Vol. 12(44), pp. 994-1003, 28 November, 2018

DOI: 10.5897/AJMR2018.8993 Article Number: 335514F59375

ISSN: 1996-0808 Copyright ©2018

Author(s) retain the copyright of this article http://www.academicjournals.org/AJMR



# African Journal of Microbiology Research

Full Length Research Paper

# Epidemiology, prevalence and antibiotic susceptibility profiles of methicillin-resistant *Staphylococcus aureus* in farm animals and farm workers in the Central Region of Ghana

Clement Yaw Effah<sup>1</sup>, Nicholas T. K. D. Dayie<sup>2</sup>, Senyo Yao Akorli<sup>1</sup> and Anthony Annan-Prah<sup>1\*</sup>

Received 1 October, 2018; Accepted 14 November 2018

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most important microorganisms which has increasingly become resistant to most commonly used antimicrobials. This research investigated the epidemiology, nasal carriage prevalence and the antibiotic susceptibility profile of MRSA among farm animals and farm workers in the Central Region of Ghana. A total of 396 nasal swabs were collected from farm animals (94.9%) and farm workers (5.1%). Antibiotic susceptibility test was carried out on Mueller Hinton agar using Kirby-Bauer disc diffusion method. Epidemiological risk factors were assessed using pre-designed questionnaires. Results showed that the overall prevalence of MRSA in the Central Region of Ghana was 49.2% (195/396). Pigs recorded the highest nasal carriage prevalence of 50.2% (157/313) followed by sheep 45.1% (23/51), goats 50% (6/12) and humans 45% (9/20). MRSA isolates were 100% susceptible to both Vancomycin and Augmentin. Epidemiological risks factors for nasal colonisation of farm workers in this study were: direct contact with pigs (p=0.000), last period of antibiotic administration (p=0.020), and the type of apparels worn (nose mask (p=0.000), and gloves (p=0.020)). Epidemiological risk factors for nasal colonisation of farm animals in this study were: the type of antibiotic administered (p=0.000) and the last period for antibiotic administration (p=0.000). Phenotypic detection of MRSA and their resistance to the tested antibiotics should be a cause of alarm in the Central Region of Ghana.

**Key words:** Methicillin-resistant *Staphylococcus aureus*, prevalence, nasal carriage, epidemiology, farm animals, workers.

# INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is of a major concern in clinical medicine due to the

importance of  $\beta$ -lactams in the therapy of staphylococcal infections. In addition, morbidity and mortality for MRSA

\*Corresponding author. E-mail: annanprah@yahoo.com. Tel: +233 24371 6979, +233 20 815 9333.

Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u>

<sup>&</sup>lt;sup>1</sup>School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana. <sup>2</sup>Department of Medical Microbiology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Ghana.

patients compared to patients infected with methicillinsusceptible S. aureus (MSSA) is on the rise (De Kraker et al., 2011). In Ghana, the children's block of the Korle-Bu Teaching Hospital (KBTH), was closed down in January, 2012 as a result of an MRSA outbreak in the ward (GNA, 2012). The epidemiology of this bacterium in animals has also gained global attention in recent years due to the spread of multidrug resistance and their increasingly evidenced zoonotic potential. In a study by Haenni et al. (2011) in France, MRSA isolates had identical characteristics to the human Geraldine clone (ST5, spatype t002, and the same virulence genes, resistance pattern, and SCCmec cassette type I). These livestockassociated (LA)-MRSA are considered to be zoonotic and people with occupational contact with livestock, for example, farmers, veterinarians, and workers at abattoirs, are frequently exposed and colonised (Cuny et al., 2013). In 2007, a report on transmission of MRSA between dairy cows and milking personnel alerted people working with dairy cattle of the occupational health risks (Juhász-Kaszanyitzky et al., 2007).

Due to the interspecies transmission of MRSA and the health risks of MRSA colonisation, there is the need to research into the roles animals play in human MRSA infections. In Ghana, very little is known about the nasal carriage prevalence of MRSA among humans (Egyir et al., 2015, Odonkor et al., 2012). In addition, there are no published data on MRSA carriage in farm animals and farm workers. Hence, this study was designed to the epidemiology determine and antimicrobial susceptibility patterns of MRSA in apparently healthy farm animals and farm workers in the Central Region of Ghana.

# **MATERIALS AND METHODS**

# Study location

The study was conducted in 9 of the 15 administrative districts in the Central Region of Ghana. These were Komenda Edina Equafo Abrem (KEEA), Cape Coast, Twifo Heman Lower Denkyira (THLD), Abura Asebu Kwamankese (AAK), Assin, Gomoa, Effutu, Mfantseman and Agona. These regions were selected to represent the North, South, East and Western zones of the region. The Central Region is located in the South-Western centre of Ghana. It is on latitude of 5° 29' 59.99 N and a longitude of -1 00' 0.00". The Central Region shares boundaries with the Ashanti Region to the north, Greater Accra Region to the south-east and to the west by Western Region. It is bound to the south by the Gulf of Guinea. It has an annual temperature range of 24 and 34°C, annual rainfall pattern averaging 800 to 1500 mm and a relative humidity of between 50 and 85%. The region has six months each of rainy and dry seasons. The population of the Central Region has been projected to be 2,521,118 in 2018 (Ghana Statistical Service, 2013).

# Sampling technique

A list of almost all farms in the districts of the region was obtained from the Ministry of Food and Agriculture. Out of this list, simple

random sampling technique was used to select the farms for this study. The districts were selected based on convenience, that is, based on the proximity of the districts to the researcher. A total of 18 livestock farms (two from each district) that reared pigs, sheep and goats were randomly selected across all the nine districts of the study area. Because most of the farmers reared pigs with goats and sheep as a supplement, pigs were allotted 80% of the total sample size. In calculating the minimum sample size for this study, the formula described by Daniel (1999) was used.

$$n = Z^2 P(1-P) / d^2$$

Where, n = sample size, Z = z statistic for a level of confidence,  $P = \text{expected prevalence or proportion (in proportion of one; if 20%, <math>P = 0.2$ ), and d = precision (in proportion of one, if 5%, d = 0.05).

For the level of confidence of 95%, which is conventional, Z value is 1.96. Also, since there was no know prevalence in the study area, the P value was perceived to be 50% (Daniel, 1999).

A total of 396 nasal samples were obtained from pigs, sheep, goats and farm workers in the study area.

# Questionnaire survey and epidemiological data

A structured questionnaire, interviews and observations were used to elicit data on potential risk factors for the colonisation of MRSA in farm animals and the farm workers. From the farm workers, demographic data (gender, age, educational background, occupation), farm- and animal-related variables (exposure to pigs, handling antimicrobial drugs to animals, use of hygiene/protective measures, and occupational activities), life style determinants (eating preferences, exposure to raw meat, smoking, contact with pets), and medical history (exposure to health care facilities, antibiotic usage) were collected for each farmer at each sampling moment. For the animals, data such as age, sex, type of antibiotics used on them and the frequency of antibiotic use were collected.

# Sample collection

From each farm, 22 nasal samples were collected from apparently healthy pigs, goats, sheep and workers. From each farm, at least one sample was taken from the farm workers. One nasal swab per individual was taken from the anterior nares of farmers and livestock using sterile swabs in liquid transport medium (ESwab, Copan, Brescia, Italy). Samples were stored at 4°C and transported to the laboratory.

# Ethics approval and consent to participate

Ethical approval was acquired from the Institutional Review Board of the University of Cape Coast, Ghana. The nasal samples from the animals were collected with the help of veterinarian.

# Isolation of MRSA

The swabs were streaked onto selective MRSA agar plates (ORSAB, OXOID, UK). These plates were incubated for 24 to 48 h at 37°C and examined for the blue, raised MRSA colonies.

# Anti-microbial susceptibility testing

The MRSA isolates were subjected to antibiotic susceptibility test using disc diffusion method by Kirby-Bauer (1966). The following antibiotics were used: penicillin (15  $\mu$ g), ampicillin (10  $\mu$ g),

District -	MRSA Isolates of species							
	Pigs [n/N (%)]	Sheep [n/N (%)]	Goats [n/N (%)]	Man [n/N (%)]	Total [n/N (%)]			
KEEA	30/39 (76.9)	0/0 (0)	0/0 (0)	2/5 (40)	32/44 (72.4)			
CC	19/28 (67.9)	6/15 (40)	0/0 (0)	0/1 (0)	25/44 (56.8)			
THLD	15/38 (39.5)	0/0 (0)	1/2 (50)	2/4 (50)	18/44 (40.9)			
AGONA	23/44 (52.3)	0/0 (0)	0/0 (0)	0/1 (0)	23/44 (52.3)			
ASSIN	21/44 (47.7)	0/0 (0)	0/0 (0)	0/0 (0)	21/44 (47.7)			
MFANTSEMAN	10/28 (35.7)	5/15 (33.3)	0/0 (0)	0/1 (0)	15/44 (34.1)			
EFUTU	7/12 (58.3)	12/21 (57.1)	5/10 (50)	1/1 (100)	25/44 (56.8)			
GOMOA	17/43 (39.5)	0/0 (0)	0/0 (0)	1/1 (100)	18/44 (40.9)			
AAK	15/37 (40.5)	0/0 (0)	0/0 (0)	3/7 (42.9)	18/44 (40.9)			
Total	157/313 (50.2)	23/51 (45.1)	6/12 (50)	9/20 (45)	195/396 (49.2)			

Table 1. Isolation rates of MRSA among different species of animals and districts in the Central Region of Ghana.

Table 2. Distribution of MRSA isolates among various Age groups and sex of animals.

	MRSA isoaltes							
Parameter	Pig		Sheep		Goat		Total No.	%
	Freq./Sample size %		Freq./Sample size %		Freq./Sample size %			
Age of animal								
<4 months	75/151	49.7	1/6	16.7	-	-	76	48.4
4-10 months	68/130	52.3	5/10	50	0/2	0	73	51.4
11-15months	8/16	50	7/12	58.3	2/4	50	17	53.1
>15months	6/16	37.5	10/23	43.5	4/6	66.7	20	44.4
Sex of animal								
Male	86/166	51.8	5/9	55.6	1/4	25	92	51.4
Female	71/147	48.3	18/42	42.9	5/8	62.5	94	47.7

tetracycline (30 µg), Cotrimoxazole (25 µg), Erythromycin (5 µg), Gentamicin (10 µg), Vancomycin (30 µg), Cloxacillin (5 µg), Cefuroxime (10 µg), Augmentin (30 µg), Meropenem (10 µg), and Ciprofloxacin (5 µg). Approximately, between 2 and 5 isolated colonies from a pure culture were emulsified in sterile nutrient broth and the turbidity of the inoculum was compared with 0.5 McFarland Standard. Mueller-Hinton Agar (Lab M Limited, Lancashire, UK) plates were prepared and a loopful of the inoculum was seeded on the surface of the media. Subsequently, a sterile cotton swab was used to spread the organism evenly on the Mueller-Hinton agar. The antibiotic discs were placed on the agar plates using sterilised forceps. The plates were read after 24 h of incubation at 37°C under aerobic condition. S. aureus ATCC 25923 was used as a quality control strain. The sensitivity of the isolates to various antibiotics were classified in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (CLSI, 2016) as susceptible, intermediate or resistance for each antibiotic tested by measuring the zone of inhibition around the antibiotic disc.

# **RESULTS**

# Nasal carriage prevalence of MRSA in livestock and farmers

A total of 396 samples were collected from farm animals

and farm workers. This comprised 313, 51, 12 and 20 nasal swabs from live pigs, sheep, goats and related farm workers, respectively from the 9 districts. In summary, 50.2% (157/313), 45.1% (23/51), 50% (6/12) and 45% (9/20) MRSA were isolated from pigs, sheep, goats and farm workers, respectively representing an overall prevalence of 49.2%. From Table 2, a total of 195 MRSA were isolated of which most were isolated from livestock between the ages of 15 months and below. The overall isolation rate of MRSA was higher among female animals. Among the various districts, KEEA district recorded the highest MRSA isolates (72.7%) with Mfantseman district recording the lowest MRSA isolates (34.1%) from both animals and farm workers (Table 1).

# **Epidemiological risk factors for MRSA colonisation**

From Table 4, it can be seen that, respondents for the potential risk factors were 5/20, 0/20, 3/20, 3/20 and 9/20 for age groups < 18 years, 18-24 years, 25-34 years, 35-44 years and > or = 45 years, respectively. Among these respondents, no MRSA isolates were isolated from age

groups 18-24 and 35-44 years. Individuals below the age of 18 years recorded the highest MRSA isolates of 4 out of 5 respondents representing 80% followed by age group 45 and above (4/9, 44.4%) and age group 25-34 (1/3, 33.3%). MRSA nasal carriage was not significantly associated with age of the respondents (p= 0.368).

From Table 4, all 20 respondents were male and 9 were MRSA carriers representing 45%. Although few, people with no educational background recorded the highest percentage of MRSA isolate (66.7%) but statistically, educational background was not significantly associated with MRSA colonisation (p= 0.189). It could be statistically deduced that, MRSA colonisation is not related to the purchase of pork, beef, chevon, mutton and chicken in this study (Table 4).

Exposure to raw meat is not significantly associated with MRSA nasal colonization (p= 0.717). Statistically, smoking was not a major risk factor for MRSA colonisation in this study (p= 0.739). Having direct contact with pigs (p= 0.000) and the last period of taking antibiotic (p= 0.020) were statistically, major risk factors for MRSA nasal colonization in farm workers.

From Table 4, it can be seen that two major factors may predispose farm animals to MRSA nasal colonisation. Statistically, it can be seen that the type of antimicrobial to treat microbial infections (p= 0.010) and the period of antibiotic administration to animals (p= 0.000) are highly associated with MRSA nasal colonisation in farm animals.

# Antibiotic susceptibility pattern of MRSA isolates

A total of 195 MRSA isolates of this study were subjected to antibiotic susceptibility test against 12 antimicrobial drugs. From Table 3, most of the MRSA isolates were sensitive to Vancomycin (100% sensitivity), Augmentin (100% sensitivity) and Ciprofloxacin (79.5% sensitivity). However, no isolate was sensitive to Penicillin or βlactam associated antibiotics. Accordingly, the highest resistance was observed for Penicillin (100%, 195), Cloxacillin (100%, 195), and Ampicillin (100%, 195). The resistance of MRSA to other antibiotics is as follows: Cefuroxime (90.8%, 177), Meropenem (86.7%, 169), Erythromycin (75.4%, 147), Gentamycin (67.7%, 132), Cotrimoxazole (57.4, 112) and Tetracycline (56.4%, 110) (Table 3). Except for Tetracycline (p=0.073), all other susceptibility test results of antibiotics against MRSA isolates were statistically significant (p<0.05).

# DISCUSSION

This is the first known study to identify MRSA colonisation in farm animals and farm workers in Ghana. The presence of MRSA in animals and humans in the study area is of veterinary and public health concern.

**Table 3.** Phenotypic Antibiotic susceptibility patterns of MRSA isolated from both animals and man in Central Region of Ghana.

Antibiotics		MRSA	- P-value		
		No.	%		
	Susceptible	-	-		
Penicillin	Intermediate	-	-	0.000	
	Resistant	195	100		
	Susceptible	-	-		
Ampicillin	Intermediate	-	-	0.000	
	Resistant	195	100		
	Susceptible	_	_		
Cloxacillin	Intermediate	_	_	0.000	
	Resistant	195	100		
	Susceptible	-	-		
Erythromycin	Intermediate	48	24.6	0.000	
	Resistant	147	75.4		
	Susceptible	-	-		
Tetracycline	Intermediate	85	43.6	0.073	
	Resistant	110	56.4		
	Susceptible	195	100		
Vancomycin	Intermediate	-	-	0.000	
	Resistant	-	-		
	Susceptible	13	6.7		
Cotrimoxazole	Intermediate	70	35.7	0.000	
	Resistant	112	57.4		
	Susceptible	5	2.7		
Cefuroxime	Intermediate	13	6.7	0.000	
	Resistant	177	90.8		
	Susceptible	30	15.5		
Gentamycin	Intermediate	33	16.9	0.000	
Containyon	Resistant	132	67.7	0.000	
	Susceptible	155	79.5		
Ciprofloxacin	Intermediate	32	16.4	0.000	
	Resistant	8	4.1		
	Susceptible	195	100		
Augmentin	Intermediate	-	-	0.000	
	Resistant	-	-		
	Susceptible	1	0.51		
Meropenem	Intermediate	25	12.8	0.000	
	Resistant	169	86.7		

Nasal carriage has an important role in the epidemiology and pathogenesis of MRSA infection in humans and animals. The overall prevalence rate of MRSA in the

**Table 4.** Epidemiologic risk factors for MRSA nasal carriage among farm workers and farm animals in the central region of Ghana.

Associated factor		Freq./Sample size		Carriers	- P-Value	
Associated factor		rreq./Sample Size	No.	%	r-value	
	< 18 years	5/20	4/5	80		
	18-24 years	0/20	-	-		
Age group	25-34 years	3/20	1/3	33.3	0.368	
	35-44 years	3/20	-	-		
	> or = 45	9/20	4/9	44.4		
Sex	Male	20/20	9/20	45		
	Female	0/20	-	-		
	None	3/20	2/3	66.7		
	Junior high	10/20	5/10	50		
Educational background	Senior high	4/20	1/4	25	0.189	
	Tertiary	3/20	1/3	33.3		
	Tornary	0/20	1/0	00.0		
	Pork					
	Yes	14/20	7/14	50	0.096	
	No	6/20	2/6	33.3	0.000	
	Beef					
	Yes	4/20	-	_		
	No	16/20	9/16	56.3	0.000	
	Mutton					
Meat Normally Purchased	Mutton Yes	2/20	1/2	50		
weat Normally Purchased			8/18		0.020	
	No	18/20	8/18	44.4		
	Chevon					
	Yes	0/20	-	-	0.000	
	No	20/20	9/20	45	0.000	
	Chicken					
	Yes	17/20	7/17	41.2		
	No	3/20	2/3	66.7	0.096	
	NO	3/20	2/3	00.7		
	Frequently	3/20	2/3	66.7		
Exposure to Raw Meat	Occasionally	8/20	3/8	37.5	0.717	
Exposure to Haw Mode	Seldom	9/20	4/9	44.4		
	Voc	E/00	A/E	00		
Smoking of Cigarette	Yes	5/20	4/5	80	0.739	
-	No	15/20	5/15	33.3		
Data of Haanitaliastics	< 3 months ago	8/20	2/8	25	0.000	
Date of Hospitalisation	> or = 3 months ago	12/20	7/12	58.3	0.096	
	2 months aga	3/20	1/3	22.2		
Last Time of Taking Antibiotic	< 3 months ago			33.3	0.020	
-	> or = 3 months ago	17/20	8/17	47.1		
Discret Ossets et a 19 Di	Yes	18/20	9/18	50	0.000	
Direct Contact with Pigs	No	2/20	0/2	_	0.000	

Table 4. Contd.

Bruises during Direct Contact	Yes	6/20	4/6	66.7	0.739
Bruises during Direct Contact	No	14/20	5/14	35.7	0.739
	4.001	40/00	4/40	40	
	1-20 hours	10/20	4/10	40	
Hours/Week in Direct Contact	21-40 hours	3/20	3/3	100	0.717
	> 40 hours	7/20	2/7	28.6	
	V	4.4/0.0	0/4.4	40.0	
Collected Blood/Urine From	Yes	14/20	6/14	42.9	0.317
Livestock?	No	6/20	3/6	50	0.011
	Boats				
	Yes	20/20	9/20	100	
			9/20	100	0.000
	No	0/20	-	-	
	Long coats				
	Yes	6/20	4/6	66.7	
	No	14/20	5/14	35.7	0.739
Apparels Worn During Contact					
	Gloves				
	Yes	0/20	-	-	0.000
	No	20/20	9/20	100	0.000
	Nose mask			-	
	Yes	0/20	-	100	0.000
	No	20/20	9/20		0.000
	Tatra avalina	205/200	440/005	20.0	
Antimicrobials to Treat Livestock	Tetracycline	285/396	110/285	38.6	0.010
Infection	Penicillin	111/396	75/111	67.6	0.0.0
Period of Antibiotic	< 3 months ago	236/396	148/236	62.7	
Administration to animals					0.000
Auministration to ariimais	> or = 3 months ago	160/396	47/160	29.4	

Central Region of Ghana was 49.2%. The prevalence of colonisation both at the farm level and the individual animal level on most farms, where MRSA was present, was striking. Most of the tested animals on many farms of this study, especially pigs, were nasally colonised by MRSA, though on some farms, only a small number of animals were nasally colonized with MRSA. It is unclear whether differences in management on farms were associated with this variation. It would be interesting to re-test low prevalence farms to determine whether the prevalence of MRSA colonisation has increased, as it is possible that the low prevalence could indicate recent introduction of MRSA.

Results from the present study showed that the carriage rate of MRSA was higher than that for Asian countries such as China, which recorded carriage rate in pigs as 58/509, 11.4% (Cui et al., 2009); Korea 21/657, 3.2% (Lim et al., 2012); Malaysia 1.4% (Neela et al., 2009); Japan 0.9% (Baba et al., 2010) and Hong Kong 16

to 21.3% (Guardabassi et al., 2009). Also, the prevalence of MRSA found in pigs (50.2%) in this study was different in comparison to other European studies. For example, in Belgium, an estimated 44% were carriers (Crombé et al., 2012); in Germany, a prevalence of 52% was reported for fattening farms (Alt et al., 2011), and there was 56% prevalence in pig holding companies in the Netherlands (Broens et al., 2011). Moreover, in La Rioja (Northern Spain), Gómez-Sanz et al. (2010), described a prevalence of 21 and 49% in fattening and suckling pigs. respectively, at the slaughterhouse level. These differences can be attributed variations to microbiological methods (sampling technique, culture and method of MRSA identification), local infection control standards, local prevalence of MRSA and husbandry methods.

This carriage prevalence in farm animals in Ghana could be attributed to the higher stocking density which was observed throughout the visit. Previous studies

indicated that higher density increased the risk of MRSA colonisation (Van Duijkeren et al., 2007; Battisti et al., 2010). S. aureus is not typically regarded as a pathogen in pigs, sheep and goat and even with a high prevalence of MRSA colonisation in these animals, clinical infections have not been widely reported in this species. However, a report by Van Duijkeren et al. (2007), implicating MRSA in exudative dermatitis in pigs raises potential pig health concerns. Although the percentage of MRSA nasal carriage in sheep was remarkably lower than those observed in pigs and goats, the reason for this is not clear but it is suggested that variation could partly be due to differences in nasal physiology and self-care behaviors of these animals. Efforts should be made to characterise possible reservoirs in order to reduce the spread of MRSA among farm animals especially pigs.

Although definite conclusions cannot be made because of small sample size, observations of this study on farm workers are consistent with numerous studies demonstrating that people working in close contact with animals colonised with MRSA have a high risk of culture positive nasal swabs (Smith et al., 2009; Weese, 2010; Wulf et al., 2008). Further confirmation of this phenomenon is unlikely to provide new insight unless accompanied by efforts to understand its biological nature and implications for occupational health.

Furthermore, although very high prevalence has been described in this study and in other cross-sectional studies of farm workers and farm animals, the routine use of multiple enrichment methods to culture samples is likely to result in detection of samples with low numbers. Quantification of MRSA from culture positive farm workers may provide a more meaningful context for evaluating contamination versus colonisation events and informing assessment of associated health risks. Transmission of methicillin resistant variant between animals and humans has been frequently reported via direct contact (Deiters et al., 2015) or indirect routes such as the environment and food chain (Peterson et al., 2012).

Although direct comparison cannot be made because a control group was not used in the assessment of human colonisation, the prevalence of colonisation in farm workers (45%) was guite high and is much greater than has been reported in some research work in the general population in Accra. For example, a study by Odonkor et al. (2012) in the Greater Accra Region of Ghana estimated a population colonisation of 84 MRSA out of the 250 S. aureus isolates, giving a prevalence rate of 33.6% of the samples collected from microbiological samples from hospital in Accra-Ghana. Further, a study on MRSA prevalence on people who visited the University of Ghana Hospital was only one (9.1%) out of 11 S. aureus positive isolates (Pesewu et al., 2014). Therefore, it is likely that personnel working with farm animals specifically pigs as seen in this study are at higher risk for MRSA colonisation compared to the

general Ghanaian population.

There were also some differences in MRSA nasal carriage rate among various districts in the Central Region, with KEEA district recording the highest and Mfantseman district recording the lowest. Reasons for these differences cannot be understood but may be attributed partly to the variation in geographical locations, the differences in the proportions of animals sampled and the extent of risky environmental exposures. Egyir et al. (2014) reported variations in the distribution of MRSA in the Northern Region of Ghana and attributed it apparently to geographical variations.

# Antimicrobial resistance profiles of the isolates from farm animals and farmers

The antimicrobial resistance profiles shown in the present study are comparable to many other studies within and outside Ghana reiterating the fact that pathogens are likely to develop resistance to most commonly used drugs (Penicillin, Ampicillin and Erythromycin) as opposed to less commonly used drugs Ciprofloxacin and Vancomycin (Verkade et al., 2014; Odonkor et al., 2012; Moyo et al., 2014). Unlike other previous studies, all the MRSA isolates were somehow resistant to all the classes of antibiotics used in the study except for Vancomycin, Augmentin and Ciprofloxacin. This study agrees with Sapkota et al. (2006), who reported that MRSA was susceptible to Ciprofloxacin and Vancomycin. The present study showed the resistance of MRSA to Penicillin (100%), Ampicillin (100%) and Tetracycline (54.6%). These findings agree with the findings of Shibabaw et al. (2013), who among others reported resistance of MRSA to Penicillin (94%) and Tetracycline (73.8%) around Addis Ababa. This confirms the previous finings that MRSA strains have been recognised to be resistant to almost all β-lactam antibiotics (Lowy, 2003). The observed resistance patterns to some of the conventional antibiotics, which are usually frequently prescribed in the study area, are alarming because of the high resistance rates among nonclinical isolates such as those obtained in this study. This further reaffirms the critical role of commensals in public health. The observed high level of resistance (65 to 100%) to almost all of the antibiotics might be due to consumption of antimicrobials (Moulin, 2001) as growth promoters (Perrier-Gros-Claude et al., 1998), used extensively in livestock husbandry in the study area.

The present study has demonstrated the existence of alarming levels of resistance of MRSA to commonly used antimicrobials (Penicillin and Ampicillin) in the farms of this study. The results were in accordance with reports from earlier studies in other countries (El-Jakee et al., 2008; Gentilini et al., 2002) suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. Vancomycin

used to be the last antibiotic for treating infections caused by such resistant isolates (Boucher et al., 2010; Fitzgerald et al., 2001; Bhalakia et al., 2006). This study reaffirms this fact as all MRSA isolates were sensitive to Vancomycin.

Meanwhile, the wide range of multiple antibiotic resistance showed a divergence between the static-use (in situation where a fixed antibiotic regimen is used) and the adaptive-use (in using varieties or wide range of antibiotics due to observed low performance of the earlier one(s)), which may imply consistent use of various antibiotics in these farms on the animal, to achieve a nonchemotherapeutic advantage (Laxminarayan et al., 2011). This implies that the organisms might have developed resistance over a period of exposure without medical prescription. Therefore, this exposure of the animal bacterial flora to antibiotics appears to be encouraging emergence of resistance across a wide range of antibiotics (Barbosa, 2000). It is, therefore, important to control the misuse or any other nontherapeutic use of antibiotics. This study is, therefore, important to the agricultural sector in Ghana as far as animal health is concerned. It is also important to the human health sector due to the possibility of zoonotic infections, even if the organism in the animals had its original source from previous human contact. Farm animal workers, especially piggery workers, should be diligently hygienic as the animal is a consistent source of MRSA. The relatively high prevalence of MRSA observed among conventional herds in the study confirms that routine antimicrobial use in farm animal especially pigs is a sufficient cause for emergence of LA-MRSA.

# Epidemiologic risk factors for MRSA colonisation among farm workers and farm animals

In this study, factors that could be associated with lower socioeconomic status were positively associated with MRSA carriage, educational status (less than high school or general educational developmental degree versus higher levels (Schinasi et al., 2013). Similarly, other studies have reported positive associations between lower socioeconomic status and MRSA (Casey et al., 2013). A recent population-based study in Pennsylvania found that community-acquired MRSA infection was associated with community economic deprivation (Casey et al., 2013). Why lower levels of educational achievement would be a risk factor, is also difficult to speculate, unless this is a surrogate for lower socioeconomic status and, therefore, crowded living conditions. Epidemiologic risk factors that have significant association with MRSA nasal carriage among farm workers and farm animals in this study were: direct contact with pigs (p=0.000), the last period of taking antibiotic (p= 0.020), type of antimicrobial to treat microbial infections (p=0.10) and the period of antibiotic administration to animals (p=0.000) and whether or not one wear apparels such as nose mask (p=0.000) and gloves (p=0.000). Similar studies showed that the rate of nasal colonisation of MRSA is high when there are predisposing risk factors like antibiotic usage in previous 4 weeks (Aiello et al., 2006; Farley et al., 2008). All respondents who wore boots were MRSA carriers. This means that wearing boots is not a sole channel or factor for assessing risk factor for MRSA nasal carriage.

It was observed that younger individuals both farm workers and farm animals had high MRSA nasal carriage. Why younger individuals and females would be at higher risk for MRSA colonisation could not be determined in this study but can be partly due to their weaker immune response. Statistically, sex was not an associated risk factor for carrying MRSA in the current study, which was in agreement to others (Graham et al., 2006; van Cleef et al., 2011). The protective measures taken by some of the farmers did not prevent them from becoming colonised with MRSA. This could be a result of breaches in adherence to these measures, e.g., poor hand hygiene after removal of gloves or the reuse of contaminated apparels, or because of contamination outside pig farms.

Although not determined, dust may also be a potential risk factor for acquiring MRSA for individuals who tested positive for MRSA and who had no direct contact with livestock. In a rural region in Lower Saxony, Germany, local residents who visited farms, e.g. to buy meat, had a 3.2-times higher risk (95% CI: 1.4-7.4) of colonisation with MRSA than did people without occupational livestock contact (Bisdorff et al., 2012).

A correlation between exposure time on farm and human colonisation has been shown elsewhere (Graveland et al., 2011), which is reaffirmed by the present results. However, a larger number of participants would be needed for more reliable results.

# **Conclusions**

The data from this study showed that MRSA is present in Central Region of Ghana and farm animals especially pigs can serve as reservoir of this multi-drug resistant organism. The carriage rate of MRSA is highest among pigs than goats, sheep and humans respectively. Antimicrobial resistance is a clear and present danger. MRSA isolates were all resistant to all the antibiotics except Vancomycin, Ciprofloxacin and Augmentin. Some epidemiological risks factors observed in this study were; direct contact with pigs, last period of antibiotic administration, type of antibiotic administered and not wearing apparels like nose mask and gloves. Based on the results, it is recommended that all MRSA isolates identified with the phenotypic method in this study should confirmed using molecular tools. Molecular be characterisation should be done to determine the genetic relatedness between MRSA strains of farm animals and

MRSA strains of farm workers in the Central Region of Ghana. In future, surveillance studies using large sample size, should be conducted so as to make the findings robust.

# **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

# **ABBREVIATIONS**

**MRSA**, Methicillin resistant *Staphylococcus aureus*; **MSSA**, methicillin susceptible *Staphylococcus aureus*; C.I., confidence intervals.

### **REFERENCES**

- Aiello AE, Lowy FD, Wright LN, Larson EL (2006). Meticillin-resistant Staphylococcus aureus among US prisoners and military personnel: review and recommendations for future studies. The Lancet Infectious Diseases 6(6):335-341.
- Alt K, Fetsch A, Schroeter A, Guerra B, Hammerl JA, Hertwig S, Senkov N, Geinets A, Mueller-Graf C, Braeunig J, Kaesbohrer A, Appel B, Hensel A, Tenhagen B A (2011). Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Veterinary Research 7(1), 69.
- Baba K, Ishihara K, Ozawa M, Tamura Y, Asai T (2010). Isolation of meticillin-resistant *Staphylococcus aureus* (MRSA) from swine in Japan. International Journal of Antimicrobial Agents 36(4):352-354.
- Barbosa TM, Levy SB (2000). The impact of antibiotic use on resistance development and persistence. *Drug resistance updates*, *3*(5), 303-311.
- Battisti A, Franco A, Merialdi G, Hasman H, Iurescia M, Lorenzetti R, Feltrin F, Zini M, Aarestrup FM (2010). Heterogeneity among methicillin-resistant Staphylococcus aureus from Italian pig finishing holdings. Veterinary Microbiology 142(3-4):361-366.
- Bauer AW, Kirby WMM, Sherris JC, Turck M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology *45*(4\_ts): 493-496.
- Bhalakia N, Morris D (2006). Isolation and plasmid analysis of vancomycin-resistant *Staphylococcus aureus*. Journal of Young Investigator 15(4):15-24.
- Bisdorff B, Scholhölter JL, Claussen K, Pulz M, Nowak D, Radon K (2012). MRSA-ST398 in livestock farmers and neighbouring residents in a rural area in Germany. Epidemiology and Infection 140(10):1800-1808
- Boucher H, Miller LG, Razonable RR (2010). Serious infections caused by methicillin-resistant *Staphylococcus aureus*. Clinical Infectious Diseases 51(Supplement\_2):S183-S197.
- Broens EM, Graat EAM, Van der Wolf PJ, Van de Giessen AW, Van Duijkeren E, Wagenaar JA, van Nes A, Mevius DJ, de Jong MC (2011). MRSA CC398 in the pig production chain. Preventive Veterinary Medicine 98(2-3):182-189.
- Casey JA, Cosgrove SE, Stewart WF, Pollak J, Schwartz BS (2013). A population-based study of the epidemiology and clinical features of methicillin-resistant Staphylococcus aureus infection in Pennsylvania, 2001–2010. Epidemiology and Infection 141(6):1166-1179.
- Crombé F, Willems G, Dispas M, Hallin M, Denis O, Suetens C, Gordts B, Struelens M, Butaye P (2012). Prevalence and antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus among pigs in Belgium. Microbial Drug Resistance:18(2):125-131.
- Cui Š, Li J, Hu C, Jin S, Li F, Guo Y, Ran L, Ma Y (2009). Isolation and characterization of methicillin-resistant Staphylococcus aureus from swine and workers in China. Journal of Antimicrobial Chemotherapy 64(4):680-683.

- Cuny C, Köck R, Witte W (2013). Livestock associated MRSA (LAMRSA) and its relevance for humans in Germany. International Journal of Medical Microbiology 303(6):331-337.
- Daniel WW (1999). Biostatistics: A Foundation for Analysis in the Health Siences. 7<sup>th</sup> edition. New York: John wiley & Sons.
- De Kraker ME, Davey PG, Grundmann H, BURDEN Study Group (2011). Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. PLoS Medicine 8(10): e1001104.
- Deiters C, Günnewig V, Friedrich AW, Mellmann A, Köck R (2015). Are cases of Methicillin-resistant *Staphylococcus aureus* clonal complex (CC) 398 among humans still livestock-associated?. International Journal of Medical Microbiology *305*(1):110-113.
- Egyir B, Guardabassi L, Esson J, Nielsen SS, Newman MJ, Addo KK, Larsen AR (2014). Insights into nasal carriage of *Staphylococcus aureus* in an urban and a rural community in Ghana. PLoS One 9(4):e96119.
- Egyir B, Guardabassi L, Monecke S, Addo KK, Newman MJ, Larsen AR (2015). Methicillin-resistant *Staphylococcus aureus* strains from Ghana include USA300. Journal of Global Antimicrobial Resistance 3(1):26-30.
- El-Jakee J, Nagwa AS, Bakry M, Zouelfakar SA, Elgabry E, El-Said WG (2008). Characteristics of *Staphylococcus aureus* strains isolated from human and animal sources. American-Eurasian Journal of Agricultural and Environmental Sciences 4(2):221-229.
- Farley JE, Ross T, Stamper P, Baucom S, Larson E, Carroll KC (2008). Prevalence, risk factors, and molecular epidemiology of methicillin-resistant Staphylococcus aureus among newly arrested men in Baltimore, Maryland. American Journal of Infection Control 36(9):644-650
- Fitzgerald JR, Sturdevant DE, Mackie SM, Gill SR, Musser JM (2001). Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. Proceedings of the National Academy of Sciences 98(15):8821-8826.
- Gentilini E, Denamiel G, Betancor A, Rebuelto M, Fermepin MR, De Torres RA (2002). Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis in Argentina. Journal of Dairy Science 85(8):1913-1917.
- Ghana Statistical Service (2013). 2010 Population and Housing Census: National Analytical Report. Ghana Statistics Service. https://www.citypopulation.de/php/ghana-admin.php?adm1id=02
- GNA (Ghana News Agency) (2012). Management of the Korle Bu Teaching Hospital has closed, copied to the Ghana News Agency (GNA) in Accra on Saturday Available at: www.ghananewsagency.org/.../K-Buchildrens-emergency-wardclosed/?[Accessed on 22 March 2017].
- Gómez-Sanz E, Torres C, Lozano C, Fernández-Pérez R, Aspiroz C, Ruiz-Larrea F, Zarazaga M (2010). Detection, molecular characterization, and clonal diversity of methicillin-resistant Staphylococcus aureus CC398 and CC97 in Spanish slaughter pigs of different age groups. Foodborne Pathogens and Disease 7(10):1269-1277.
- Graham PL, Lin SX, Larson EL (2006). A US population-based survey of Staphylococcus aureus colonization. Annals of Internal Medicine 144(5):318-325.
- Graveland H, Wagenaar JA, Bergs K, Heesterbeek H, Heederik D (2011). Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PloS One 6(2):e16830.
- Guardabassi L, O'Donoghue M, Moodley A, Ho J, Boost M (2009). Novel lineage of methicillin-resistant *Staphylococcus aureus*, Hong Kong. Emerging Infectious Diseases 15(12):1998.
- Haenni M, Galofaro L, Ponsin C, Bes M, Laurent F, Madec J-Y (2011). Staphylococcal bovine mastitis in France: enterotoxins, resistance and the human Geraldine methicillin-resistant *Staphylococcus aureus* clone. Journal of Antimicrobial Chemotherapy 66(1):216-218.
- Juhász-Kaszanyitzky É, Jánosi S, Somogyi P, Dán Á, van der Graafvan Bloois L, van Dulijkeren E, Wagenaar JA (2007). MRSA transmission between cows and humans. Emerging Infectious Diseases 13(4):630-632.

- Laxminarayan R, Klugman KP (2011). Communicating trends in resistance using a drug resistance index. BMJ Open 1(2):e000135.
- Lim SK, Nam HM, Jang GC, Lee HS, Jung SC, Kwak HS (2012). The first detection of methicillin-resistant *Staphylococcus aureus* ST398 in pigs in Korea. Veterinary Microbiology 155(1):88-92.
- Lowy FD (2003). Antimicrobial resistance: the example of Staphylococcus aureus. The Journal of Clinical Investigation 111(9):1265-1273.
- Moulin G (2001). Surveillance of antimicrobial consumption: activities in France (Agence Nationale du Médicament Vétérinaire). In 2nd International Conference of the Office International des Epizoosties.
- Moyo SJ, Aboud S, Blomberg B, Mkopi N, Kasubi M, Manji K, Lyamuya EF, Maselle SY, Langeland N (2014). High nasal carriage of methicillin-resistant *Staphylococcus aureus* among healthy Tanzanian under-5 children. Microbial Drug Resistance 20(1):82-88.
- Neela V, Zafrul AM, Mariana NS, van Belkum A, Liew YK, Rad EG (2009). Prevalence of ST9 methicillin-resistant *Staphylococcus aureus* among pigs and pig handlers in Malaysia. Journal of Clinical Microbiology 47(12):4138-4140.
- Odonkor ST, Newman MJ, Addo KK (2012). Prevalence and antibiotic susceptibility profile of methicillin resistant *Staphylococcus aureus* in Accra, Ghana. Microbiology Research 3(2):20.
- Perrier-Gros-Claude J, Courrier P, Bréard J, Vignot J, Masseront T, Garin D, Thierry J, Garrabé E (1998). Entérocoques résistants aux glycopeptides dans les viandes. Bulletin Epidemiologique Hebdomadaire 12:50-51.
- Pesewu GA, Dogbe R, Asmah RH, Olu-Taiwo MA, Adjei DN (2014). Prevalence and susceptibility profiles of methicillinresistant *Staphylococcus aureus* (mrsa) in the University of Ghana Hospital, Legon, Accra, Ghana. International Journal of Pharma and Bio Sciences 5(3):(B)185-193.
- Peterson AE, Davis MF, Awantang G, Limbago B, Fosheim GE, Silbergeld EK (2012). Correlation between animal nasal carriage and environmental methicillin-resistant Staphylococcus aureus isolates at US horse and cattle farms. Veterinary Microbiology 160(3-4):539-543.
- Sapkota K, Basnyat SR, and Shrestha CB (2006). Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in clinical specimens from patients and screening of nasal carriage of MRSA from medical staffs of Bir Hospital. A dissertation submitted to the Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.
- Schinasi L, Wing S, MacDonald PD, Richardson DB, Stewart JR, L Augustino K, Nobles DL, Ramsey KM (2013). Medical and household characteristics associated with methicillin resistant Staphylococcus aureus nasal carriage among patients admitted to a rural tertiary care hospital. PloS one 8(8):e73595.

- Shibabaw A, Abebe T, Mihret A (2013). Nasal carriage rate of methicillin resistant Staphylococcus aureus among Dessie Referral Hospital health care workers; Dessie, Northeast Ethiopia. Antimicrobial Resistance and Infection Control 2(1):25.
- Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, Capuano AW, Herwaldt LA, Diekema DJ 2009). Methicillin-resistant Staphylococcus aureus (MRSA) strain ST398 is present in midwestern US swine and swine workers. Plos one 4(1):e4258.
- van Cleef BA, Graveland H, Haenen AP, van de Giessen AW, Heederik D, Wagenaar JA, Kluytmans JA (2011). Persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* in field workers after short-term occupational exposure to pigs and veal calves. Journal of clinical microbiology 49(3):1030-1033.
- Van Duijkeren E, Jansen MD, Flemming SC, de Neeling H, Wagenaar JA, Schoormans AH, van Nes A, Fluit AC (2007). Methicillin-resistant *Staphylococcus aureus* in pigs with exudative epidermitis. Emerging Infectious Diseases 13(9):1408.
- Verkade E, Kluytmans J (2014). Livestock-associated *Staphylococcus aureus* CC398: animal reservoirs and human infections. Infection, genetics and evolution 21:523-530.
- Weese JS (2010). Methicillin-resistant *Staphylococcus aureus* in animals. ILAR Journal 51(3):233-244.
- Wulf MWH, Sørum M, Van Nes A, Skov R, Melchers WJG, Klaassen CHW, Voss A (2008). Prevalence of methicillin-resistant Staphylococcus aureus among veterinarians: an international study. Clinical Microbiology and Infection 14(1):29-34.