. Recommendations

Improvement of hygiene by the farmers will eliminate presence of this bacteria in the farming environment. This can be achieved through proper housing, hygienic milking practices, teat dips and farm biosecurity measures. Prompt treatment of teat injuries can help reduce incidences of bacteria entry through the teat canal therefore reducing risk of infections. Farmers' education on early detection of mastitis can help in early treatment of cases to avoid overuse and misuse of drugs. Regulation of drug availability should be

addressed as this is a hotbed for creating resistance. A one health approach to prevention of infections, antimicrobial resistance and prevention of zoonotic spread of bacteria and drug resistance genes across the food chain would go a long way in helping to curb resistance. Use of diagnostic laboratories by the farmers and animal health providers can help in to identifying the correct drug to administer thereby preventing occurrence of resistance.

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7. References

1. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management, Clin Microbiol Rev. 2015;28(3):603-661. doi:10.1128/CMR.00134-14.
2. Kalayu AA, Woldetsadik DA, Woldeamanuel Y, Wang SH, Gebreyes WA, Teferi T. Burden and antimicrobial resistance of S. aureus in dairy farms in Mekelle, Northern Ethiopia16(1). BMC Vet Res. 2020;16(1):20. doi: 10.1186/s12917-020-2235-8. PMID: 31969151.
3. Hussein A, Teshome NY, Tilahun G. Prevalence of mastitis in different local and exotic breeds of milking cows, Ethiop. J Agric. Sci. 1997;16:53-60.
4. Esther Kamau, Mburu Kimani *et al*. Risk Factors Associated with Occurrence of Mastitis in Mathira East in Nyeri County, Kenya. EC Veterinary Science. 2020;5(9):142-149. https://www.semanticscholar.org/paper/Risk-Factors-Associated-with-Occurrence-of-Mastitis-Kamau-Kimani/182486704d768f0f690a6c444952b9ac5a2aef02, 2020.
5. CLSI. Clinical Laboratory Standards Institute (CLSI), Performance standards for antimicrobial susceptibility testing, 24th informational supplement. M100-S23, Wayne PA, USA, 2014.
6. WHO, Global action plan on antimicrobial resistance, 2015.
7. Mbindyo CM, Gitao GC, Plummer PJ, Kulohoma BW, Mulei CM, Bett R. Antimicrobial Resistance Profiles and Genes of Staphylococci Isolated from Mastitic Cow's Milk in Kenya, Antibiotics. 2021;10:772. https://doi.org/10.3390/antibiotics1007077.