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METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CARRIAGE IN PIGS AND CATTLE FROM KIAMBU COUNTY, KENYA: A POSSIBLE SOURCE OF INFECTION TO FARM ANIMAL HANDLERS

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METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CARRIAGE IN PIGS AND CATTLE FROM KIAMBU COUNTY, KENYA: A POSSIBLE SOURCE OF INFECTION TO FARM ANIMAL HANDLERS

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ABSTRACT

***Objective*: To determine the potential zoonotic transmission of MRSA from farm animals to human beings.**

***Design*: Lot Quality Assurance Sampling (LQAS) was used to sample the subjects where nasal swabs were collected to culture *S. aureus*. Susceptibility testing was performed, and inhibition zones recorded.**

***Setting*: Small scale farms in Landless Estate, Komu Location in Kiambu County, Kenya.**

Subjects /Participants: Animal handlers, pigs and cattle selected at random from households that keep cattle and/or pigs.

***Interventions*: Mannitol salt, Staphaurex, DNAse agar and one molar HCL were used to identify *S. aureus*. Antimicrobial susceptibility of *S. aureus* isolates was conducted using the Kirby-Bauer disk diffusion method to test several antibiotics. Polymerase chain reaction (PCR)was used to detect the *mec-A* gene.**

***Main Outcome Measures*: The prevalence of MRSA in humans is higher compared to the study animals.**

***Results*: MRSA was highest in animal handlers (18.6%) and lowest in pigs (9.6%). Tetracycline resistance was higher in Cattle (38.3%), compared to Pigs (35.3%) and least among animal handlers (27.3%). Ampicillin resistance was highest in animal handlers (72.7%) and lowest in pigs (29.4%). No resistance to Ciprofloxacin and Chloramphenicol was recorded.**

***Conclusion*: More stringent health and hygienic measures should be taken to help curb the possibility of zoonosis.**

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a critical human and animal pathogen that has recently presented itself as a health concern [1]. MRSA is less frequent than regular *S. aureus*, even though it can still be found in 1-3% of healthy nares [2]. MRSA is resistant to all antibiotics in the penicillin family, as well as several other antibiotics, including cefoxitin. Most MRSA-related diseases are skin infections and abscesses or boils, but MRSA can also result in more complicated infections like 'flesh-eating disease' [3]. There has been a noteworthy concern relating to the emergence of MRSA in food animals over the past five years. MRSA is a critical cause of food poisoning, pneumonia, postoperative wound infections, and nosocomial bacteremia [4]. Human isolates of *S. aureus*, unlike animal isolates, are normally resistant to the Penicillinase-resistant penicillin [5].

MRSA occurs in diverse animal species such as dogs, cats, dairy cattle, veal calves, pigs, and exotic species, both as a cause of infection and residing in healthy carriers [1]. MRSA of animal origin may show a genetic connection to the MRSA obtained from humans [5]. MRSA in companion animals has also been described as a source of infection for animals and humans [6].

Staphylococcus aureus causes severe animal diseases. These diseases include suppurative disease, mastitis, arthritis, and urinary tract infection. The diseases are attributed to an array of virulence factors, which include the production of extracellular toxins and enzymes [5].

Methicillin resistance is a result of a modified penicillin-binding protein PBP2a, encoded by the *mec-A* gene, located on one of six types of staphylococcal chromosomal cassettes (SCCs), which highly differ in size. Except for isolates of sequence type (ST) 22, SCCs in Hospital-Acquired–MRSA (HA-MRSA) strains normally possess supplemental genetic material, including genes encoding resistance to multiple classes of antimicrobials [4]. These SCC elements play the role of agents in the transfer of various genetic markers, including genes mediating antibiotic resistance or virulence. This potential role of SCC is awaiting further investigation[7]. Bacterial strain typing is crucial to the probing of MRSA outbreaks, evaluation of the transmission of MRSA strains, and to studying evolution.

Currently, MRSA from pig and cattle reservoirs are responsible for 20% of all human MRSA infections in the Netherlands [8]. These new MRSA strains also seem to be evolving in animals. This poses a possible threat to the health of people through occupational exposure and facileness of proliferation during the high levels of movement of livestock and people working in farms, calling for research on animal MRSA [4]. In Kenya, there is a meagerness of data on MRSA infections in animals and documentation of zoonosis. Limited information is available on the prevalence, reservoirs, and patterns of transmission of MRSA in Kenyan hospitals or in the community requiring extensive research to enhance information for further understanding and effective response.

Considering how humans socially interact at close range with animals, it is anticipated that contact with human carriers such as during the veterinary practice or while handling farm animals would be associated with MRSA acquisition by animals [9].

This study sought to determine the potential zoonotic transmission of MRSA isolates from farm animals in a small-scale farming area of Thika town, 50km north of Nairobi City.

MATERIALS AND METHODS

Study Design: Lot Quality Assurance Sampling (LQAS) was used to sample the subjects where nasal swabs were collected to culture *S. aureus*. Susceptibility testing was

performed, and inhibition zones recorded.

Study Setting: This study was carried out in small scale farms in Landless Estate, Komu Location, Makongeni Division, Thika West District in Kiambu County, Kenya. Households keeping cattle and/or pigs were randomly selected from the local veterinary officer's records.

Sample Size: To get the sampling size the Lot Quality Assurance Sampling (LQAS) was used. LQAS is a sampling methodology that employs the use of small sample sizes when conducting surveys in small geographical or population-based areas or lots. Sampling stops when the maximum sample size is reached.

It was computed as:

Sampling size (n) = Clusters (Households) × Number of cattle/pigs/animal farm handlers per household (at random)

Therefore,

For cattle, the sample size was 80 × 4 = 240

For Pigs, the sample size was 80 × 2 = 160

For animal farm handlers, the sample size was 80 × 1 = 80

Small scale farmers have about 1-10 pigs or cattle in their farms.

Inclusion criteria: All small-scale farms that have more than 2 pigs or cattle

Cattle or pigs that are healthy and not under any antibiotic treatment

All animal farm handlers should be above the age of 21

Animal farm handlers directly linked to cattle or pigs

Animal farm handlers found in the homesteads with the pigs and cattle in farms.

Exclusion criteria: All small-scale farms that have more than 30 pigs or cattle

Unhealthy cattle or pigs under antibiotic treatment

Animal farm handlers under the age of 21

Animal farm handlers who do not directly deal with cattle or pigs

Anyone who is not a farm handler in the homesteads with pigs and cattle in farms

Sampling Procedure: Nasal swabs were obtained from Animal handlers, pigs and cattle. On average, swabs were taken from one animal handler, three cattle and three pigs per farm. The swabs were placed into 3 ml of Mannitol salt enrichment broth and incubated for 24 hours at 35°Cin order to isolate *S. aureus*. The yellow colonies on mannitol salt agar were inoculated onto anew Mannitol-salt agar and incubated at 35°C for 24 hours. The samples that showed significant small, glossy, yellow concave colonies were identified as being possible *S aureus* isolates. The yellow colonies were then tested for production of catalase-using 5% hydrogen peroxide. The catalase-positive isolates were then tested for production of coagulase using a drop of Staphaurex. The coagulase-positive isolates were confirmed by inoculation on DNAse agar and incubation at 35°C for 24 hours, followed by flooding of the DNAse agar plate with one molar HCl. Isolates that caused a clear zone around the inoculums were identified as *S. aureus*.

Data collection: A consent form and a questionnaire were administered to the farm manager and the animal farm handler. A nasal swab was then taken from the selected pigs, cattle, and animal farm handlers. The swab was taken by inserting a cotton-tipped swab approximately 1 cm into each nostril, with the help of a veterinarian. The swabs were then placed in liquid Stuart medium and maintained at 4°C until processing.

Data Analysis: The data collected were analyzed by SPSS software statistical application version 20 (SPSS INC, Chicago, IL, USA). The study findings are displayed in tables and Figure.

Testing for antimicrobial susceptibility of S. aureus isolates

Antimicrobial susceptibility was of the isolates tested using the Kirby-Bauer disk diffusion method in line with the Clinical Laboratory Standards Institute guidelines. *S. aureus* ATCC 25923 was used as a quality control organism. Antibiotics used were Oxacillin 1ug, Erythromycin 5ug, Tetracycline 30ug, Vancomycin 5ug, Augmentin 20ug, Ampicillin 10ug, Trimethoprim/Sulfamethoxazole 25ug, Ciprofloxacin 30ug, Chloramphenicol 30ug, and Cefoxitin 30ug.

Detection of mec-A gene using PCR amplification assay

Detection of the *mecA* gene was done by polymerase chain reaction (PCR) assay. The PCR amplification was carried out in a total volume of 25 µl containing 1 µl of DNA template, 0.2 µl each for the forward and reverse primers, *mecAF*, and *mecAR*, 1 µl of 10x PCR buffer, Ready to go beads (Amersham biosciences) containing 0.8 µl of 0.125 mM of dNTPs, 1.2 µl of 0.125 mM MgCl2, 0.4 µl of Amplitaq®) *Taq*polymerase, and 23 µl of PCR water, depending on the master-mix. The PCR amplification was done using a DNA Engine DYAD™ Peltier Thermal Cycler (MJ Research) using the following cycling conditions Initial denaturation step at 94°C for 3mins followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec and a final extension step at 72°C for 10 min.

DNA fragments were separated by use of agarose gel electrophoresis and thereafter visualized on a UV lightbox and photographed with a Polaroid camera lens with an aperture set at f/11 and exposure time of 30 milliseconds. The MRSA *mecA* bands from the target bacteria, *S aureus*, were read in comparison with those in the ladder.

Ethical Approval

The study was cleared by Board of Post-graduate Studies (BPS) of Jomo Kenyatta University of Agriculture and Technology (JKUAT), Scientific Steering Committee (SSC) of Kenya Medical Research Institute (KEMRI), and the National Ethical Review Committee and was assigned SSC No. 2088.

RESULTS

Distribution and Characteristics of study units

A total of 49 households were visited. Out of 40 (82%) reared cattle only, 8(16%) reared pigs while only one household (2.5%) had both cattle and pigs. Among the 40 households rearing cattle, the mean number of cattle reared was 4.8. In total, 237 cattle were screened. For the nine households rearing pigs, the mean number of pigs reared was 13.2. In total, 177 pigs and 225 cattle were screened (Table 1).

Table 1

Number of Animal handlers, cattle, and pigs per household

Sample size	Household (n)
One human and cattle	31
One human and pigs	7
Two humans and cattle	7
Two humans and pigs	1
Human, pigs and cattle	1
Cattle only	1
Pigs only	1

Level of education and duration of employment

Animal handlers with primary education as their highest level of qualification accounted for (30)61.2% of the households sampled with a mean employment duration of 15.18

months. Those with secondary education as their highest level of qualification accounted for (11)22.4% of the households sampled with a mean employment duration of 13.5 months.

Risk factors associated with MRSA isolation

Delivery within the last one year in both cows (O.R=1.022, 95%CI,[0.221-4.455], p=1), and pigs (95%CI,[0.523-Inf], p=0.0667) respectively was not associated with MRSA isolation. Milking was also not associated with MRSA infection in animal handlers (O.R=0.919, 95%CI,[0.073-7.558], p=1).

Awareness and hygienic practices observed by animal handlers

Nineteen (48%) of animal handlers indicated that they were aware of diseases animals can transmit to humans. Boiling of milk was the most (93.9%) hygienic way of preparing milk in households. Only four (10%) households admitted that cows were currently under treatment for diarrhea, respiratory disease or worms (Figure 1).

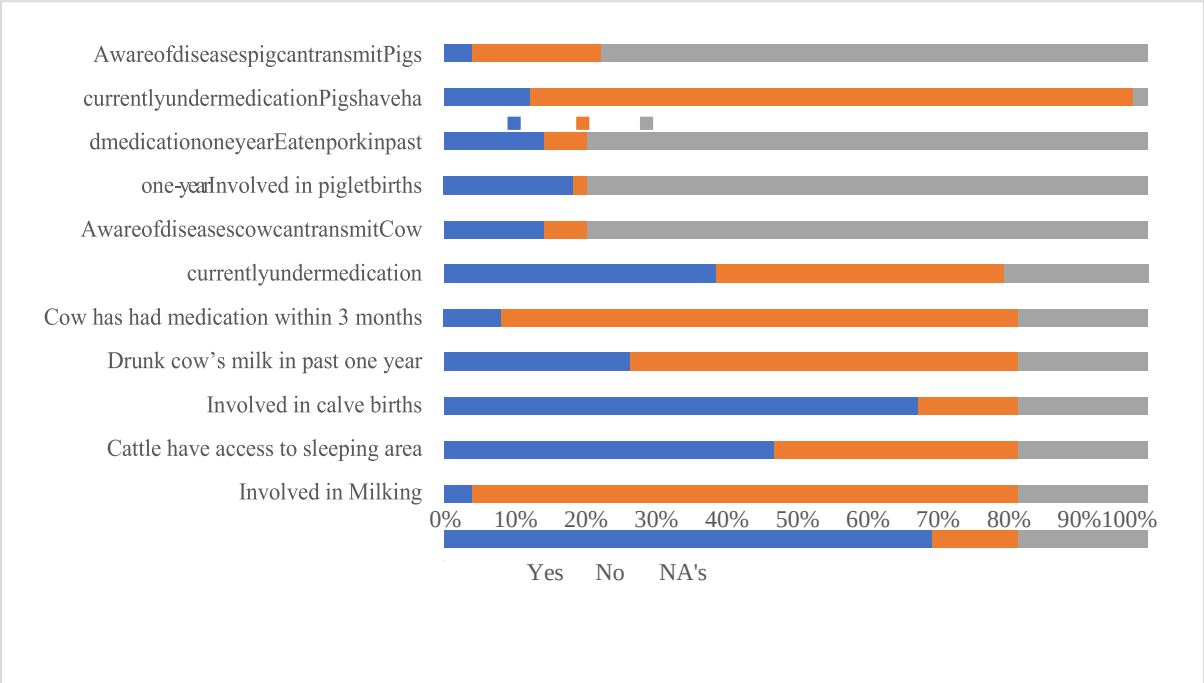

Figure 1: Awareness and hygienic practices observed by animal handlers

Methicillin Resistance for Staphylococcus aureus prevalence

Considering both cattle and pigs, MRSA had 1.50 times higher probability of occurring in humans compared to cattle and pigs combined (OR=1.50; 95% CI [0.73 – 3.05]; p=0.266). MRSA was highest in animal handlers, 18.6%, and lowest in pigs, 9.6%. Interestingly, MRSA was 2.16 times more likely to occur in humans compared to pigs (OR=2.16; 95% CI [0.95 – 4.92]; p=0.068).

Antimicrobial Susceptibility Patterns

Figure 3 presents the distribution of samples showing resistance or intermediate resistance to the various drugs by category of the population. The best-performing drugs are Chloramphenicol and Ciprofloxacin, both showing nil with respect to resistance or intermediate resistance to either of the study subjects. The worst performing drugs are Tetracycline, 27.3% with animal handlers, 38.3% with cattle, and 35.3% with pigs; and Ampicillin 72.7% with animal handlers, 31.6% with cattle, and 29.4% with pigs.

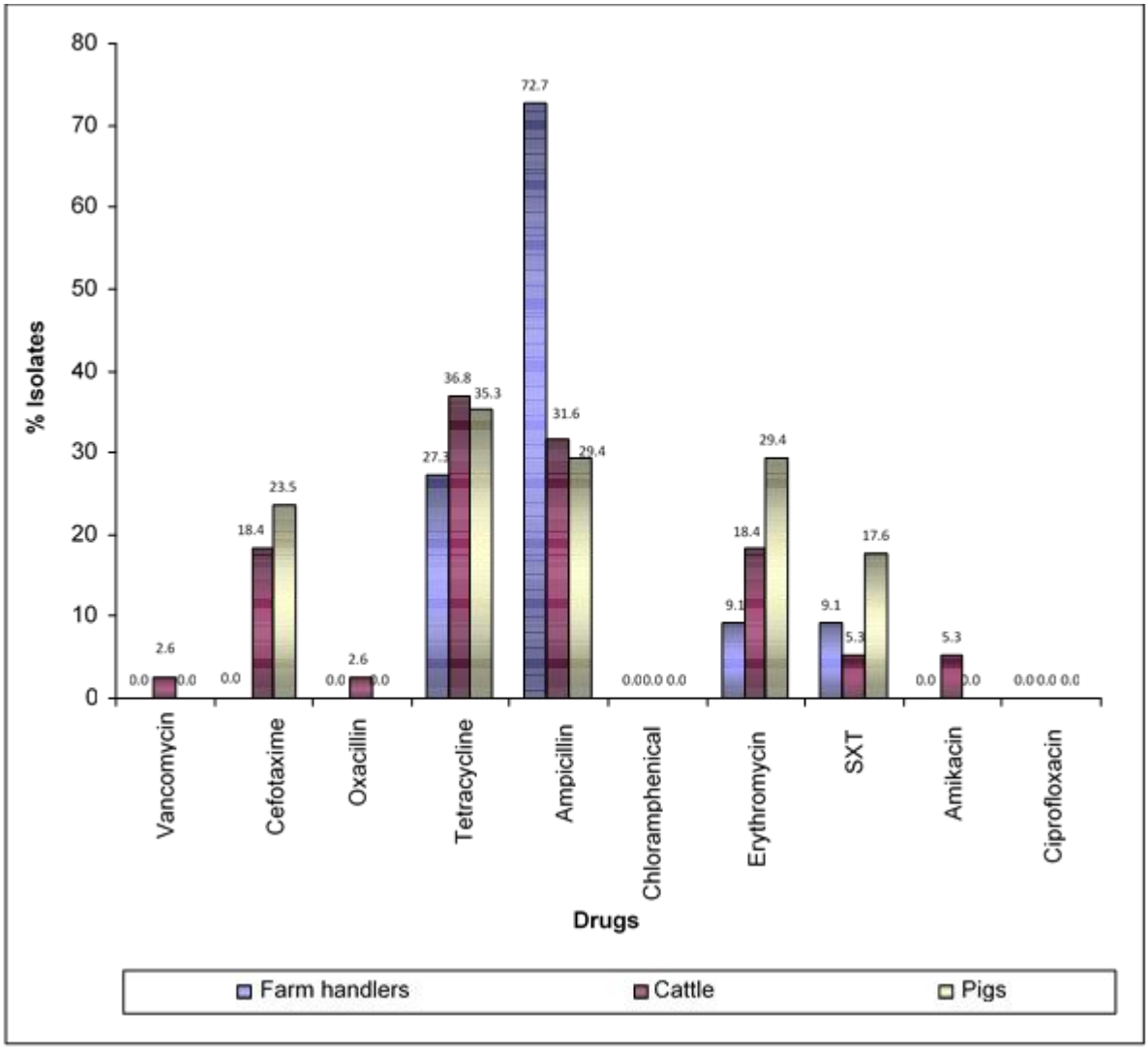

Figure 2: Resistance or intermediate resistance to various drugs by Category of population

MRSA among animal handlers in relation to MRSA in livestock

A relationship between occurrences of MRSA in animal handlers in relation to occurrences of MRSA in livestock was as presented in Table 2. There was no significant relationship between the occurrence of MRSA in animal handlers and the occurrence of MRSA in animals ($p>0.05$). However, MRSA was relatively high among animal handlers rearing cattle, pigs, and/or cattle and pigs, with at least one animal having MRSA compared to those rearing cattle with none of the animals having MRSA.

Table 2

Comparison of MRSA by Category of population

Variables	Positive for SAU (n=66)		Negative for SAU (n=407)		OR	95%CI		p value
	n	%	n	%		Lower	Upper	
Category of population								
Farm handlers	11	18.6	48	81.4	2.16	0.95	4.92	0.068
Cattle	38	16.0	199	84.0	1.80	0.98	3.30	0.059
Pigs	17	9.6	160	90.4	1.00			
Category of population								
Farm handlers	11	18.6	48	81.4	1.20	0.57	2.52	0.629
Cattle	38	16.0	199	84.0	1.00			
Category of population								
Farm handlers	11	18.6	48	81.4	2.16	0.95	4.92	0.068
Pigs	17	9.6	160	90.4	1.00			
Category of population								
Farm handlers	11	18.6	48	81.4	1.50	0.73	3.05	0.266
Cattle and/or pigs	55	13.3	359	86.7	1.00			

Identification of MRSA mec-A gene

Detection of the *mec-A* gene was performed with a PCR assay. Strains yielding the *mec-A* gene were amplified with the *mec-A* gene primers *mec A*-F and *mec A*-R (F is forward, and R is reverse), which amplify the region between the 5′-CS and the 3′-CS (variable region harboring gene cassettes), yielding products of various sizes, depending on the number and length of the inserted gene cassettes. Out of a total 66 isolates that were used, 65 were positive for the *mec-A*gene (Figure 3).

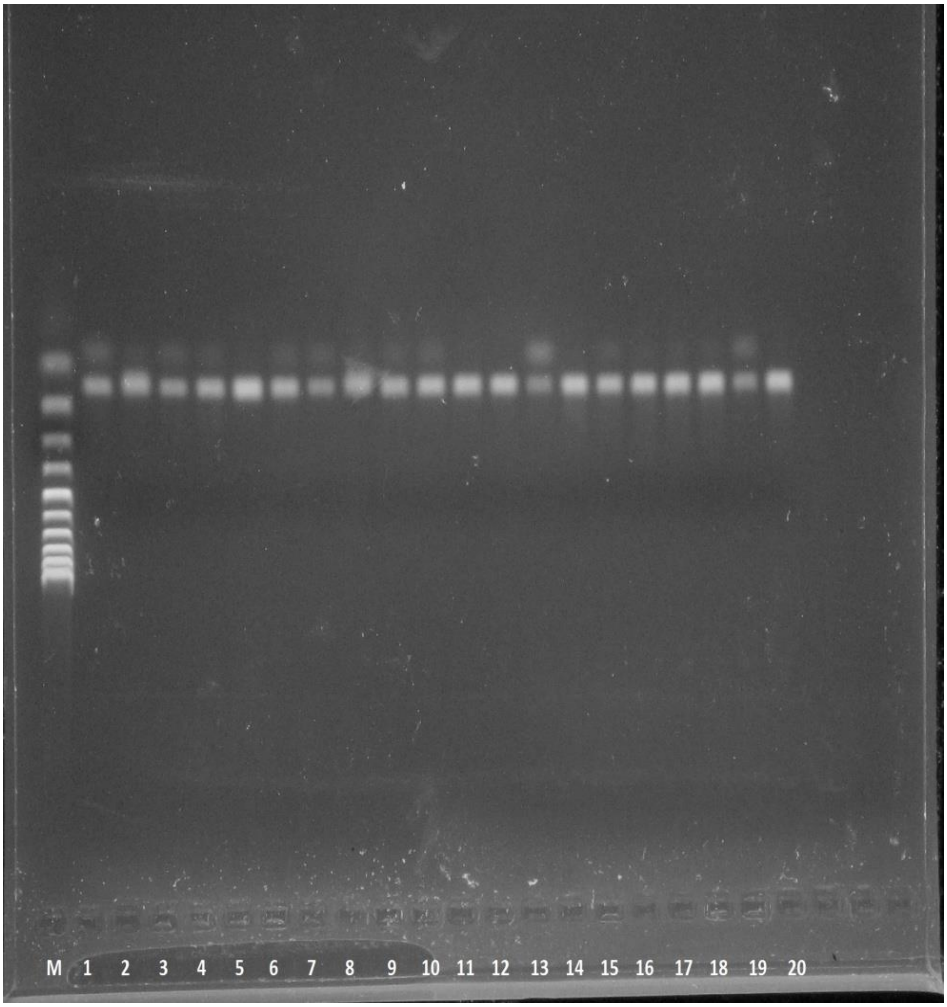

Figure 3: Agarose Electrophoresis gel illustrating the mec-A positive isolates. H- Humans, C- Cattle and P-Pigs.
M: 100-160 bps Ladder; 2: H-07; 3: H-22; 4: H-24; 5: H-31; 6: C-36; 7: C-55; 8: C-134; 9: C-210; 10: C-174; 11: C-12; 12: C-86; 13: C-192; 14: P-103; 15: P-64; 16: P-178; 17: P-112; 18: P-162; 19: P-79; 20: P-93; 21. P-16

DISCUSSION

Staphylococci are widespread in nature, although they majorly occur on the skin, mucous membranes (nose and mouth), and skin glands of mammals and birds, without causing illness. Until recently, MRSA was close to a hospital-acquired bacterium, but in the 1990's it more steadily led to illness in persons who had no contact with hospitals. So-called 'community-acquired' MRSA infections became visible in the US, Britain, Canada, among other countries [13]. For many years, MRSA was perceived to occur only in humans, until documentation of MRSA infection in a dairy cow was made in 1972.

The results also showed that MRSA is 1.2 times more probable to appear in humans compared to cattle, while MRSA is 2.16 times more likely to occur in humans compared to pigs and 1.50 more likely to occur in humans compared to cattle and pigs. This leads to the conclusion that the prevalence of MRSA in humans is higher in comparison to the study animals. The likely hood of MRSA in humans than in animals is high. Zoonosis, therefore, is likely to occur from humans to the animals rather than animals to humans.

As stated above, it is 1.23 times more likely for a farm handler to have MRSA in a homestead with MRSA positive cattle, 2.14 times more likely with pigs, or 1.15 times more likely with both pigs and/or cattle. This ascertains the previous statement above of humans being more likely the source of MRSA than animals.

Considering the close social interaction of animals with humans, it is anticipated that the association with human carriers such as at the veterinary practice or while handling farm animals would be connected to MRSA acquisition by animals [9].

Epidemiological studies have revealed that Livestock Associated-MRSA (LA-MRSA) besides colonizing livestock also overcome the species barrier leading in zoonotic transmission to persons who are directly exposed to livestock. Consequently, nasal colonization or contamination was found in 23–86% of all pig farmers and veterinarians [14], and in 1–5% of persons with indirect animal exposure such as the family members of farmers and farm visitors [15].

In Ontario, Canada, 45% of pig farms, 24.9% of pigs, and 20% of pig farmers were colonized by MRSA (predominantly ST398) in 2007 [2]. In an archetypal intensive pig breeding farm in the US Midwest, holding 60,000 pigs at a given time, 49% of the pigs and 45% of the laborers in the farm were colonized by MRSA ST398 [16]. MRSA ST398 has been identified in cows with mastitis or in their milk in Switzerland, Germany, and Belgium [17-19]. In an examination of three dairy herds in southwest Germany, milk samples from 5% to 17% of the cows and 100% of high-volume tank milk samples were found to be positive for MRSA ST398.

Furthermore, nasal swabs revealed that 47% of cows, 57% of calves, and 78% of the workers bore MRSA [19]. Of 102 Dutch veal calf farms studied in 2007-2008, 88% of the farms and 26% of the calves were positive for MRSA, nearly all of these were the ST398 strain. At the maximum, 100% of the calves on a farm were positive. Of the people in contact with the calves, 33% of the farmers, 8% of their family members, and 26% of the people working for them were also positive for MRSA ST398. The research established that the probability of people being colonized by MRSA was 'strongly linked to the intensity of animal contact and with the number of MRSA positive animals on the farm [20].

When MRSA also emerged in veal farms, it came to light that calves on large farms were 'notably more often colonized (by MRSA) compared to calves from smaller farms [20].

Chloramphenicol and Ciprofloxacin showed the best effectiveness among all isolates, both animals and humans. They had no form of resistance or intermediates among the isolates tested. Sulfamethoxazole/Trimethoprim and Amikacin, together with Vancomycin and Oxacillin, were effective with intermediates and resistance in cattle of 5.3% and 2.6%, respectively. Cattle isolates showed more resistance towards the antibiotics used. Among the two most important antimicrobials Oxacillin and Vancomycin, there was one cattle isolate that showed resistance not only to these two antibiotics but also in the other antimicrobials used.

With the results above, more studies should be done on the various causes of MRSA as there is clearly more that should be done in this area. With very minimal data locally (in Kenya and Africa at large), more diverse projects should be done to curb MRSA and zoonosis.

With MRSA being more prevalent in humans than animals, animals stand a risk of obtaining MRSA from humans. Cattle also showed more of its isolates being resistant to various drugs. There stands a risk of transfer of resistance between these isolates and other isolates in humans and animal species. Therefore, more awareness should be done to farmers and their handlers on zoonotic diseases, prevention measures for both the

animal handlers and animals. Also, different hygiene measures should be taken as some of the farms animal sheds were not hygienic, a factor that could be contributing to the spread of MRSA.

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