

Original Research Article

Prevalence, risk factors and antibiogram of bacteria isolated from skin of human having close contact with dairy cows in small-holder farms in Gatundu Sub-County, Kenya

Felix O. Otenga¹, John M. Kagira^{2*}, Kenneth Waititu³, Daniel M. Kiboi¹,
Maina Ngotho⁴, Naomi Maina¹

¹Department of Biochemistry, ²Department of Animal Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

³Department of Animal Science, Institute of Primate Research, Nairobi, Kenya

⁴Department of Clinical Studies, University of Nairobi, Nairobi, Nairobi, Kenya

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*Correspondence:

Dr. John M. Kagira,

E-mail: jkagira@jkuat.ac.ke

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ABSTRACT

Background: Antimicrobial resistance is a global health crisis which needs surveillance especially in people having close contact with animals.

Methods: A total of 120 skin swabs from around the neck region were obtained from human respondents and subjected to bacteriological analyses. Antimicrobial susceptibility test was done using Kirby Bauer disk diffusion method and results were interpreted according to the Clinical and Laboratory Standard Institute guidelines.

Results: The results showed that the skin was colonized by *Staphylococcus aureus* (49.4%), coagulase-negative staphylococci (CoNS) (16.9%), *Pantoea* spp. (13%), *Serratia* spp. (13%), *Bukholderia cepacia* (3%), *Enterobacter* spp. (3%), *Yersinia enterocolitica* (1.3%) and *Pasteurella aerogenes* (1.3%). The CoNS were mostly resistant to gentamycin (100%), clindamycin (84.6%), erythromycin (84.6%) and ciprofloxacin 84.6% and sensitive to sulphamethoxazole-trimethoprim (76.9%). For *S. aureus*, most isolates were resistant to gentamycin (86.8%), oxacillin (86.8%) and vancomycin (73.7%) but susceptible to amoxycillin-clavulanic acid (89.5%) amongst other antibiotics. The multiple antibiotic resistance index for *S. aureus* and CoNS was 0.92 and 0.92, respectively. Respondents aged more than 40 years had higher burden of AMR compared to the other respondents ($p = 0.011$, $OD=1.745$). Similarly, the AMR burden was higher in respondents who had previous history of using medication compared to those who had not ($p=0.025$, $OD=0.204$).

Conclusions: The study showed a high prevalence of antibiotic resistance in CoNS and *S. aureus* isolates from skin of people having regular contacts with dairy cows in the study area. Interventions strategies to stem the emergence of AMR should be undertaken.

Keywords: Prevalence, Human, Risk-factors, Antibiotics, Antimicrobial resistance, *Staphylococci* spp., *Enterobacteriaceae*

INTRODUCTION

For decades, antibiotics have played a critical role in the treatment and management of bacteria-associated

infections in human and animals. However, indiscriminate use of antibiotics in agriculture, veterinary and medical sectors drive the selection-pressure of bacteria and this has led to emergence of antibiotic-

resistant bacteria.¹ Currently, due to high burden in antibiotic resistance, there has been a decrease in treatment options for patients.^{2,3} A recent systematic review showed that the global deaths associated with microbes having antimicrobial resistance was estimated to be at 4.95 million people in 2019.⁴

The death rate due to antimicrobial resistance in Sub-Saharan Africa was the high with 7.3 deaths per 100,000 people.⁴ Although there is paucity regarding antimicrobial resistance in human beings in Kenya, a few studies show the prevalences antimicrobial resistance to be high. In developing countries like Kenya, most people have close interactions with livestock where they derive their livelihoods from products such as milk and meat. However, the interactions with animals can expose them to zoonotic bacteria from animals and some of these microbes could be resistant to antibiotics due to over-use of the drugs in the livestock sector. One of the most common zoonotic bacteria are *Staphylococcus* spp. which causes a various infection in both human and livestock.⁵ In humans, Staphylococci infections range from mild skin infections to life threatening conditions such as bacteremia, endocarditis, necrotizing pneumonia and toxic shock syndrome.⁶ In dairy animals, *Staphylococcus* spp. causes mastitis amongst other diseases. It has been noted, *Staphylococcus* spp. is among the highly adaptive bacteria which can establish is new hosts and in humans it ends up colonizing the skin. A recent Kenyan study showed that consumption of raw milk was associated with protracted healing of skin wounds in patients, raising a possibility of cross-transmission of bacteria from animals to man.⁷

In Africa, *S. aureus* is the most common bacteria associated with skin wounds with the prevalence ranging from 1.4% to >35% and a large proportion of *S. aureus* from septic wounds have high resistance to commonly used antibiotics.^{4,8,9} Other bacteria which are found on the skin include coliforms such as *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*.³ In humans, these coliforms can cause diseases such as diarrhea and food poisoning.¹⁰ There is paucity of data on the occurrence of bacteria on the skin in people who are predominantly livestock keepers.

A study undertaken in Uganda, showed that the skin of milkmen were colonized with *Staphylococci* spp. (35%) and *Enterococci* spp. (6%).¹¹ The Ugandan study found that *Staphylococci* spp. were resistant to ampicillin (100%), cefoxitin (63.6%), oxacillin (63.6%), tetracycline (63.6%) and ciprofloxacin (27.7%).¹¹ The current study sought to determine the risk factors, prevalence and antibiogram of bacteria isolated from skin swab of human beings having frequent and close contact with the dairy cows in Gatundu Sub-County, Kenya. These farmers closely interact with their animals through provision of feeds, milking and cleaning the dairy shed, milking parlor and handling of the animal's manure.

METHODS

Study area

The study was carried out in selected administrative Wards (Chania, Mang'u, Ng'enda, Wamwangi) which have a large proportion of farmers involved in small scale dairy farming in the Gatundu Sub- County, Kiambu County, Kenya. The sub-county is located 40km from Nairobi City (1°1'S 37°5'E). Its rainfall pattern is bimodal, ranging from 500 to 1300 mm and has an average temperature of 18.7°C. The major economic activities are agriculture and trade. The human population of the sub-county is 231,978 while the livestock population in Gatundu Sub-County is 31,229.¹²

Study design, and sample size determination

A cross-sectional study was undertaken using a sample size calculated according to the Fishers formula.¹³ Using the latter, the minimum a sample size was 120 respondents. The sampling unit of interest was a farmer keeping lactating dairy cows. Since there was no formal list of dairy cows' farmers in the study area, the snowball technique and sampling to redundancy method was used as a sampling strategy to locate the farmers. The initial farmers were identified with the help of the local veterinary officer. Thereafter, these farmers helped in further identification of other farmers with lactating dairy cows. Using this strategy, a total of 120 farms were identified and visited.

Risk factors

The risk factors which have previously been associated with the occurrence of antibiotic resistance and zoonotic transmission were determined through a questionnaire that was administered to the respondents. The specific questions addressed the demographics, recent ailments, usage of antibiotics, frequency of handling dairy cows, and types of medicine used and frequency of visiting a hospital in the last 3 months.

Collection of samples, bacterial culturing and identification

The skin swab was applied to the household head who had regular interaction with the dairy animals. Briefly, a sterile swab was wet using normal saline and rubbed on the skin (neck region) for 15 seconds. The swabs were then aseptically inserted in a cryotube having Stuart transport media, labelled and then placed in a cooler box containing ice packs and transported to the JKUAT Microbiology laboratory. In the laboratory, the content of each swab was inoculated into sheep blood agar and MacConkey agar.³ The morphological characteristics of the resulting bacterial colonies were assessed for shape, color, size, and texture as well as lactose fermentation ability on MacConkey plates and hemolysis patterns on sheep blood agar plates. Gram staining was done to

distinguish between Gram-positive and Gram-negative isolates. A catalase test using 3% hydrogen peroxide was performed on all Gram-positive isolates to identify *Staphylococcus* spp. *Staphylococcus* spp. were also identified based on their growth and fermentative attributes on mannitol salt agar and the results of an oxidase test. *S. aureus* was differentiated from coagulase-negative staphylococci (CNS) using tube coagulase tests that used rabbit plasma.¹⁵ The oxidase test was performed on all Gram-negative isolates to identify Enterobacteriaceae, and those isolates that were oxidase negative were subsequently sub-cultured on MacConkey agar to assess lactose fermentation. Further identification on Enterobacteriaceae was done using API 20E kit.¹⁶

Antibiotic susceptibility test

The *Staphylococci* spp. isolates were sub-cultured on nutrient broth at 37°C for 24 hours to revive them. The turbidity standard equivalent to 0.5% McFarland was determined before inoculation on Mueller-Hinton agar.¹⁷ The antibiotic susceptibility test was formed using the Kirby Bauer disk diffusion method.¹⁸ Seventeen antibiotics were investigated and these included: tetracycline, mecillinam, erythromycin, sulphamethoxazole-trimethoprim, ampicillin, kanamycin, ciprofloxacin, gentamicin, oxacillin, aztreonam, amoxillin-clavulanic acid, vancomycin, clindamycin, cefoxitin, meropenem, cefotaxime, and chloramphenicol. These drugs were randomly selected from different antibiotics classes commonly used in Kenya to treat bacterial infections in both animals and humans. The disks were gently pressed on the agar, incubated for 24 hours at 37°C for the examination of zones of inhibition and the results were interpreted according to Clinical and Laboratory Standard Institute protocols.¹⁹ *E. coli* strain ATCC 25922 and *S. aureus* strain ATCC 25923 were used as quality-control strains for the antimicrobial susceptibility testing.²⁰

The occurrence of Multiple Antibiotic Resistance (MAR) index was calculated using the formula:

$$MAR\ index = a / b$$

Where 'a' is the total number of antibiotics to which a particular bacterium was resistant and 'b' is the total number of antibiotics against which the bacterium was tested.²¹

Statistical analysis

The coded data was entered into MS Excel (Microsoft, USA) spreadsheet and exported to SPSS v26 (Microsoft, USA) for data analysis. Descriptive statistics were expressed as percentages and frequencies, and data were presented in tables and histograms.²² Logistic regression was used to test the relationship between occurrence of resistance and specific risk factors ($p < 0.05$). The odds

ratio were used to determine the strength of associations identified in the logistic regression.²³

RESULTS

Characteristics of the respondents

The 120 respondents consisted of males (63.3%) and females (37.7%). Most of the respondent were more than 50 years of age (32.5%), while the rest ranged between 30-40 years (21.7%) and 40-50 years (20.0%). All of them kept are dairy cows which were reared in a small-holder zero grazing system. The respondents interacted closely with livestock through provision of feeds, milking and cleaning of the animal housing. Most (84.2%) indicated they had suffered various ailments in the past three months before the study. The most common ailment suffered in the last three months included boils (5.4%), arthritis (1.4%), chest-pain (20.9%), influenza (33.3%), skin rashes (3.4%), stomachache (12.8%), open-wounds (7.4%) and diarrhea (3.4%). The respondents used various drugs for management of the various ailments whose names they could not remember.

Prevalence of bacteria colonizing the skin of the respondents

Bacteria were isolated from all (100%) the respondents. A total of 77 bacteria were isolated and identified from the skins of the respondents. The most prevalent bacteria were *S. aureus* (49.4%) followed by coagulase-negative staphylococci (16.9%) and *Pantoea* spp. (13.0%). The least isolated bacteria were *Yersinia enterocolitica* (1.3%) and *Pasteurella aerogenes* (1.3%) (Table 1).

Table 1: Prevalence of bacteria isolated from human respondents in small scale farms in Gatundu Sub-County, Kenya.

Bacterial species	N	%
<i>Staphylococcus aureus</i>	38	49.4
CoNS	13	16.9
<i>Pantoea</i> spp.	10	13.0
<i>Serratia</i> spp.	10	13.0
<i>Bukholderia cepacian</i>	2	3.0
<i>Enterobacter</i> spp.	2	3.0
<i>Yersinia enterocolitica</i>	1	1.3
<i>Pasteurella aerogenes</i>	1	1.3

CoNS - coagulase negative staphylococci

The overall prevalence of antibiotic resistance among *S. aureus* isolates was 37.3%. In descending order, the *S. aureus* were resistant against gentamicin (86.8%), followed by oxacillin (86.8%), vancomycin (73.7%) and ampicillin (60.5%). Most *S. aureus* were sensitive to amoxycillin-clavulanic acid (89.5%), chloramphenicol (73.7%), cefoxitin (65.8%) and ciprofloxacin (65.8%) (Table 2).

Table 2: Susceptibility profile of *S. aureus* isolated from human having close contact with dairy cows in small scale farms in Gatundu Sub-County, Kenya.

Antibiotic	Antibiogram for <i>S. aureus</i> (n=38)					
	Resistant		Intermediate		Susceptible	
	N	%	N	%	N	%
GEN	33	86.8	0	0	15	39.5
DA	11	28.9	20	52.6	7	18.4
E	10	26.3	25	65.8	3	7.9
TE	11	28.9	8	21.1	19	50
C	10	26.3	0	0	28	73.7
AMC	1	2.6	3	7.9	34	89.5
AMP	23	60.5	0	0	15	39.5
MEL	15	39.5	6	15.8	17	44.7
SXT	0	0	9	23.7	29	50
OX	33	86.8	0	0	5	13.2
VA	28	73.7	8	21.1	2	5.3
FOX	13	34.2	0	0	25	65.8
CIP	13	34.2	0	0	25	65.8

GEN-gentamycin, DA-clindamycin, E-erythromycin, TE-tetracycline, C-chloramphenicol, AMC-amoxycillin, AMP-ampicillin, MEL-mecillinum, SXT-sulphamethoxazole-trimethoprim, OX- oxacillin, VA-vancomycin, FOX- cefoxitin, CIP- ciprofloxacin.

Antibiogram for CoNS

The overall prevalence of antibiotic resistance in coagulase negative *Staphylococci spp* was found to be 63.1 %. Most CoNS were resistant to gentamycin (100%), clindamycin (84.6%), erythromycin (84.6%) and ciprofloxacin 84.6%. The CoNS were most sensitive to sulphamethoxazole-trimethoprim (76.9%) and chloramphenicol (69.2%).

Table 3: Antibiogram of coagulase negative *Staphylococci* (CoNS) isolated from skin swab from human respondents having close contact with dairy cattle in the small-scale farms in Gatundu Sub-County, Kenya.

Antibiotic	Antibiogram of CoNS (n=13)					
	Resistant		Intermediate		Susceptible	
	N	%	N	%	N	%
Gen	13	100.0	0	0	0	0
DA	11	84.6	2	15.4	0	0
E	11	84.6	0	0	2	15.4
TE	8	61.5	1	7.7	4	30.8
C	4	30.8	0	0	9	69.2
AMP	11	84.6	0	0	2	15.4
OX	8	61.5	0	0	5	38.5
FOX	5	38.5	0	0	8	61.5
CIP	11	84.6	0	0	2	15.4
SXT	0	0	3	23.1	10	76.9

Association of antimicrobial resistance and risk factors

The burden of Association of Antimicrobial Resistance (AMR) was higher in males (59.2%) than females

(52.3%). However, the relationship between antimicrobial resistance and gender was statistically insignificant ($p=0.463$, $OD=-0.121$). Most of the respondents having AMR isolates were in the age categories of >50 years old (76.9%) and 40-50 years old (75%). The prevalence of AMR isolates was significantly ($p=0.011$, $OD=1.745$) related to age of the respondents. The AMR burden was higher in respondents who had previous history of usage of medications compared to those who had not ($p=0.025$, $OD=0.204$). The current study also sought to find out on the recovery of the respondents who have protracted use of drugs. Among the respondents, 52.5% did not recover and 47.5% recovered. Further, 65.1% of who had not recovered had AMR resistant isolates while 56.1% of those recovered also had AMR resistant isolates ($p=0.859$, $OD=1.105$).

Over half (54.1%) of the participants who had visited the healthcare center for treatment had AMR resistant isolates. The relationship between the prevalence of antimicrobial resistance and health centre/hospital visitation was not statistically significant ($p=0.287$, $OD=0.577$) (Table 4).

Table 4: Association between risk factors and proportion of antimicrobial resistance (AMR) isolates in respondents from Gatundu Sub-County, Kenya.

Risk factor	N	%	P value	OD
Gender				
Male	45/76	59.2	0.463	-0.121
Female	23/44	52.3		
Age (years)				
≤20	9/16	56.3	0.011	1.745
21-30	7/15	46.7		
30-40	10/26	38.5		
40-50	18/24	75.0		
>50	30/39	76.9		
Medication usage				
No	25/53	47.3	0.025	0.204
Yes	37/67	55.2		
Recovery				
No	41/63	65.1	0.859	1.105
Yes	32/57	56.1		
Hospital visitation				
No	45/59	76.3	0.287	0.577
Yes	33/61	54.1		

Occurrence of MAR

The MAR Index for *S. aureus* and CoNS was 0.92 and 0.92, respectively. This shows that both for both bacteria, the MAR index was greater than 0.2. Occurrence of multidrug resistance (MDR) was noted amongst the various *S. aureus* and CoNS isolates (Table 5). For *S. aureus*, the MDR was expressed in the following antibiotics: 66 (14.7%) isolates expressed MDR to gentamycin and oxacillin, 26 isolates (5.8%) were resistant to cefoxitin and ciprofloxacin, 22 isolates (4.9%)

were resistant to clindamycin and tetracycline while 20 isolates (4.4%) were resistant to chloramphenicol and erythromycin. For CoNS, 44 (33.8%) isolates expressed MDR to clindamycin, erythromycin, ampicillin and ciprofloxacin, and 16 (3.6%) isolates were resistant to tetracycline and oxacillin.

Table 5: Multiple resistance patterns of *S. aureus* and CoNS isolated from human in small-holder farms in Gatundu Sub-County, Kenya.

Bacteria	Antibiotic	N	%
<i>Staphylococcus aureus</i>	GEN & OX	66	14.7
	FOX & CIP	26	5.8
	TE & DA	22	4.9
	E & C	20	4.4
CoNS	DA, E, AMP & CIP	44	33.8
	TE, OX	16	3.6

DISCUSSION

Information about the current magnitude of the burden of bacterial AMR, trends in different parts of the world, and the leading pathogen–drug combinations contributing to bacterial AMR burden is crucial.²⁴ With minimal surveillance, the spread of AMR could make many bacterial pathogens much more lethal in the future than they are today.⁴ The current study investigated the emergence of AMR among human having close contact with dairy cattle in small-scale set-ups in Gatundu sub-county, Kenya. The findings of the current study showed the farmers had previous skin infections such as boils, skin rashes and open wounds, which they treated using various drugs. Most could not remember the specific names of the drugs. The presence of some of these ailments could have led to the high burden of coagulase negative *Staphylococci* spp. and *S. aureus* colonizing the skins of the farmers. Other bacteria isolated included *Pantoea* spp., *Serratia* spp., *Bukholderia cepacia*, *Enterobacter* spp., *Yersinia enterocolitica* and *Pasteurella aerogenes*. These results are consistent with a study conducted in milkmen from Uganda, where the prevalence of *S. aureus* and CoNS was high (71%).¹¹ The present study also agrees to a study conducted in western Romania which found *S. aureus* and CoNS being dominant bacteria colonizing the skin, representing, 48% and 64%, respectively.²⁵ *Staphylococci* spp. are skin commensals and very adaptive on mucous membrane of various animals.

The present study found high percentage of respondents having *S. aureus* resistant to gentamycin, oxacillin and vancomycin. This could be attributed to high prescription of these antibiotics in the study area. The highest susceptibility of *S. aureus* to antibiotics used in this study was observed in amoxycillin-clavulanic acid, chloramphenicol, ciprofloxacin and ceftiofur. The result from the current study are consistent with a study by others.¹¹ Further studies are needed to determine the causes of the high burden of resistance which was

observed. This study observed a high percentage of CoNS resistant to gentamycin, clindamycin, erythromycin, ampicillin and ciprofloxacin. A similar study conducted in Egypt found prevalence of CoNS antibiotic resistant to vary by site of isolation.²⁵ The prevalence of resistant CoNS from the nasal and hands of butchers were 20.0%, 13.5%, respectively. The percentage of CoNS milker's hand was 4.0%.²⁵ In the Egyptian study, CoNS had lower resistance to gentamycin (3.6%), ciprofloxacin (3.6%), sulphamethoxazole-trimethoprim (17.9%), chloramphenicol (25.2%) and ceftiofur (60.7%).²⁵ The observed prevalences could be related to selection pressure due to specific antibiotics used in a given area. Overall, the current study found that large proportion of *S. aureus* and CoNS isolates were exhibited multidrug resistant trait, which agrees with studies done elsewhere.^{22,25} The Ugandan study reported that all *S. aureus* isolates from milkmen's hands were resistant to a combination of ceftiofur, gentamycin, tetracycline, erythromycin and chloramphenicol.¹¹ Multidrug drug resistant was also reported other countries like Ethiopia, where MDR of 7 of 9 (77.8%) antimicrobial classes was noted in CoNS.²² This resistance can be attributed to consistent therapeutic and/or indiscriminate use of these antimicrobials in these study areas. Age and drug usage were found to be potential risk factors to prevalence of antibiotic resistant bacterial pathogen to human who frequently and closely handle dairy cows in the study area. Older respondents had higher burden of AMR in the study area and this could be due to accumulation of resistant bacterial strains due to longer interaction with animals. On the other hand, respondents who has protracted drug usage had higher burden of AMR strains of bacteria. Although the names and classes of drugs used by the respondents could not be ascertained, studies have shown that antibiotic use in both Kenyan inpatient and outpatient settings is more than 80% and is rarely backed by culture and sensitivity data.²⁵ It is hypothesized that over-use and mis-use of antibiotics could be the cause of high burden of AMR bacteria in the study area.

Limitations

The current study was cross-sectional and therefore we could not ascertain whether the isolated bacteria were of human or animal origin. In addition, the antimicrobial sensitivity study only focused on *Staphylococci* spp. The study did not differentiate the species of Coagulase negative *Staphylococci*. Studies should be undertaken to determine the origin of these resistant bacteria with an intention of advocating for the judicious use of available antibiotics.

CONCLUSION

The present study showed a high prevalence of CoNS and *S. aureus* among the human respondent which could be a potential risk to human health. Further, high prevalence of antimicrobial resistance was noted in these isolates. Further studies should be undertaken to determine the

cause for the high burden of AMR, the factors causing its emergence and whether the bacteria could be spreading from animals and the implications in human and veterinary medicine.

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