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Integrating multi-wet laboratory diagnostics to study staphylococci in animals in Uganda

Steven Kakooza[1*], Wilfred Eneku[2], Esther Nabatta[3], Eddie M. Wampande[2], Paul Ssajjakambwe[2], Mariam Wanyana[1], Damien F. N. Munyiirwa[1], Dickson Ndoboli[1], Dorcus Namuyinda[1], Grace Athieno[1], Edrine Kayaga[1], Rodney Okwasiimire[1], Sayaka Tsuchida[4], Kazunari Ushida[4], Ken'ichi Sakurai[5] and Francis Mutebi[1]

Abstract

Background Several diagnostic environments in Uganda lack real-time, robust and high-throughput technologies for comprehensive typing of microbes, which is a setback to infectious disease surveillance. This study combined various wet laboratory diagnostics to understand the epidemiology of pathogenic staphylococci isolated from animals in Uganda and the implications for global health security priorities.

Methods A retrospective study was conducted employing records and pathogenic staphylococci (from animals) archived at the Central Diagnostic Laboratory (CDL), Makerere University, Uganda, between January 2012 and December 2019. The bacteria were speciated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and tested for virulence factors [beta lactamases, lecithinase, deoxyribonuclease (DNase), haemolysins] and resistance to ten antimicrobials of clinical and veterinary relevance. Tetracycline and methicillin resistance genes were also tested.

Results The prevalent diseases were mastitis in cattle and skin infections in dogs. Of the 111 staphylococci tested by MALDI-TOF MS, 79 (71.2%) were *Staphylococcus aureus,* 27 (24.3%) were *Staphylococcus pseudintermedius* and 5 (4.5%) were *Staphylococcus schleiferi.* All these strains expressed haemolysins. The prevalence of strains with lecithinase, penicillinase, cephalosporinase and DNase was 35.9% (14/39), 89.7% (35/39), 0.0% (0/39) and 87.2% (34/39), respectively. Staphylococci were primarily resistant to early penicillins (over 80%), tetracycline (57.7%), and chloramphenicol (46.2%). Minimal resistance was noted with cloxacillin (0.0%), ciprofloxacin (9.6%), and cefoxitin (3.8%). The prevalence of multidrug resistance (MDR) was 78.8% for general staphylococci, 82.2% for *S. aureus*, 73.1% for *S. pseudintermedius*, and 60.0% for *S. schleiferi.* Multidrug resistant staphylococci were significantly more prevalent in the cattle isolates than in the dog isolates ($P < 0.05$). The prevalence of methicillin-resistant staphylococci (MRS) tested by resistance to cefoxitin and *mecA* carriage was 3.8%. These four strains were all isolated from dog skin infections. The *tetK* gene was the most predominant (35.4%), followed by *tetM* (25.0%).

Conclusion In resource-constrained settings, the approach of integrated diagnostics promises sustainable disease surveillance and the addressing of current capacity gaps. The emergence of MRS (zoonotic bacteria) in companion animals creates a likelihood of reduced treatment options for related human infections, a threat to global health.

*Correspondence:
Steven Kakooza
ksteven310@gmail.com
Full list of author information is available at the end of the article

Keywords Staphylococci, Virulence, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), Global health security priorities

Introduction

Staphylococci are gram-positive, aerobic, ubiquitous bacteria that can cause a wide range of opportunistic infections in both humans and animals [1, 2]. In Uganda, staphylococcal infections have been recognized as a significant concern in livestock, pets, and wildlife. Some of the common diseases caused by staphylococci in animals include bovine mastitis, canine pyoderma [3–5], surgical site infections, osteomyelitis, pneumonia [6], endocarditis [7], and joint infections [8].

The ability of staphylococci to cause disease is mediated by a number of virulence properties, including biofilm formation; capsules that increase bacterial resistance to phagocytosis; protein A, which is an immunoglobulin G-binding protein found on the cell wall of *S. aureus* that can assist the bacterium in avoiding phagocytosis by polymorphonuclear leukocytes; enzymes including coagulase, deoxyribonuclease (DNase), hyaluronidase, staphylokinase, and proteases; endotoxins such as haemolysins (alpha, beta, gamma, delta), Panton-Valentine leukocidin (PVL) (the main reason for necrotic skin lesions in humans and animals), toxic shock syndrome toxin-1, and enterotoxins; antimicrobial degrading enzymes (penicillinase); and resistance genes [9–11]. The escalating bacterial diseases in animals have triggered the inappropriate use of antimicrobials for treatment and prophylaxis [12], causing a rise in antimicrobial resistance (AMR). Some noxious staphylococci include methicillin-resistant *S. aureus* (MRSA), which can pose a zoonotic risk, potentially infecting humans in close contact with infected animals.

Bacterial characterization facilitates epidemiological investigations [13], and can take several directions including, species identification, detection of virulence factors or antimicrobial resistance genes, among others. The commonly used methods are usually grouped into phenotypic-, genotypic- and serological-based typing, which can be performed manually or using automated machines. While phenotypic methods are cost-effective and rapid, genotypic techniques, such as polymerase chain reaction (PCR) and sequencing, provide enhanced discriminatory power. Automated microbiology technologies, including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), BD phoenix, and Biomérieux Vitek 2, offer efficient strain identification. Complementing phenotypic methods with genotypic techniques can overcome limitations in discriminatory power and provide a more robust understanding of microbial populations and their characteristics. However, genomic technologies may also require specialized equipment, expertise in data analysis and longer turnaround times for some techniques. Standardization efforts are also crucial to ensure reliability [14].

Precise characterization of staphylococci strains is crucial for effective management and control, yet several diagnostic environments in Uganda often lack the capacity to provide robust and high-throughput tools for comprehensive study of microbes. This ultimately hampers accurate identification of emerging strains so as to inform targeted interventions. Despite the setbacks, we explored integrating multiple wet laboratory diagnostics (Fig. 1), combined with disease records, as an approach to enhance our understanding of the epidemiology of pathogenic staphylococci in Uganda's animal health context and its implications for global health security priorities.

Materials and methods

Study design and ethical considerations

This was a retrospective study conducted on disease-causing staphylococci diagnosed in animal cases handled at the Central Diagnostic Laboratory (CDL), College of Veterinary Medicine, Animal Resources and Bio-Security (COVAB), Makerere University, Uganda, between January 2012 and December 2019. Permission to conduct research on the archived biological samples and the generated animal disease data sets was obtained from the management of the Central Diagnostic Laboratory, COVAB, Makerere University.

Bacterial strains

All staphylococci that had been archived after cultures were performed on samples from the clinical cases of animals presented to the laboratory from 2012 to 2019 were included in this study. The recovery was performed with modification of the methods explained in a previous study [12]. A sterile wire loop was used to scrap ice flakes of approximately 0.2 g from the top of the bacterial stocks. The flakes were then inoculated in 2 ml of brain heart infusion broth (Oxoid, United States of America) and incubated at 37 °C for 24 h. The overnight broth was streaked onto 5% sheep blood agar (Conda laboratories, Spain), and further confirmation of bacterial species was performed using MALDI-TOF MS.

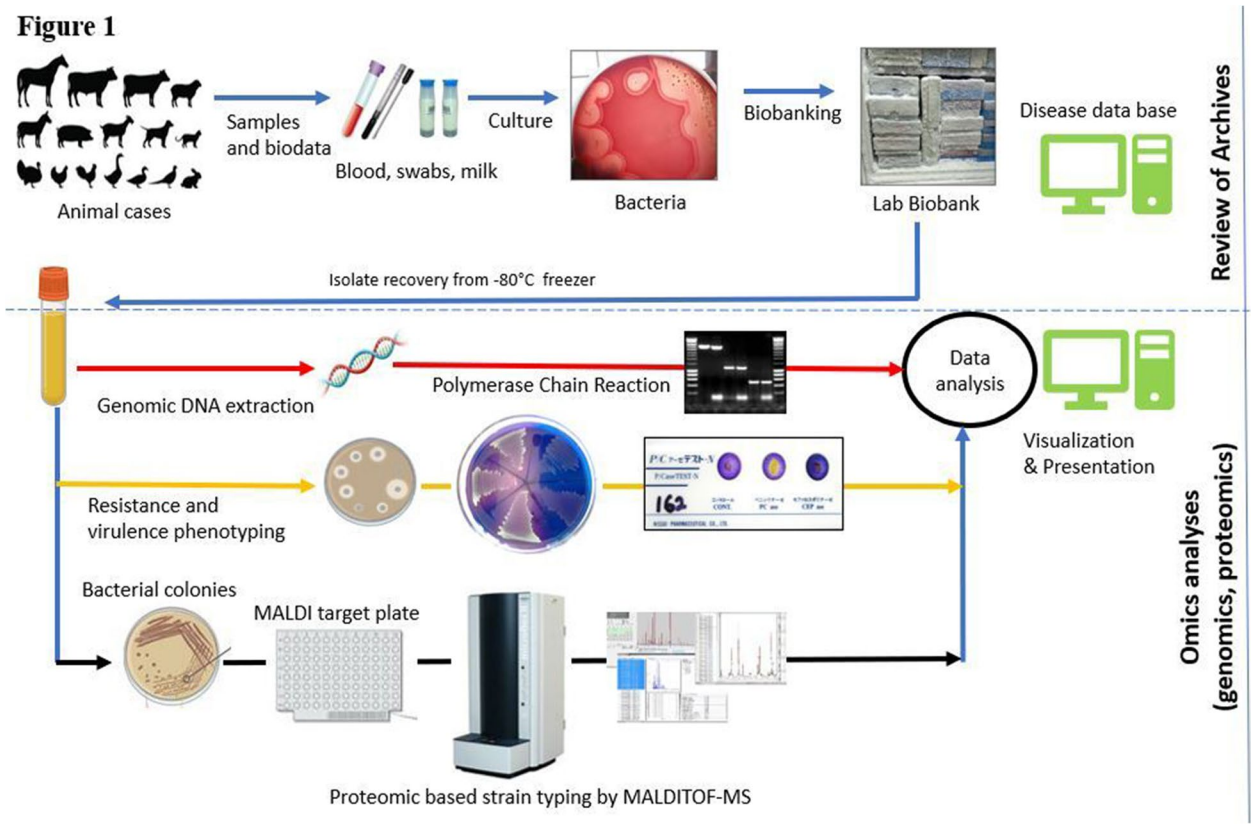


Fig. 1 Explains the framework of integrated diagnostics used in this study. The process involved a major review of archives (records, bacteria) and performance of multiple analyses (phenotyping, genomics and proteomics)

Speciation of isolates by MALDI-TOF MS

Species level identification was performed by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS, MicroFlex™, Bruker Daltonics, United States), as described in previous studies [15, 16]. Freshly grown bacterial colonies were spread on a ground steel reusable MALDI target plate (with 96 sample positions) using a sterile disposable inoculation loop. The bacterial smears (preferably thin) were then air-dried and overlaid with 1.0 µL of Bruker IVD Matrix HCCA, followed by further air-drying for five minutes at room temperature. The target plate was subsequently inserted into the Bruker MicroFlex instrument, operated using FlexControl 3.0 software (Bruker Daltonics). External calibration (quality control) of the instrument was conducted using Bruker's Bacterial Test Standard (BTS, Bruker Daltonics). The identification results obtained through MALDI-TOF MS were evaluated according to Bruker's guidelines, either at the genus or species level. A high-confidence identification agrees with a score within the 2.00—3.00 range, indicating reliable species-level identification. In contrast, low-confidence identifications are accepted at the genus level, with scores falling between 1.70 and 1.99. Scores below 1.70 are considered unreliable for identification at any taxonomic level [17].

Antibiogram profiling

Susceptibility testing on the isolates was performed on Mueller Hinton agar (Conda laboratories, Spain) using the disc agar diffusion method according to the Clinical Laboratory Standards Institute (CLSI) 2022 guidelines. A bacterial suspension of fresh culture colonies was prepared, equivalent to 0.5 McFarland standard. The agar surface was then evenly swabbed with the suspension, and the plates were allowed to dry before introducing the antimicrobial discs. The bacteria were tested against a panel of 9 antimicrobial agents of both human and veterinary relevance. These included: penicillinase-labile penicillins (ampicillin 10 µg, penicillin 10 U), second-generation cephalosporins (cefoxitin 30 µg), penicillinase-stable penicillins (cloxacillin 5 µg), aminoglycosides (gentamicin 10 µg), tetracyclines (tetracycline 30 µg), newer quinolones (ciprofloxacin 5 µg), potentiated sulphonamides (trimethoprim/sulphamethoxazole 1.25/23.75 µg), and phenicols (chloramphenicol 30 µg). The plates were then incubated at 37 °C for 24 h.

For quality control, *S. aureus* ATCC 25923 was used as a reference strain. Upon incubation, the diameter of the zone of inhibition around the disc was measured using a ruler, and the results were interpreted according to the 2022 CLSI guidelines. Multidrug resistance was defined as resistance to at least three different classes of antimicrobials [18].

Phenotypic detection of virulence factors

The factors detected were haemolysins, DNase, lipase and beta lactamases.

Haemolysins

The haemolysins were characterized based on methods presented in previous studies [19]. Bacteria were streaked in triplicate on blood agar supplemented with 5% sheep blood and incubated at 37 °C for 24 h. The lysis zones were observed for each isolate on the plates (Fig. 2C and D).

Deoxyribonuclease (DNase) activity

Fresh cultures were inoculated onto DNase agar (Nissui, Japan) with toluidine blue O and incubated at 37 °C for 24 h. The presence of pink clear zones around the streak (Fig. 2B) indicated a positive result [20, 21].

Lecithinase activity

Fresh cultures were inoculated onto mannitol salt agar (Nissui, Japan) supplemented with egg yolk (added to achieve a 3% final concentration) and incubated at 37 °C for 24 h [22, 23]. Lecithinase production was detected by observance of opaque white zones around colonies that produced this enzyme (Fig. 2A).

Production of beta lactamases

Beta lactamase testing was performed on the basis of acidometry by using a commercial test product [P/C アーゼテスト-N (P/Case TEST-N); Nissui Pharmaceutical, Japan]. Three indicator discs [including a control (CONT.)], were premoistened with 30 µl of sterilized distilled water. Then, bacterial cultures (three pure colonies) were rubbed in the centre of only three panels—the CONT: control, P Case: penicillinase and CEP ase: cephalosporinase. The discs were then kept at room temperature and observed for color changes for 30 min. Enzyme production was detected as a colour change from purple

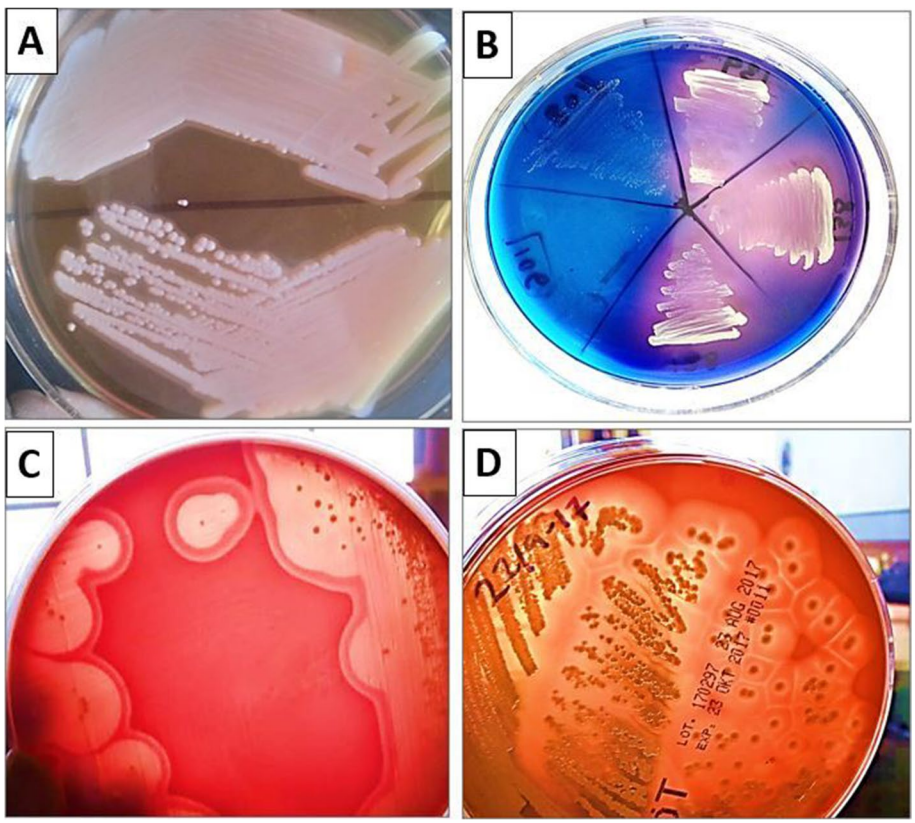


Fig. 2 Shows a positive lecithinase test (**A**), positive DNase test: colonies surrounded by a pink zone (**B**), production of haemolysins tested using 5% sheep blood agar (**C** and **D**)

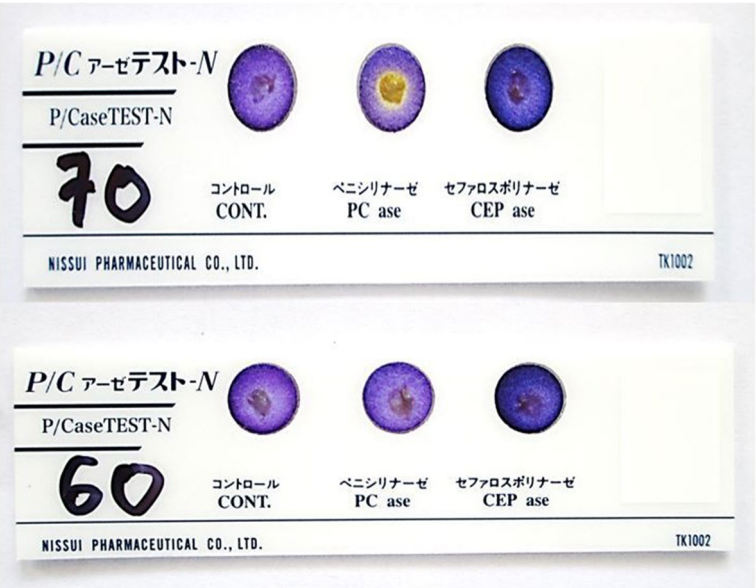
to yellow, whereas the negative tests panels retained the purple color (Fig. 3).

Genotypic detection of AMR genes

Conventional colony PCR [24], using gene-specific primer sets and reaction conditions borrowed from previous studies [25] was performed. Staphylococci isolates, both susceptible and resistant to either tetracycline and sulphonamides, were tested. The procedure involved recovery of bacteria in nutrient broth, DNA extraction, conduction of PCR, agarose gel electrophoresis, and visualization of PCR products. Known positive DNA containing the targeted genes served as a positive control during PCR runs, with products visualized on 2% agarose gels. Isolates that were phenotypically resistant to cefoxitin were further confirmed as methicillin-resistant staphylococci (MRS) by PCR detection of the *mecA* gene, as described in a previous study [26].

Data analysis

The data obtained were entered into Microsoft Excel and analysed using R software. Descriptive statistics were utilized to summarize the data, using percentages to inform the prevalence. For each bacterial group, the prevalence of multidrug resistance (MDR) was calculated as a proportion of isolates resistant to at least three different classes of antimicrobials out of the total number of isolates. The multiple antibiotic resistance index (MARI) for each isolate was calculated using the formula *c/d,* where *c* represents the number of antibiotics to which the isolate was resistant and *d* is the total number of antibiotics tested against the isolates [27]. The Pearson chi-square (X^2) test was used to assess significant differences ($p < 0.05$) between the proportions of resistance phenotypes and the variables such as bacteria, animal and infection type. However, Fischer's Exact was used for some cases where the expected cell counts were less than five. Bivariate logistic regression modelling was conducted to demonstrate associations between independent variable levels [(breed, region) and bacteria, animal, infection)] and the likelihood of dichotomous dependent variables [(staphylococcal mastitis) and (resistance phenotypes outcomes)]. The logistic regression odds ratios (OR) and their associated 95% confidence intervals were estimated to quantify the strength and direction of the associations with significance levels set at $p < 0.05$. To evaluate the appropriateness of the logistic regression model in predicting the binary outcomes, goodness of fit assessments were done using the Hosmer–Lemeshow test. A non-significant result ($p > 0.05$) indicated a good fit of the logistic regression model to the data. A one-way analysis of variance (ANOVA) test at 5% level of significance was done to compare the means of the MARI among more than two groups whereas the Mann–Whitney U test was



CONT. – control, PC ase – Penicillinase, CEP ase – Cephalosporinase

Fig. 3 Two strains identified numerically as 70 and 60 were subjected to the P/C アーゼテスト-N (P/Case TEST-N) assay for the detection of penicillinase (PC ase) and cephalosporinase (CEP ase). A yellow color change indicated a positive result as seen with penicillinase production by strain 70 whereas purple indicated negative penicillinase production as detected with strain 60. Both strains were negative for cephalosporinase production as depicted by no color change (disc remained purple). Observing no color change in the control verified that the test functioned as designed and can be relied on to interpret the results in the other panels (penicillinase and cephalosporinase)

used to compare the means of the MARI between two groups instead of the Student's t—test. Before selecting the Mann–Whitney U test, the assumptions such as homogeneity of variances (tested using Levene's test) and normality assessments (done using the Shapiro–Wilk test, visual inspection with box plots and the bell-shaped curve on histograms) were tested and found to be violated by the data. The Levene's statistic showed that the independent group variances were not equal ($p < 0.05$) and also the *p*-values for the Shapiro–Wilk tests were less than 0.05 depicting that the data was not normally distributed for the MARI between the two groups of the animal variable.

Results

Descriptive epidemiology of common staphylococcal diseases of animals in Uganda

Staphylococcal mastitis in cattle

A total of 299 clinical mastitis cases were presented to CDL accompanied by the following common clinical signs: milk discolouration (bloody, yellow) (Fig. 4C), reddened teats, swollen udder and teats, decreased milk production, difficulty in milk letting down, foul smelling discharge from quarters, and fever. Other complaints included decreased milk shelf life and milk rejection at the bulk tank milk collection centres. In some instances, the farmers had also tried treatment, but the outcomes were negative.

Of these cases, 149 (49.8%) originated from central Ugandan districts (Buikwe = 2, Kalangala = 1, Kampala = 9, Kiboga = 4, Luweero = 1, Masaka = 13, Mityana = 1, Mpigi = 3, Mukono = 6, Wakiso = 107), 122 (40.8%) from western Uganda (Kabarole = 121, Kiruhura = 1), 6 (2.0%)from northern Uganda (Arua = 3, Gulu = 3) and 3 (1.0%)from eastern Uganda (Jinja = 2, Manafwa = 1). Nineteen cases (6.4%) were from unidentified districts or regions. The cattle breeds were crosses, local and exotic.

From the 299 milk samples submitted, 237 (79.3%) were culture positive for various pathogens. The prevalence of staphylococcal mastitis among the samples was 62.2% (186/299). The most commonly identified bacteria were *S. aureus* and coagulase-negative staphylococci (CNS).

The prevalence of staphylococcal mastitis varied by breed: 75.0% (12/16) in crosses, 56.8% (113/199) in exotic breeds, 25% (1/4) in local breeds and 75% (60/80) in other unidentified breeds. There was a significant association between breed and the occurrence of staphylococcal mastitis ($X^2 = 11.528$, df = 3, $p = 0.009$). However, the logistic regression analysis found no association between the breed variable levels and the likelihood of contracting staphylococcal mastitis. The cross breeds had an OR of 9.0 [$p = 0.089$, 95% CI = 0.72–113.02] and exotics had an OR of 3.9 [$p = 0.238$, 95% CI = 0.40–38.56] when compared to the locals (reference breed).

The prevalence of staphylococcal mastitis by region was 51.7% (77/149), 33.3% (1/3), 50.0% (3/6), 75.4% (92/122) and 68.4% (13/19) in samples from central, eastern, northern, western and unidentified regions, respectively. There was a significant association between region and the occurrence of staphylococcal mastitis ($X^2 = 17.828$, df = 4, $p = 0.001$). By logistic regression, the western region cattle had 3.7 times higher odds [$p < 0.001$, CI = 2.13–6.32] of contracting staphylococcal mastitis compared those in the central region (reference region). In contrast, cattle in the eastern region had an OR of 0.6 [$p = 0.665$, 95% CI = 0.05–6.61], and no statistic could be computed for cattle in the northern region [$p = 0.999$, 95% CI = not applicable].

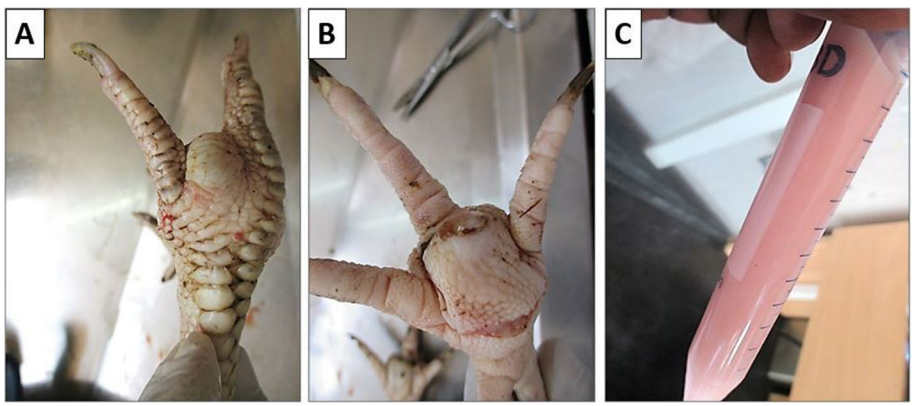


Fig. 4 Shows a bird case of bumble foot (plantar pododermatitis) in chicken characterized by massive swelling and abscesses in the digital pads (**A**, **B**) and abnormal colouration (bloody milk) of a milk sample submitted for diagnosis of mastitis causative bacteria by culture (**C**)

Cases of bumble foot (plantar pododermatitis) in poultry

In this study, we identified three cases of bumble foot out of 873 poultry cases submitted for laboratory diagnosis during the study period. On October 18, 2019, three live broiler chickens were submitted to the CDL from a commercial poultry farm in Mukono, where they were part of a flock of 30,000 birds. Although the chickens did not exhibit serious clinical signs, they showed reduced activity. No prior treatment had been administered. During necropsy, all birds were found to have massive swelling and abscesses in the digital pads of both legs (Fig. 4A and B). The swollen sites were surgically opened, and swab samples were collected for bacterial culture and sensitivity, which confirmed the presence of *S. aureus.*

Staphylococcal diseases in companion animals

Cats – Records from 19 cases were reviewed. Two cases submitted samples from which coagulase-negative staphylococci (CNS) was isolated. In both cases, the cats lived with owners residing in Kampala. Case one: A 6-year-old male cat of unknown breed, with an aspirate sampled from an unspecified site. Case two: A domestic short hair cat, with unknown sex, from which a lymph node aspirate, ear swab, and skin scrapping were sampled. No clear history or clinical signs were provided by the sample submitters.

Dogs – records from 137 cases were reviewed. The majority were male, with breeds including basenji, boerboel, German shepherd, labrador retriever, local, maltese and pitbull. All dog owners were based in Kampala. The prevalence of staphylococcal skin, ear, vaginal, nasal, eye, gastrointestinal, and nonhealing wound infections was 19.7% (27), 5.8% (8), 1.5% (2), 0.7% (1), 1.5% (2), 1.5% (2) and 0.7% (1), respectively. The commonly isolated bacteria in all these infections were *S. aureus*, followed by CNS.

Skin infections were accompanied by clinical signs, including alopecia, pruritus, sores and pus from wounds due to scratching. For diagnosis, skin or wound swabs and skin scrapping samples were cultured. Ear infections were accompanied by clinical signs of irritation, pruritus, and secretion of abnormal discharge from the ears. Diagnosis was confirmed through ear swab cultures. In the cases of suspected vaginal infection, dogs presented with abnormal discharge from the vagina, and vaginal swabs were collected for diagnosis. In the cases of suspected nasal infection, dogs had abnormal bloody discharge from the nose, and nasal swabs were collected for culture. Eye infections were accompanied by clinical signs of major creamy white ocular discharges, and eye swab samples were cultured to ascertain the causative pathogens. In the cases of suspected gastrointestinal disease, dogs presented with diarrhea, and fecal samples were cultured. Unidentified staphylococci were isolated from cases of skin, ear, and vaginal infections.

Infection management with antimicrobials

Mastitis records indicated that on some occasions, farmers had used single antibiotic (penicillin or gentamicin) and combination antibiotic therapies (penicillin-tetracycline or neomycin-gentamicin or penicillin-gentamicin or penicillin–streptomycin or gentamicin-penicillin–streptomycin) to manage the infections.

For the other diseases in companion animals, we did not find records of previous case management with antimicrobials.

Proteomic-based bacterial identification by MALDI-TOF MS

Out of the 111 staphylococci tested by MALDI-TOF MS, 79 (71.2%) were identified as *S. aureus,* 27 (24.3%) as *S. pseudintermedius* and 5 (4.5%) as *S. schleiferi* (Table 1). These bacteria were diagnosed from cases of mastitis by milk culturing, skin infections using skin swabs sampled from the infected sites, ear infections using ear swabs and vaginal infections after culturing vaginal discharge from the animals.

Expression of virulence factors

All the strains cultured showed haemolysis on 5% sheep blood agar. The prevalence of strains exhibiting beta haemolysis was 4.5% (5/111), whereas 106 strains (95.55%) expressed both alpha and beta haemolysins. Lecithinase

Table 1 Bacterial isolates distribution by host, clinical case and sample type

Variable	*S. aureus* (*n*=79)	*S. pseudintermedius* (*n* = 27)	*S. schleiferi* (*n* = 5)
Sample type			
Ear swab	3	3	0
Milk	60	4	1
Skin swab	16	18	4
Vaginal discharge	0	2	0
Animal species			
Cattle	60	4	1
Dog	19	23	4
Clinical condition			
Skin infection	16	18	4
Mastitis	60	4	1
Ear infection	3	3	0
Vaginal infection	0	2	0

activity was detected in 35.9% (14/39) of the isolates. The prevalence of beta-lactamases was 89.7% (35/39) for penicillinase and 0.0% (0/39) for cephalosporinase. The prevalence of isolates expressing DNases was 87.2% (34/39).

Antimicrobial resistance in pathogenic staphylococci

Among the general staphylococci, the majority of the strains were resistant to penicillin (88.5%), ampicillin (88.5%), tetracycline (57.7%), and chloramphenicol (46.2%). Minimal resistance was noted with cloxacillin (0.0%), ciprofloxacin (9.6%), and cefoxitin (3.8%). The prevalence of MDR was 78.8% for general staphylococci, 82.2% for *S. aureus*, 73.1% for *S. pseudintermedius*, and 60.0% for *S. schleiferi*. The X^2 test revealed a significant difference between the proportions of penicillin resistant isolates by species of *Staphylococcus* ($p=0.007$). *Staphylococcus aureus* were 7.4 and 11.5 times more likely to be ampicillin and penicillin resistant respectively, when compared to *S. schleiferi* (Table 2). The average MARI value of the staphylococci was 0.366, and a non-significant difference (F = 2.527, df between groups = 2, $p=0.085$) was found between the mean MARI of *S. aureus* (0.366), *S. pseudintermedius* (0.396) and *S. schleiferi* (0.220) isolates.

Antimicrobial resistance in pathogenic staphylococci from cattle and dogs

The prevalence of penicillin, ampicillin, and multidrug resistant staphylococci was significantly higher in the cattle isolates compared to the dog isolates, whereas trimethoprim sulphamethoxazole resistance was higher in the dog isolates ($p<0.05$). Staphylococci from cattle were 7.8 and 4.6 times more likely to be ampicillin and multidrug resistant, respectively, when compared to the dog strains. Also, cattle isolates cases were 0.3 times less likely to show trimethoprim sulphamethoxazole resistance compared to the dog bacteria (Table 3). To compare the mean MARI between cattle staphylococci (mean: 0.374) and the dog isolates (mean: 0.357), a Mann–Whitney U test was performed, revealing a non-significant difference ($p=0.579$).

Antimicrobial-resistant staphylococci causing mastitis and skin, ear and vaginal infections in animals

The X^2 test revealed a significant difference between the proportions of ampicillin, trimethoprim sulphamethoxazole, penicillin, and multidrug resistant staphylococci isolated from the various clinical conditions ($p<0.05$). Staphylococci from skin infections were 0.2 times less likely to be ampicillin and multidrug resistant when compared to the mastitis isolates. Also, isolates from skin infections were 2.6 times more likely to be trimethoprim sulphamethoxazole resistant compared to the mastitis causing strains (Table 4). The one-way ANOVA revealed a non-significant difference (F = 1.087, df between groups = 3, $p=0.358$) in the mean MARI of staphylococci isolated from mastitis (0.374), skin (0.347), ear (0.350) and vaginal (0.550) infections.

Prevalence of methicillin-resistant staphylococci (MRS)

Of the 111 isolates, the prevalence of MRS detected by phenotypic resistance to cefoxitin was observed in 4 strains (3.8%). Among these, methicillin-resistant *S. aureus* (MRSA) accounted for 1 strain (1.4%) out of 73 *S. aureus* isolates, and methicillin-resistant *S. pseudintermedius* (MRSP) accounted for 3 strains (11.5%) out of 26 *S. pseudintermedius* isolates. All the MRS strains were isolated from cases of skin infections in dogs.

Bacterial genetics of tetracycline and methicillin resistance

A total of 48 genomic DNA samples were screened for *tet* genes (*M, K, O, L*). Of the tested strains, 43.8% (21/48) had one gene, 6.3% (3/48) had 2 *tet* genes and none had more than 2 genes detected. Among the 30 that showed tetracycline phenotypic resistance, 17 (56.7%) tested positive for the selected *tet* genes, while 13 (43.3%) did not have *tet* genes. The *tetK* gene was the most predominant, with a 35.4% (17/48) prevalence, followed by *tetM* with a 25.0% (12/48) prevalence. The least frequently detected genes were *tetL* and *tetO,* with a 0.0% prevalence. The *tetM* gene was also more prevalent in cattle isolates (29.2%, 7/24) than in dog isolates (20.8%, 5/24). The highest prevalence of the *tetM* gene was found in the isolates from mastitis (29.2%, 7/24), followed by the dermatitis isolates (22.2%, 4/18). The highest prevalence of *tetM* was found in the mastitis condition isolates (45.8%, 11/22), followed by skin infection-causing staphylococci (27.8%, 5/18). The *tetK* gene was also more prevalent in cattle isolates (45.8%, 11/24) than in dog isolates (25.0%, 6/24).

Only four isolates that were resistant to cefoxitin in the primary collection of staphylococci were screened for the *mecA* gene. All these strains tested positive, yielding 3.8% MRS, in concordance with the phenotypic screening results. Agarose gel pictures of amplified AMR genes are presented in Additional file 1.

Discussion

Diagnostics in disease surveillance

Infectious diseases place an offensive social economic burden on low-middle income countries (LMICs); thus, national disease control programmes have the hard task of allocating limited budgets for interventions in their context [28]. Diagnostic interventions have emerged as a cornerstone in infectious disease surveillance, and these employ both dry and wet laboratory techniques to understand pathogen features and molecular characteristics.

Table 2 Antimicrobial resistant bacteria in the archived staphylococci collection

Antibiotic	Bacteria	Resistance[a]	X^2 *p*-value[b]	Odds ratio (OR)	95% CI for OR
Ciprofloxacin	Staphylococci	10 (9.6)	0.342		
	S. aureus	9 (12.3)		N/A	N/A
	S. pseudintermedius	1 (3.8)		N/A	N/A
	S. schleiferi	0 (0.0)		1.0	Ref
Cloxacillin	Staphylococci	0 (0.0)	c	C	c
	S. aureus	0 (0.0)		C	c
	S. pseudintermedius	0 (0.0)		C	c
	S. schleiferi	0 (0.0)		C	c
Ampicillin	Staphylococci	92 (88.5)	0.077		
	S. aureus	68 (91.8)		7.4	1.03-53.62
	S. pseudintermedius	22 (84.6)		3.7	0.45-29.42
	S. schleiferi	3 (60.0)		1.0	Ref
Trimethoprim sulphamethoxazole	Staphylococci	40 (38.5)	0.050		
	S. aureus	26 (35.6)		N/A	N/A
	S. pseudintermedius	14 (53.8)		N/A	N/A
	S. schleiferi	0 (0.0)		1.0	Ref
Tetracycline	Staphylococci	60 (57.7)	0.111		
	S. aureus	41 (56.2)		5.1	0.55-48.12
	S. pseudintermedius	18 (69.2)		9.0	0.86-93.83
	S. schleiferi	1 (20.0)		1.0	Ref
Chloramphenicol	Staphylococci	48 (46.2)	0.653		
	S. aureus	32 (43.8)		1.2	0.18-7.43
	S. pseudintermedius	14 (53.8)		1.8	0.25-12.28
	S. schleiferi	2 (40.0)		1.0	Ref
Penicillin	Staphylococci	92 (88.5)	0.007*		
	S. aureus	69 (94.5)		11.5	1.48-89.65
	S. pseudintermedius	20 (76.9)		2.2	0.25-12.28
	S. schleiferi	3 (60.0)		1.0	Ref
Gentamicin	Staphylococci	35 (33.7)	0.505		
	S. aureus	22 (30.1)		0.6	0.10-4.15
	S. pseudintermedius	11 (42.3)		1.1	0.16-7.74
	S. schleiferi	2 (40.0)		1.0	Ref
Cefoxitin	Staphylococci	4 (3.8)	0.062		
	S. aureus	1 (1.4)		N/A	N/A
	S. pseudintermedius	3 (11.5)		N/A	N/A
	S. schleiferi	0 (0.0)		1.0	Ref
MDR	Staphylococci	82 (78.8)	0.355		
	S. aureus	60 (82.2)		3.1	0.47-20.31
	S. pseudintermedius	19 (73.1)		1.8	0.25-13.21
	S. schleiferi	2 (60.0)		1.0	Ref

MDR multidrug resistance, *N/A* statistics not presented where model did not fit the data well

[a] The proportions were computed from a total of 104 staphylococci, 73 *S. aureus, 26 S. pseudintermedius* and 5 *S. schleiferi* isolates, X^2 - Chi square test

[b] general staphylococci are excluded from this analysis

[c] no statistic computed

*statistically significant at $p < 0.05$

It is believed that new innovations in the use of metadata generated by high-throughput technologies could enhance our understanding of public health events.

High-throughput technologies generate large-scale data related to omics analyses, such as genomics, transcriptomics, proteomics, phenomics and metabolomics

Table 3 Comparison of AMR in pathogenicstaphylococcifrom cattle and dogs

Antibiotic	Animal	Resistance, n (%)[a]	X^2 *p*-value	Fisher's exact *p-value*	Odds ratio (OR)	95% CI for OR
Ciprofloxacin	Cattle	8 (13.8)	c	0.179	3.5	0.71-17.46
	Dogs	2 (4.3)			1.0	Ref
Cloxacillin	Cattle	0 (0.0)	c	c	C	c
	Dogs	0 (0.0)			C	c
Ampicillin	Cattle	56 (96.6)	0.004*	c	7.8	1.61-37.57
	Dogs	36 (78.3)			1.0	Ref
Trimethoprim sulphamethoxazole	Cattle	16 (27.6)	0.010*	c	0.3	0.15-0.79
	Dogs	24 (52.2)			1.0	Ref
Tetracycline	Cattle	36 (62.1)	0.310	c	N/A	N/A
	Dogs	24 (52.2)			1.0	Ref
Chloramphenicol	Cattle	25 (43.1)	0.483	c	0.8	0.34-1.64
	Dogs	23 (50.0)			1.0	Ref
Penicillin	Cattle	58 (100.0)	< 0.001*	c	N/A	N/A
	Dogs	34 (73.9)			1.0	Ref
Gentamicin	Cattle	19 (32.8)	0.496	c	1.1	0.48-2.48
	Dogs	16 (34.8)			1.0	Ref
Cefoxitin	Cattle	0 (0.0)	c	0.035*	N/A	N/A
	Dogs	4 (8.7)			1.0	Ref
MDR	Cattle	52 (89.7)	0.002*	c	4.6	1.63-13.08
	Dogs	30 (65.2)			1.0	Ref

MDR multidrug resistance, *N/A* statistics not presented where model did not fit the data well

[a] The proportions were computed from 58 cattle and 46 dog isolates, X^2 - Chi square test

[c] no statistic computed

*statistically significant at $p < 0.05$

[29]. These technologies complement the dry laboratory approaches, which utilize computational approaches such as bioinformatics, that also relies on sequencing data [30]. However, these may not be widely adopted in LMICs public health management systems due to resource constraints.

As we progress step by step, diagnostic settings could adopt the approach of combined wet laboratory phenotypic and genotypic-based technologies to understand the epidemiology of infections in both animals and humans, as demonstrated by this study. This is the first study to showcase the application of MALDI-TOF MS proteomics technology (an emerging real-time tool) in identifying pathogenic staphylococci in Uganda.

Staphylococci infections, hosts and strains

The study revealed several staphylococcal diseases of animals, including mastitis in cattle, podo dermatitis in chickens and skin, ear, vaginal, nasal, eye, gastrointestinal, and nonhealing wound infections in companion animals (dogs and cats). The most prevalent diseases were mastitis in cattle and skin infections in dogs. Previous studies have primarily focused on diseases caused by *S. aureus* in animals, consistently finding that mastitis and skin infections, such as pyoderma, are prevalent. The ability of staphylococci to cause infections is mediated by a variety of virulence factors. Staphylococci, particularly *S. aureus,* are known to produce DNase which hydrolyses extracellular host DNA into smaller fragments, thus enhancing their capability to colonize, invade, and persist inside tissues. The enzyme also promotes biofilm disruption, immune evasion, tissue invasion, and modulation of the host immune response. Over 80% of the strains expressed DNases, as seen with isolates tested in a previous study [31]. Lecithinase enhances staphylococci virulence by breaking down host cell membranes, thus facilitating tissue invasion. It also destroys immune cells and inhibits phagocytosis, aiding immune evasion. Additionally, it supports biofilm formation by improving adhesion and providing a protected environment, leading to persistent and chronic infections. A slightly lower prevalence of lecithinase producers was detected compared to a previous study [23]. This could be due to differences in the settings or environments in which the strains were isolated and the methods used. The production of haemolysins is another vital virulence factor that enables the bacterium to obtain nutrients such as iron from host blood, cause tissue damage and invasion, evade

Table 4 Antimicrobial resistance in staphylococcicausing mastitis and skin, ear and vaginal infections in animals

Antibiotic	Clinical condition	Resistance[a]	X^2 *p*-value	Odds ratio (OR)	95% CI for OR
Ciprofloxacin	Mastitis	8 (13.8)	0.416	N/A	N/A
	Skin infection	2 (5.3)		N/A	N/A
	Ear infection	0 (0.0)		N/A	N/A
	Vaginal infection	0 (0.0)		1.0	Ref
Cloxacillin	Mastitis	0 (0.0)	c	c	c
	Skin infection	0 (0.0)		c	c
	Ear infection	0 (0.0)		c	c
	Vaginal infection	0 (0.0)		c	c
Ampicillin	Mastitis	56 (96.6)	0.002*	1.0	Ref
	Skin infection	31 (81.6)		0.2	0.03-0.81
	Ear infection	3 (50.0)		0.0	0.00-0.30
	Vaginal infection	2 (100.0)		c	c
Trimethoprim sulphamethoxazole	Mastitis	56 (96.6)	0.036*	1.0	Ref
	Skin infection	31 (81.6)		2.6	1.11-6.19
	Ear infection	3 (50.0)		2.6	0.48-14.38
	Vaginal infection	2 (100.0)		c	c
Tetracycline	Mastitis	36 (62.1)	0.393	N/A	N/A
	Skin infection	19 (50.0)		N/A	N/A
	Ear infection	3 (50.0)		N/A	N/A
	Vaginal infection	2 (100.0)		1.0	Ref
Chloramphenicol	Mastitis	25 (43.1)	0.058	1.0	Ref
	Skin infection	16 (42.1)		0.8	0.05-12.71
	Ear infection	6 (100.0)		0.7	0.04-12.52
	Vaginal infection	1 (50.0)		c	c
Penicillin	Mastitis	58 (100.0)	< 0.001*	N/A	N/A
	Skin infection	29 (76.3)		N/A	N/A
	Ear infection	3 (50.0)		N/A	N/A
	Vaginal infection	2 (100.0)		1.0	Ref
Gentamicin	Mastitis	19 (32.8)	0.168	N/A	N/A
	Skin infection	11 (28.9)		N/A	N/A
	Ear infection	3 (50.0)		N/A	N/A
	Vaginal infection	2 (100.0)		1.0	Ref
Cefoxitin	Mastitis	0 (0.0)	0.065	N/A	N/A
	Skin infection	4 (10.5)		N/A	N/A
	Ear infection	0 (0.0)		N/A	N/A
	Vaginal infection	0 (0.0)		1.0	Ref
MDR	Mastitis	52 (89.7)	0.009*	1.0	Ref
	Skin infection	25 (65.8)		0.2	0.08-0.65
	Ear infection	3 (50.0)		0.1	0.02-0.71
	Vaginal infection	2 (100.0)		c	c

MDR multidrug resistance, *N/A* statistics not presented where model did not fit the data well

[a] The proportions were computed from 58 mastitis, 38 skin, 6 ear, 2 vaginal infection isolates, X^2 - Chi square test

[c] no statistic computed

*statistically significant at $p < 0.05$

the host immune response, and exert cytotoxic effects on host cells [32]. All strains secreted at least one of the beta or alpha haemolysins. In this study, we only tested a few properties due to resource setbacks; thus, further studies could venture into either PCR-based detection or high-throughput sequencing of pathogenic strains to give more robust data on virulence genes of local staphylococci isolates.

The strains identified by MALDI-TOF MS technology were isolated from cattle and dogs. *Staphylococcus aureus* from mastitis was the prevalent pathogenic staphylococci of cattle in the isolates collection, a finding consistent with recent research [33, 34]. *Staphylococcus pseudintermedius* was the common perpetrator of dog skin infections, as explained elsewhere [4]. This study also detected *S. pseudintermedius* in cases of mastitis, although this strain is primarily hosted by dogs [35]. This could suggest possible spillovers between companion and food animals at homestead interfaces. We also detected *S. schleiferi* in clinical infections of dogs, although it is a rare bacterium compared to commonly identified species such as *S. pseudintermedius* and *S. aureus* in cats and dogs [36].

The emergence of AMR in bacteria can be associated with the irrational use of antimicrobials in humans and animals [37]. Multiple antibiotic resistance indexing has been shown to be a cost-effective and valid method of bacterial source tracking [38]. The average MARI value across the general and various staphylococci groups was above 0.2, a signal that most strains originated from potentially dangerous sources where antibiotics are regularly used. The majority of the staphylococci were resistant to penicillins, as reported in other studies [39, 40]. This could be attributed to penicillinase (detected in over 80% of the isolates), which confers resistance to beta-lactam antibiotics (such as penicillin and ampicillin) by enzymatically inactivating these antibiotics [41, 42]. Minimal to no resistance to cloxacillin (0.0%), ciprofloxacin (9.6%) and cefoxitin (3.8%) was observed. This could be due to the uncommon use (misuse or overuse) of these drugs in cattle and dogs, specifically in infection management.

Among staphylococci from dogs, AMR was high against commonly used antimicrobials, such as penicillins, tetracycline and trimethoprim sulphamethoxazole, similar to findings from previous studies [43, 44]. Among the dog collection, the prevalence of MDR was higher than in other studies [40, 43], particularly because those studies worked with nonclinical strains.

The majority of the cattle isolates were *S. aureus* from cases of mastitis. In this group, resistance was also high against commonly used antimicrobials, such as penicillins, tetracycline and trimethoprim sulphamethoxazole, a finding consistent with previous studies [33, 45–47].

This study detected community-associated MRS in dog isolates. The rise of community- or hospital-associated MRSA and MRSP has a significant health impact on veterinary health practitioners and pet owners [48]. However, the study lacked human isolates, preventing the execution of comparative genetic relatedness tests essential for fully elucidating the likelihood of transmission between humans and animals. Although the occurrence of MRSA and MRSP in humans and animals has been reported in many other countries, information on animals such as dogs in Uganda is limited, if not absent. To the best of our knowledge, this is the first report of pathogenic MRSP and MRSA affecting dogs in Uganda.

The prevalence of MRSP in this study was higher than that reported by Rana et al. [48] but lower than that from previous studies [40, 49]. The prevalence of MRSA in dogs was lower than rates reported in previous studies [48, 50]. These variations could plausibly be explained by the difference in geographical locations. In this study, we did not follow up to screen MRS colonization in humans living in proximity to infected dogs, which further studies can study to fill gaps in zoonotic transmission dynamics.

The observed putative discrepancy between cefoxitin and cloxacillin resistance in our study unveils the complexity of MRS dynamics. While cefoxitin resistance serves as a surrogate marker for MRS [51], strains resistant to cefoxitin surprisingly exhibited susceptibility to cloxacillin. This suggests that the conventional assumption of perfect concordance in predicting methicillin resistance with different Isoxazolyls or penicillinase-stable penicillins (like oxacillin, cloxacillin, dicloxacillin, nafcillin and flucloxacillin) may not hold true in all cases. The presence of *mecA*, the primary genetic determinant of methicillin resistance [52], did not uniformly confer resistance to cloxacillin, despite the fact that strains having *mecA* are often resistant to almost all beta lactams [53, 54]. This can be attributed to factors such as genetic variability among strains, heterogeneous resistance [55], variability in penicillin binding protein 2a expression levels and protection towards various antibiotics [56], the presence of other unknown resistance mechanisms [57] and limitations of invitro testing methods.

Future research should delve into the molecular underpinnings of this phenomenon, extending beyond *mecA*, to refine diagnostic and therapeutic strategies in the face of evolving antibiotic resistance in staphylococci.

The tetracycline resistome is characterized by over 40 determinants that fall into three major categories: ribosomal protection proteins, active efflux pumps, and enzymatic inactivation. Amongst staphylococci, resistance can be mediated by ribosome protection encoded by *tetM*, *tetO*, *tetS*, and *tetW* genes, by efflux pumps encoded by *tetK*, *tetL*, *tet38*, and *tet42* genes, and by an unknown mechanism encoded by *tetU* [58]. In this study, we investigated tetracycline resistance genes encoding ribosome protection and efflux pump proteins among the strains. The most prevalent genes were *tetK* and *tetM*, a similar finding to previous studies [59, 60].

Implications for global health priorities

Pathogens can spread rapidly through the globalized system of travel, trade, and food distribution, thus posing a threat to the entire world [61]. They recognize no borders. "A threat anywhere is a threat everywhere", as Kathleen Sebelius, former United States Secretary of Health and Human Services, aptly stated. Supporting this, a previous report explains how various MRS clones have spread between communities, hospitals and countries [62].

According to the Global Health Security (GHS) Index released in November 2019, the first detailed assessment and benchmarking of 195 countries, less than 20% of countries were fully prepared to detect and respond to disease threats [63]. This prompted the launch of the new Global Health Security Agenda (GSHA) to support non-compliant countries. The agenda aims to prevent, detect, and respond to infectious disease threats, thus promoting security as an international priority. Among the global health priorities of the GHSA include AMR and zoonoses control (more details concerning the GHSA can be found at https://globalhealthsecurityagenda.org/).

The carriage of critically important antimicrobial-resistant bacteria in animals, such as MRS (also zoonotic), detected in this study is a threatens global health. The World Health Organization reports worrying statistics: "people with MRSA are 64% more likely to die than people with a non-resistant form of the infection." To control these noxious bugs, country health teams should invest in strengthening resistance tracking and laboratory capacity, which is still lacking in LMICs, including Uganda. In this manuscript, we recommend using integrated diagnostics (a cost effective option) to better understand the epidemiology of infectious diseases and their causative microbes, as we work towards the acquisition of currently fancied high-throughput technologies.

Conclusions

The present study highlighted some of the major staphylococcal diseases affecting animals in the Ugandan context. Mastitis emerged as the most prevalent among the food animal category (specifically cattle) while staphylococcal skin infections predominated among companion animals (particularly dogs). Using a cocktail of diagnostic tools, we enhanced our understanding of circulating pathogenic staphylococci strains in Uganda's animal population. This approach proves more sustainable for conducting infectious disease surveillance in resource-constrained environments. From a One Health perspective, the emergence of lethal strains of resistant bacteria, such as MRS in companion animals justifies the need for continuous AMR surveillance but also threatens global health due to a likelihood of reduced treatment options in the case of spillover of staphylococcal zoonoses into human populations.

Abbreviations

CDL	Central Diagnostic Laboratory
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
DNase	Deoxyribonuclease
GHS	Global health security
GHSA	Global health security agenda
MARI	Multiple antibiotic resistance index(es)
MDR	Multidrug resistant (resistance)
MRS	Methicillin resistant staphylococci
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSP	Methicillin resistant *Staphylococcuspseudintermedius*
AMR	Antimicrobial resistance
PCR	Polymerase chain reaction
COVAB	College of Veterinary Medicine, Animal Resources and Bio-Security
CLSI	Clinical Laboratory Standards Institute
MARI	Multi antibiotic resistance index
X^2	Chi-squared
ANOVA	Analysis of variance
CNS	Coagulase negative staphylococci

Supplementary Information

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Additional file 1. Agarose gel pictures of amplified AMR genes.

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Authors' contributions

This work was done in collaboration amongst all authors. Steven Kakooza (SK), Esther Nabatta (EN), Wilfred Eneku (WE), Paul Ssajjakambwe (PS), Eddie M. Wampande (EMW), Dickson Ndoboli (DN), Sayaka Tsuchida (ST), Kazunari Ushida (KU), Ken'ichi Sakurai (KS) and Francis Mutebi (FM) conceptualized and designed this study. Esther Nabatta (EN), Mariam Wanyana (MW), Damien F. Munyiirwa (DFN), Dorcus Namuyinda (DN), Grace Athieno (GA), Edrine Kayaga (EK), Rodney Okwasiimire (RO) performed the laboratory experiments. SK conducted data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

All the data presented by this study have been submitted with this research paper. Raw data and any other forms data generated by this research can be obtained from the corresponding author upon request by e-mail.

Declarations

Ethics approval and consent to participate

Permission to access the Central Diagnostic Laboratory bacteria bank and the disease database was granted by the laboratory management committee.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Central Diagnostic Laboratory, College of Veterinary medicine, Animal resources and Biosecurity, Makerere University, Kampala, Uganda. [2]Department of Veterinary Pharmacy, Clinics and Comparative Medicine, College of Veterinary Medicine, Animal Resources and Bio-Security Makerere University, Kampala, Uganda. [3]National Animal Disease Diagnostic and Epidemiology Centre, Entebbe, Uganda. [4]College of Bioscience and Biotechnology, Biotechnolorere University, Aichi, Japan. [5]Faculty of Life and Environmental Sciences, Department of Animal Sciences, Teikyo University of Science, Tokyo, Japan.

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