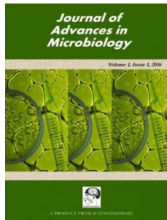

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# High Rates of Methicillin-Resistant *Staphylococcus aureus* Colonization of Domesticated Swine of Kabale District – Southwestern Uganda

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author BA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AK and OT managed the analyses of the study. Author BJ managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** *S. aureus* is a commensal mammalian pathogen which can establish itself as part of the skin flora. However, can eventually cause infections and invasive diseases in both hospital and community settings. Livestock-associated Methicillin-resistant *S. aureus* remains a major concern to public health. This study investigated the rates of methicillin resistance *S. aureus* (MRSA) colonization and respective antibiotic resistance profiles in domestic pigs in Kabale District - South Western Uganda.

**Method:** This was a cross-sectional study conducted between June 2016 and February 2017 in which nasal swabs from 585 pigs from 147 homesteads were collected and cultured using standard

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microbial techniques to isolate *S. aureus* and phenotypically screen for MRSA using Cefoxitin disc. MecA polymerase chain reaction (PCR) was used to confirm MRSA. Antimicrobial susceptibility testing was performed using the Kirby Bauer technique to determine antimicrobial susceptibility pattern among MRSA towards to the commonly used antibiotics in the region.
**Result:** From the five hundred and eighty-five (585) pigs, 172 (29.4%) were MRSA. There was high antibiotic resistance among MRSA isolates was observed against Sulfamethoxazole – trimethoprim was 170(99%), Erythromycin; 154(89%), Ciprofloxacin 124(72%), Clindamycin; 121(70), Tetracycline; 121(70%), Gentamycin; 84(49%), Rifampicin; 40(23%); Cefipime; 40(23%) and Vancomycin; 03(2%).
**Conclusion:** The observed high rate of MRSA colonization among domestic pigs is of a significant public health concern in Kabale region. A greater number of MRSA isolates were highly resistant to commonly used antibiotics.

*Keywords: MRSA; Swine; Uganda.*

## 1. INTRODUCTION

*S. aureus* is a commensal mammalian pathogen, which establishes itself to the skin as a flora, and eventually causes infections and invasive diseases to both hospital and community settings [1,2]. *S. aureus* is never an absolute commensal since it turns to be pathogenic to man and many homoeothermic species [3]. The invasiveness of this species is enhanced by its capacity to produce a complex arsenal of toxins and ability to acquire resistance to multiple antimicrobials [4,5]. *Staphylococcus aureus* acquisition of mobile genetic element, staphylococcal cassette chromosome (SCC*mec*) carrying *mecA* gene which encodes for an altered PBP – PBP2a/PBP2′ which results into a high level of resistance to β-lactam antibiotics in MRSA [6]. The expressed protein, which is a transpeptidase, catalyzes cell-wall crosslinking in the face of the challenge by β-lactam antibiotics. This results into a reduced affinity for β-lactam antibiotics and therefore, cell wall biosynthesis in MRSA strains continues even in the presence of otherwise inhibitory levels of β-lactam antibiotics. The activity of this protein is regulated by allostery at a site 60 Å distant from the active site, where crosslinking of cell wall takes place [7]. Methicillin-resistant *S. aureus* (MRSA) is of increasing importance in hospitals [8] the community [9] and livestock farming [10]. To date, livestock-associated MRSA (LA-MRSA) has been found worldwide, particularly among people who are involved with livestock farming [11]. Of particular concern, is the MRSA which causes difficult-to-treat infections leading to increased length hospitalization and mortality [12,13]. The widespread emergence of MRSA has continued to be a serious global public health concern especially in hospitals [14] communities [15] and in animals as livestock-associated Methicillin-resistant *S. aureus* (LA – MRSA) [16]. Animal-to-human transmission of MRSA have been reported and the high prevalence of MRSA puts the community at high risk to invasive and hard to treat infections [17]. Although there is an increasing level of swine domestication as a source income in Uganda [18,19]. There is no data on colonization of these animals with S. aureus including MRSA. This study was conducted to determine the colonization rates of *S. aureus* including MRSA and to determine the resistance patterns of the bacterial strains to the common antibiotics used to treat human infections.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

This was a cross-sectional study conducted between June 2016 and February 2017 and involved147 homesteads in Kabale district with domestic pigs. After farmers consented, nasal swabs from 585 pigs were collected using sterile cotton-tipped swabs and transported in Amie's transport medium (Hi-Media, Mumbai) for bacteriological analysis in Microbiology laboratory located at Uganda national health laboratories (UNHLS), formerly Central public health laboratories (CPHL).

### 2.2 Bacteriological Analysis

This involved immediate inoculation of the swabs into 7.5% NaCl (w/v) in Nutrient broth (NB) (Difco, Detroit, MI) with a slight modification of Chatterjee et al. methodology (20) and incubated overnight for enrichment to *S. aureus.* A discrete colony growth on the NB was sub cultured onto mannitol salt agar (Difco, Detroit, MI) and further incubated aerobically at 37ºC for 24 hours.

*S.aureus* growth was identified as red colonies [21], which were further characterized by catalase, tube coagulase [22,23] and DNase test [24,25] (Hi-Media, Mumbai). API STAPH (bioMerieux, Inc., Durham, NC) was used following manufacturer's instructions [26] to phenotypically confirm *S. aureus.* The isolates were screened for methicillin resistance using Cefoxitin 30 ug disks diffusion techniques as described in the CLSI, 2015 guidelines [27]. To achieve this, a suspension of *S. aureus* isolates equivalent to 0.5 McFarland was inoculated onto Mueller-Hinton agar followed by application of Cefoxitin (30 µg) disk and the inoculated plates were incubated aerobically at 35ºC for 18-24 hours. The zone of inhibition diameter around Cefoxitin (30 µg) disk was measured using a Vernier calliper and results recorded and compared with the CLSI, 2015 standards. Any *S. aureus* isolates with zone diameter of ≤ 21 mm was considered to be MRSA while similar isolate with ≥ 22 mm was considered as methicillin sensitive *S.aureus* (MSSA) [27].

## 2.3 Molecular Testing

Phenotypic MRSA isolates were confirmed by PCR assay to detect mecA gene [28]. DNA was extracted from all the phenotypic MRSA previously sub-cultured on brain heart infusion (Difco Laboratories) agar plates as purity plates and incubated at 37$^{o}$C, after 24hours. One colony was suspended in 25 µl of sterile distilled water and the suspension was then placed in a 100ºC heat block for 15 min according to Pérez-Roth et al. [29]. A 5-µl volume was directly used as a template for PCR amplification. For MecA, the following primers were used ''MecA F; 5' TCC AAT TAC AAC TTC ACC AGG 3' and "MecA R; 5' CCACTTCATATCTTGTAA CG 3'" as described by Simone *et al* (30). PCR products were detected by gel (electrophoresis followed by visualization using UV transilluminator (Fig. 1).

## 2.4 Antimicrobial Susceptibility Testing

Isolates were tested for their antimicrobial susceptibility by disk diffusion test (31). The drugs tested were those commonly used in the Kabale region and recommended in the Uganda clinical guideline(UGC)(32), including Ciprofloxacin (CIP), chloramphenicol (C), clindamycin (CL), Erythromycin (E), gentamicin (CN), Rifampin (RIF), tetracycline (TE), Sulfamethoxazole - trimethoprim (SXT), Ceftaroline and vancomycin (V). A 0.5 McFarland standard suspension of all isolates was prepared and a lawn culture of bacteria seeded on to Mueller-Hinton agar plates (Difco, Detroit, MI) where a panel of antibiotic disks was placed according to Kirby Bauer disk diffusion technique

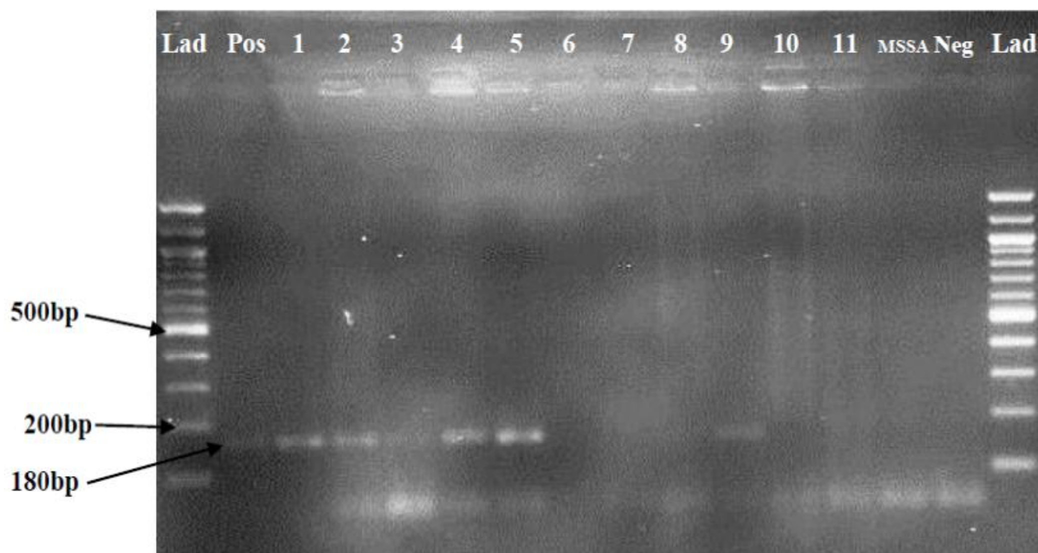


**Fig. 1. Results of mecA gene PCR where an Amplicon of approximately 180bp was expected. The lad is a 100bp ladder, Lane Pos. contains the Positive control (MRSA ATCC 43300), and Lanes 1, 2,3,4,5, and 9 contain MRSA isolates from swine. Lane 6, 7,8,10 and 11 were MSSA isolates from swine. MSSA contains a Methicillin-Susceptible S. aureus strain ATCC 25923, whereas Neg. is the Negative amplification control**

following recommendations in the Clinical Laboratory Standard Institute (CLSI 2015)(33) at 37ºC [27]. In order to be able to detect inducible clindamycin resistance (iCR), Clindamycin (2 ug) and Erythromycin (15ug) antimicrobial disks (Oxoid™) were positioned at a distance of 15mm (edge to edge) from each other during every run of Kirby Bauer disk diffusion test to determine induced clindamycin resistance among isolates. Resistance pattern was confirmed with VITEK® 2 (BioMerieux France) an automated instrument for identification and antibiotic sensitivity testing [34,35]. Every isolate that was vancomycin resistance by disk diffusion technique, was further suspended to 1.0 McFarland Turbidity Standard (Fisher Scientific, R20411) in 0.85% NaCl – saline (BioMerieux France) and tested using FilmArray Blood Culture Identification Panel following manufacture instruction to confirm the presence of vanA/B -vancomycin-resistance genes [36,37].

### 2.5 Quality Control

Only in-house prepared media was used in this study*, and S. aureus (*ATCC™ 6538), *S. epidermidis (*ATCC 12228), *Proteus mirabilis (*ATCC 12453), and *Escherichia coli (*ATCC 25922) were used following manufacturer's instructions for every new batch of MSA [38] prepared before use. MSSA (ATCC 25923) and *MRSA*) ATCC 43300 were used as negative and positive controls respectively for every new batch of Mueller-Hinton agar plates (Difco, Detroit, MI) prepared before antibiotic susceptibility testing. All antibiotic used were procured from Oxoid and underwent quality control according to the manufactures instructions.

### 2.6 Ethics Statement

The Institutional Review committee of Mbarara University of Science and Technology (MUST) and the Uganda National Council of science and technology (UNCST) approved the protocols (Number 13/08 – 15) prior to the initiation of the study. All the farmers consented before the samples were collected from their animals.

## 3. RESULTS

Of the five hundred and eighty-five (585) pigs that were sampled, 254 (43.4%) were found to be colonized by S. *aureus*. MRSA was confirmed in 172(29.4%) by cefoxitin disk diffusion test and *mecA* gene detection by PCR. As shown in Fig. 2, the MRSA isolates were phenotypically highly resistant to erythromycin, Cotrimoxazole, ciprofloxacin, clindamycin and tetracycline. Of note, three isolates (1.9%) were resistant to vancomycin. Antibiotic resistance was high among MRSA compared to MSSA isolates (Fig. 2) where the resistance against Sulfamethoxazole – trimethoprim was 170(99%), followed by Erythromycin; 154(89%), Ciprofloxacin 124(72%) and Clindamycin; 121(70), Tetracycline; 121(70%), Gentamycin; 84(49%), Rifampicin; 40(23%); and Cefipime; 40(23%). Of note, 03(2%) of the MRSA isolates were resistant to Vancomycin. However, among the MSSA, there was low antibiotic resistance compared to MRSA (Fig. 2).

## 4. DISCUSSION

The present study provides novel information about *S. aureus* including MRSA *carriage* rate among pigs in Kabale district- South Western Uganda. Currently, Pig farming is an important activity that provides local population with an opportunity to generate house hold income [39]. Increasing demand for pork has resulted in fast-growing market for this animal, consequently, many families are acquiring these animals as an income generating venture. However, this might come with public health cost because of the potential to act as a reservoir for drug-resistant human pathogens. The zoonotic spread of MRSA to humans through direct animal contact, environmental contaminations are of great concern [40,41] where such phenomenon could undermine existing MRSA control programs. The close proximity nature of farmers and domestic pigs in their respective homesteads provides high chances of close contact which may result into cross-transmission [6,15,20,42,49]. However, until recent focus has been researching on *S. aureus* in swine neglecting MRSA and its ancestral origin [43] as this may be too costly in resource-limited settings. Consequently, this study, only did a limited molecular analysis to establish *mecA* gene but not phylogenetics and therefore difficult to establish the origin of the colonizing type which would have given insight as to whether it was livestock-associated MRSA or the human type and likely route of transmission.

MRSA colonization has recently been identified in pigs and people that work with pigs, raising concerns about the role of pigs as reservoirs of MRSA for human infection [44,17]. Mounting concerns regarding the occupational and public health implications of MRSA in pigs and other

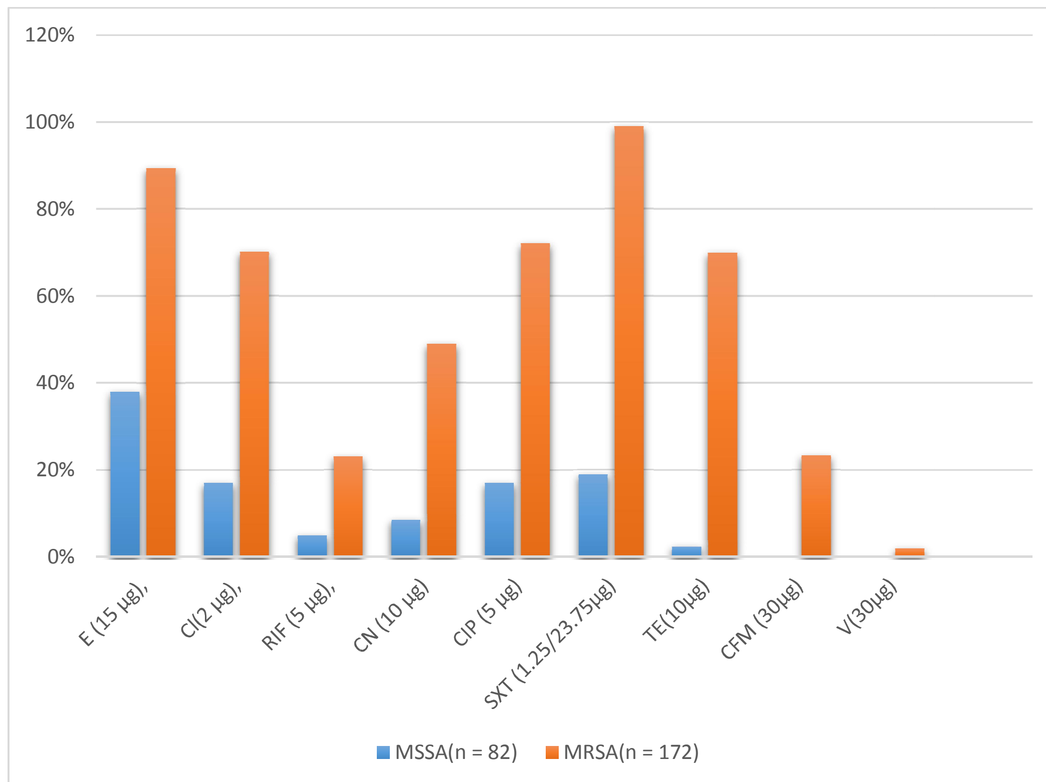


**Fig. 2. The resistant pattern of *S. aureus* isolates**

*Note: n= number; MSSA = Methicillin-susceptible S. aureus; MRSA = Methicillin resistance S. aureus, S = Sensitive, R =Resistant, E = Erythromycin, CL= Clindamycin, RIF = Rifampicin, CN = Gentamycin, CIP = Ciprofloxacin, SXT = Sulfamethoxazole – trimethoprim, TE = Tetracycline, CFM =Cefipime, V = Vancomycin*

livestock populations requires intensive research of MRSA in animals, and particularly pigs which are increasingly reared as a source of income in Uganda. For instance, the colonization rate of 29.4% is alarmingly high, and this is consistent with other studies which demonstrated similar colonization rates of this pathogen in swine population [45,46]. In addition, this also similar to Mroczkowska et al. [47] and Van Cleef et al. [48] who reported carriage of MRSA to be 29% and 30% in Demark and Thailand respectively. The reasons for this high prevalence are unknown, but might be related to the altered interplay of the host, the microorganism, and the environment. In addition, this may demonstrate the abuse of the antibiotics that are supposed to be used in human but are now freely and generously used in animals [49,50]. The use of antibiotics in livestock farming is routinely described as probably the major contributor to the antimicrobial resistance phenomenon being witnessed today in human medicine [50]. The MRSA prevalence reported in this study is much higher than that previously reported in Dakar – Senegal, United states (USA) [51] and the Netherlands [52]. However, the prevalence of MRSA in pig herds varies widely (0 to 50%) among European countries [52,53]. The pig herd prevalence of MRSA in North America is uncertain, but appears lower than in many European countries [54]. The prevalence and spread of MRSA in Africa is unclear, in contrast to the rest of the world [55]. However, by Kalule et al. (2014) reported MRSA prevalence rate of 64% among *S.aureus* isolated from pigs in Uganda [56].

Alarmingly, studies about the epidemiology of S. aureus and MRSA associated with swine has been remarkably neglected and considerable uncertainty remains regarding MRSA prevalence among pigs in Uganda. The high prevalence of MRSA in the pigs demonstrates a potential public health threat with great risks of zoonotic transmission of the MRSA to humans and vice versa. MRSA strains are always highly resistant to multiple antibiotics [46,57]. The high resistance rate among MRSA towards commonly

used antibiotics (Fig. 2) is of great concern. Today, MRSA isolates often resistant to several antibiotics [58]. Significant resistance was observed in case of Sulfamethoxazole – trimethoprim, erythromycin, Clindamycin, ciprofloxacin, tetracycline and gentamicin. This is probably attributed to prolonged antibiotic usage in animal feeds resulting in enhanced selection pressure [59,60]. Because of the widespread use of antibiotics, especially in developing countries, the resistance profile of MRSA and other microorganisms [61] is changing, evidenced by increasing occurrences of high antibiotic resistance among MRSA populations. Additionally, The high resistance rates observed in this study is consistence with what has been reported in other studies elsewhere [62,63].

Vancomycin is a glycopeptide antibiotic used for the treatment of Gram-positive bacterial infections including MRSA. The appearance of MRSA strains resistant to vancomycin has been perceived as a formidable threat in the therapeutic field, as this antibiotic is traditionally used as a drug of last resort [64]. However, the presence of Vancomycin resistance among of MRSA strains isolated from pigs raises more questions and warrants further investigation.

## 5. CONCLUSION

There is a very high colonization rate of the swine with *S. aureus* including MRSA which is very resistant to the commonly used human antibiotics. The data from this study showed that MRSA is present in swine population which can serve as a reservoir. The rate of spread of MRSA and its unique ability to acquire antibiotic resistance calls for urgent and well-coordinated surveillance programme to combat this situation.

## 6. FUNDING

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## COMPETING INTERESTS

There are no competing conflicts of interest concerning this article.

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