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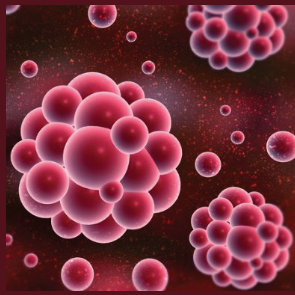
RAS MICROBIOLOGY AND INFECTIOUS DISEASES



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Research Article: Prevalence of methicillin resistant *Staphylococcus aureus* and risks factors in pig farmers in rural West region, Cameroon

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Abstract:
Background: Animal farming can be a potential reservoir of microbial resistance. Little data and information are documented on the prevalence and spread of methicillin resistant *Staphylococcus aureus* (MRSA) in remote and rural and semi-urban settings in developing countries. Yet, the increasing spread of antibiotics resistance either in the community or at the hospital has become a major public health problem in Cameroon. This study aimed to determine the prevalence of nasal methicillin resistant *Staphylococcus aureus* carriers among pig farmers and breeders in the West Region of Cameroon.

Methods: A total of 103 nasal scraping samples were collected in four distant division of Bamboutos, Nkoung-Khi, Menoua and Mifi for microbiological, biochemical and sensitivity testing according to the standard protocol of CASFM 2018.

Results: A high presence of *Staphylococcus aureus* of 29.13% was documented. *S. aureus* resistant showed 93.3%, to the ß-lactamases family, with MRSA prevalence at 27.18%. Resistance to glycopeptides (vancomycin) was 60%, macrolides-lincosamides-streptogramines mainly resistance to erythromycin 90% and clindamycin 30.6%, tetracycline 83.3%, fusidic acid 96.6% and to aminosides (tobramycin and gentamycin 76.6%). All isolated samples were sensitive to fluoroquinolones, notably to ofloxacin (80%) and to ciprofloxacin (86.6%).

Conclusion: There is an urgent need to implement a robust and sustainable MRSA and AMR surveillance in curbing the growing threat locally.

Keywords: Methicillin Resistant *Staphylococcus aureus*, Carriage, Prevalence, Pig Farmers

Introduction

Staphylococcus aureus is commensalism on skin and nasal track of humans and animals [1]. It's a Gram-positive coccus, both aerobic and anaerobic. It is responsible for septicemia, endocarditis, pneumonia, furuncle linked to nosocomial infections and also responsible for most cases of intoxications [2]. Farm animals, mainly pigs are the reservoir of these bacteria [3]. The prevalence of methicillin-resistant *S. aureus* (MRSA)was detected first in France in 2005. MRSA transmission was demonstrated during professional's exposure in pigs farmers and was responsible for the increasing *S. aureus* morbidity and mortality, prior to the discovery and use of antibiotics [4,5]. Nowadays, with the spread of antibiotics resistance in community/hospital worldwide, which represents a major public health concern. *S aureus* has been shown to progressively develop resistance to several antibiotics including the ß-lactamase family (> 99%) and MRSA resistance emergence [6]. MRSA in humans is associated with contact with infected pigs fed with supplement antibiotics [7], but can also occur in healthy people at risk such as new-born elderly people and pig farmers [8].
It is recommended to treat *Staphylococcus* infections based on susceptibility testing results [9]. Several studies worldwide have shown the prevalence of MRSA in pig farmers. In Holland, 20% of MRSA cases have been responsible for human pathologies and about 30% in Denmark [10]. A recent study has shown that 24-86% of nasal samples in pig farmers were diagnosed positive

as MRSA[11]. A study in West Africa showed the prevalence (30.8%) and risk factors of MRSA on 52 pigs farmers examined in Dakar, Senegal in 2012 [19].

Previous studies have reported the prevalence of MRSA as between 35-51% in piggeries and pig farmers in Europe [10]. MRSA prevalence was reported at 14% in pig farmers in France [11]. A prevalence of MRSA of 38.8% was documented in pig slaughterhouse in Canada [12]. MRSA piggery strains linkind carriage and infection were reported in the Netherlands and raised the question of potential transmission to Humans [11]. MRSA of piggery origin made up 20% and 30% of human pathologies in the Netherlands and Denmark respectively [13]. A recent study reported 26.6% of nasal samples from piggery farmers tested positive for MRSA[14]. The prevalence of MRSA in West Africa was reported greater than 30% in 2007 [15]. Similarly, a study reported MRSA prevalence of 19.3% in Ivory Coast [16], and a low rate of MRSA in Burkina Faso [17]. Likewise, MRSA carriage from nasal samples was estimated at 46,9% at Tlemcen, Algeria in 2013[18]. The prevalence and predicting factors associated with MRSA in pigs were examined and reported from 52 pig farmers, also found a 30.8% colonization by MRSA strains in Dakar, Senegal in 2012 [19]. However, there is scarcity of data on the prevalence of MRSA in pig farming in Cameroon, this study aimed at determining the prevalence of resistant nasal *S. aureus* to methicillin amongst pig farmers in the west region of Cameroon. This is important for evidence based quality and hygienic measures improvements in reducing MRSA transmission dynamics and infections in pig farmers and their neighborhood in Cameroon.

Methods

Study site and population

We carried out a descriptive and analytical cross-sectional study on the nasal carriage

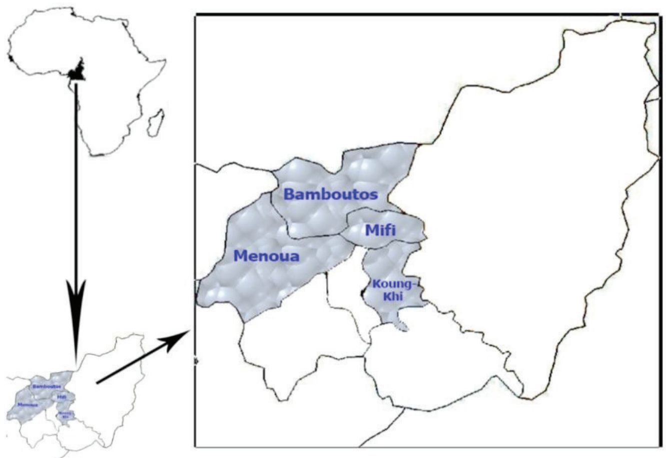

Figure 1.Study sites in the West region of Cameroon

Criteria and data collection

Included in our study were all modern or semi-modern pig farmers whose names were on the list of the Ministry of Livestock, Fisheries and Animal Husbandry of the Western Region, and farms with at least 50 pigs. Those who received antibiotic treatment ≤72 hours before and whose activity time in the company was less than three months were excluded from the study. After filling out the informed consent form, participants were subjected to astandardized questionnaire and once completed, samples were collected from the nasal cavity using a sterile swab and transported in a cooler containing cold accumulators to the laboratory of the Protestant Hospital of Mbouo in Bandjoun.

Sample analysis

Culture and identification

Once in the laboratory, the Mannitol Salt Agar and Mueller Hinton culture media were prepared according to the manufacturer's instructions. Fertility, specificity and sterility tests were performed for quality control of the media using reference strains ATCC 29213 *S.aureus*. Samples were plated according to the quadrant method on Mannitol Salt Agar and incubated for 24 hours at 37ºC. The biochemical identifications was done by catalase and coagulase tests performed with rabbit serum. The confirmation of *S.aureus* was done using the Api Staph gallery (Biomérieux SA, Lyon,France)

Antimicrobial Susceptibility Testing

This was performed by disc the disc diffusion method on Mueller Hinton agar according to the recommendations of *Comité d'Antibiogramme de la Société Francaise de Microbiologie (CASFM*,2018) [21]. Detection of the phenotype of methicillin resistance was achieved by two methods. The disc diffusion method of oxacillin disk (1 µg) on Mueller Hinton agar and that of thecefoxitin disc (30µg). The strains were considered resistant for a diameter of inhibition (< 20mm, < 27mm respectively) for MRSA and sensitive for a diameter (≥20 mm and ≥27mm respectively) as Methicillin Sensitive *Staphylococcus aureus* (MSSA) [21].

The data was collected with Excel 2013 and Startview 5.1. was used to process and analyze data. Fisher tests were used toevaluate and establish correlations between variables. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated in unavailable test and multivariate analysis for possible presumptive variables. P-value less than 0.05 was considered as statistically significant.

Ethical clearanceand research authorization

The ethical approval N0 2018/177/UdM/PR/CIEwas received from the ethical review board of the Université des Montagnes. We had the authorization of MINEPIA N0 71/18/L/DREPIA-O/SRAG before collecting the samples.

Results

Distribution of MRSA carriage prevalence and search for associated factors in univariate analysis.

The prevalence of MRSA in breeders was 27.8%. This study presents the prevalence of MRSA and the predictive factors according to place of residence, age group and the means of protection used. MRSA carriers 42.42% were more common among farmers in Bamboutos division compared to 34.78% in Menoua and 18.52% in Koung-Khi and Mifi 5% division. In univariate analysis, the fact of residing in Mifi or Nkoung-Khi was negatively associated with nasal carriage of MRSA respectively (OR = 0.07 ± 0.59, P= 0.01 and OR = 0.31 ± 0.93;P = 0.05). Carrying MRSA was less common among breeders who used a gown or overall to protect themselves, 10% versus 56.16% for those who did not use it. Furthermore, in univariate analysis, it was noted that wearing a gown or overall was associated with a low incidence of nasal MRSA carriage (OR = 0.11 ± 0.26; P = 0.0001). Pig farmers who used boots to protect themselves had a low frequency of *Staphylococcus aureus* (18%) compared to 35.85% in pig farmers who did not protect themselves with boots. In univariate analysis, this difference was statistically significant (OR = 0.39 ± 0.82, P = 0.04) (Table I).

Table I. Univariate analysis of risk factors for nasal carriage of MRSA

Variables	Total (N)	Prevalence of MRSA n(%)	OR [95% CI]	P-value
Residence division				
Bamboutos	33	14(42,42%)	1	Ref
Mifi	20	1(5%)	0,07 [0,01 ; 0,60]	0,01
Menoua	23	8(34,78%)	0,72 [0,24 ; 2,18]	0,56
Koung-Khi	27	5(18,52%)	0,31 [0,09 ; 1,02]	0,05
Age range				
>50 years	18	5(27,78%)	1	Ref
[19 years ; 50 years]	74	21(28,38%)	1,03 [0,33 ; 3,25]	0,96
[8 years ; 18 years]	11	2(18,18%)	0,58 [0,09 ; 3,66]	0,56
Wearing of protective overall				
No	75	22(56,16%)	1	Ref
Yes	28	6(10%)	0,11 [0.04 ; 0,30]	0,0001
Wearing protective shoe				
No	75	19(35,85%)	1	Ref
Yes	28	9(18%)	0,39 [0,16 ; 0,98]	0,04

Distribution of prevalence of MRSA carriage and search for associated factors in multivariate analysis.

The bacteriological analysis of the samples revealed to us, on the one hand, that 30 breeders were carrying *S. aureus* or a prevalence of nasal carriage of 29.13% ± 8.77. On the otherhand, 73(70.87%) of breeders had nasal carriage of *Staphylococcus* coagulase negative and other germs. The susceptibility testing results showed that 28 pig farmers were carriers of MRSA, with a respective prevalence of nasal carriage of 93.33% and 6.67%. In multivariate analysis, resident in Mifi and blouse wearing or overall coats were significantly associated with protection from MRSA nasal carriage (OR = 0.07 ± 0.23, P = 0.0001) (Table II).

Table II. Multivariate analysis of risk factors for nasal carriage of MRSA

Variables	Adjusted OR	95%CI	P-value
Residence division			
Bamboutos	1	/	Ref
Mifi	0,05	[0.009 ; 0,49]	0,01
Menoua	1,37	[0,35 ; 5,41]	0,65
Nkoung-Khi	0,26	[0,06 ; 1,14]	0,07
Age range			
>50 years	1	/	Ref
[19 years ; 50 years]	2,08	[0,46 ; 9,42]	0,34
[8 years ; 18 years]	1,37	[0,13 ; 14,43]	0,79
Wearing of protective overall			
No	1	/	Ref
Yes	0,07	[0,02 ; 0,25]	0,0001
Wearing protective shoe			
No	1	/	Ref
Yes	1,13	[0,33 ; 3,90]	0,84

Evaluation of the activity of MRSA and MSSA to antibiotics
We had a high resistance of MRSA with the production of penicillin G (100%), Amoxicillin(100%), ticarcillin (100%), oxacillin (93.3%), cefoxitin (93.3%) and cefotaxim (93.3%), the group of vancomycin (60%), to eryromycin (90%) and clindamycin, tobramycin (76.6%) and gentamycin (76.6%). The tetracycline (83.3%), ofloxacin (80%), ciprofloxacin (86.6%), fusidic Acids (96.6%) and chloramphenicol (56.6%) (Table III).

Table III. Antibiotic resistance profile of MRSA and MSSA

Strains		**MRSA**	**MSSA**
Antibiotics family	**Antibiotic**	**Frequence(%)**	**Frequence(%)**
ß-lactams	Penicillin G	100	91.3
	Amoxicillin	100	80
	Ticarcillin	100	80
	Oxacillin	93.3	91.3
	Cefoxitin	93.3	80
	Cefotaxim	93.3	80
Glycopeptides	Vancomycin	60	91.3
MLS	Erythromycin	90	93.3
	Clindamycin	30.6	93.3
Aminoglycosides	Tobramycin	76.6	59
	Gentamycin	76.6	90
Tétracyclines	Tetracyclin	83.3	29.6
Fluoroquinolones	Ciprofloxacin	13.3	76.6
	Ofloxacin	20	76.6
Various	Chloramphenicol	56.6	56.6
	Fusidicacid	96.6	13.3

MLS : Macrolides-lincosamides-Streptogramines

Discussion
In order to produce epidemiological data and to show the risks related to pig farming, we conducted a study on the prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* in pig farming in the Western Region of Cameroon. A total of 103 pig farmers in 4 departments were selected at random. The survey prevalence of nasal carriage of *S. aureusin* pig farmers was 29.13%. This result is similar to the 24% reported by Aubry *et al* in France in 2001 [9]. Among the 29.13% of *S. aureus* obtained, we noted a prevalence of 93.33% of MRSA nasal carriage. This prevalence is significantly higher to the 44.6% reported by Aubry et al in 2001 in France [9], 30% and 20% obtained by Van loo *et al* in Denmark and the Netherlands respectively [13], 86% obtained by Cuny *et al* in 2010 in Germany [20], 30.8% of Cheikh *et al* in 2012 in Senegal [19], and 26.6% reported by Jayaweera *et al* in 2017 in Anuradhapura [14]. The increased of nasal colonization may be due to precarious living conditions, poorhygiene. Our findings showed that farmers in the division of Bamboutos were the most colonized by MRSA with 42.42% than those in other departments. This difference could be explained by the non-respect of hygiene and the non-usage of protection measures and guidelines by pig farmers in the Bamboutos. During the univariate and multivariate analysis, we were able to show that the wearing of gowns / overall coat by breeders was statistically significant and proves to be a protective factor against MRSA (OR adjusted = 0.12 ± 0.34, P= 0.0003). Antimicrobial sensibility testing results revealed multiresistance of *S. aureus* to several families of antibiotics including vancomycin 60%, erythromycins (90%)and for clindamycins (30.6%), tetracyclines (83.3%), Fusidic acids (96.6%), tobramycin and gentamicin (76.6%). These results are similar to thoseobtained by Cuny et al in 2010 in Germany [20] showing resistance to clindamycin (30%). There is also a much higher resistance of 46% to tobramycin and erythromycin as reported byVan loo et al in 2007 in Denmark [13], and to 27.4% (tetracycline) reported by Cheikh et alin Senegal in 2012 [19]. These levels of resistance could be related not only to automedication of antibiotics in piggery production, but also to the inappropriate use or misuse of antibiotics in pig farming. Nevertheless, these strains weresensitive to fluoroquinolones, particularly ofloxacin (80%) and ciprofloxacin (86.6%). This result is well above the 3% observed by Van loo in 2007 in Denmark [13]. This could meanthat this family of antibiotics is little used by breeders. We also highlighted the resistance profile of MRSA and MSSA to other families of antibiotics. Most of these strains were multiresistant to Glycopeptides, macrolide-lincosamides-streptogramins, tetracyclines, Fusidic acid and aminoglycosides. At present, farmers have a habit of administering antibiotics to animals (pigs) as food additives, as apreventive or as a curative gesture. In both cases, they almost

never consult veterinary doctors because they are expensive. In addition, the uncontrolled consumption of inexpensive (generic) antibiotics increases resistance and drastically reduces treatment options for the treatment of MRSA and MSSA infections.

Conclusion

Our results from 103 pig farmers collected in 4 Grass field departments showed 30 (27.8%) *S.aureus* carriers. In addition, we documented increasing multiresistance to others antibiotics including methicillin resistance (MRSA) 29.13% for 28 breeders. *S. aureus* isolates were predominantly resistant to ß-lactam, Glycopeptides, Macrolides, Lincosamides, streptogramins, tetracyclines and Fusidic acids, as well as the rapid emergence of new antibiotic resistance. There is an urgent need to develop and implement innovative and contextual approaches in antimicrobial resistance awareness and community-based stewardship programs, and appropriate antibiotic use amongst farmers in rural settings in Cameroon and Africa.

Authors' contributions

Ingrid Cécile Djuikoue designed the study and developed it with, Célestin Tagne Kamga, Gildas Tazemda, Judith Magne, Suzie Lacmago, Danielle Wonkam, Sonia Anouwou, conducted the laboratory aspect of the study with the contribution of SuzieLacmago and Olivier Pompte. Célestin Tagne Kamga wrote the article with contributions from Kengne Michel, Ingrid Cécile Djuikoue. All authors have reviewed the article.
All authors read and approved the final manuscript.

Conflict of interest

Authors declare no competing interest.

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