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Methicillin Resistant *Staphylococcus aureus* in Pigs and Pig Breeders in Three Regions of Cameroon

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Abstract

The spread of antibiotic resistance, whether in the community or in the hospital, has recently become a major public health problem. Moreover, livestock seems to be a reservoir of resistant microorganisms such as porcine methicillin resistant *Staphylococcus aureus* (P-MRSA) whose carriage and transmission was mainly demonstrated in persons with occupational exposure to pigs. Much uncertainty remains about the public health implications of P-MRSA. To address concerns that exist regarding the zoonotic risk that pig carriers pose to breeders, the prevalence of P-MRSA in pigs and pig breeders was determined among 152 pig breeders in three regions of Cameroon. **Materials and Method:** Participants in this study came from Adamawa, Far North and Littoral regions of Cameroon. A total of 152 pig breeders or farm workers participated in this study. After having collected some social and cultural data with the aid of a questionnaire, nasal swabs were collected from pigs (n = 275) and their breeders using the standard collection procedures and placed in cooler containing cold accumulators. For each sample, microbiological assays were done as well as antibiotic susceptibility tests. **Results:** MRSA was isolated from 25 out of 275 pigs sampled (9.09%) and from 32 out of 152 (21.05%) pig breeders sampled; 33 breeders (21.71%) were carriers of *S. aureus* including 32 MRSA and one MSSA. The prevalence of MRSA nasal carriage was 21.05% ± 6.48. A significant negative association between body protection and nasal carriage of MRSA (OR = 0.29, 95% CI [0.093; 0.93]; p = 0.04) was observed. In each region, similar strains of MRSA were isolated both in pigs and their breeders with the same antibiotic resistant profile. **Conclusion:** The prevalence of MRSA though not high compared to European countries warrants further research as data on this zoonosis is scarce in our context. As such, transmission of MRSA from pigs to pig breeders or vice-versa constitutes a real danger, and this relationship may be a starting point for MRSA contamination in the community. Moreover, proper use of body protections and antibiotic medications as recommended will be a better protective measure against nasal MRSA carriage.

Keywords

Methicillin Resistant *Staphylococcus aureus*, MRSA, Pig Breeders, Pigs, Cameroon

1. Introduction

Antimicrobial resistance is a major public health problem. However, several bacterial species have developed resistance to a multitude of antibiotics like *Staphylococcus aureus* (*S. aureus*). Methicillin, a penicillinase-resistant *β*-lactam, was used from 1960; *S. aureus* rapidly developed resistance to this antibiotic in 1961, hence the term methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. Since the mid-1990s, there has been an explosion in the number of MRSA infections among populations that have not been exposed to the risk factors for acquiring MRSA-H (hospital-related) [2]. This increase has been associated with the identification of new strains of MRSA, known as community-related MRSA-C and Livestock-related MRSA-L, which is particularly prevalent in livestock. Livestock breeding is favorable for the emergence of the resistance of bacteria to certain antibiotics [3] [4]. Livestock, especially pigs, are a reservoir for this bacterium [5]. Porcine methicillin-resistant *Staphylococcus aureus* (MRSA) carriage was reported for the first time in France in 2005, and porcine MRSA transmission was mainly demonstrated in persons with occupational exposure to pigs [6]. Also, the use of antibiotics for prophylactic and therapeutic purposes or as promoters of growth is frequent in France and on the other hand, the frequent indiscriminate interaction between the animals as well as with the breeder favors the exchanges of bacteria and the diffusion of genes coding for antibiotic resistance [7]. In addition, the dispersion of large amounts of resistant bacteria through feces can also promote this spread in the environment as well as in humans [7]. It is especially in pigs that the description of strains of MRSA has made the human medical community aware of the existence of this pathogen in animals, as they receive curative or preventive treatment with more than nine families of antibiotics [8]. These dangerous practices are empirically observed in Cameroon known to be the greatest pig production basin in Central Africa.

The One Health approach prioritizes that zoonotic diseases is of greatest concern that should be jointly addressed by human, animal, and environmental health sectors in a particular community. There is paucity of literature on the impact of MRSA in communities; hence more information is needed on control measures to reduce the impact of the emerging MRSA on public health and how these can be developed and implemented.

Some African countries have reached very high prevalence levels, particularly in Algeria (in Tlemcen) with a prevalence of approximately 52% [9]. In Cameroon, a recent study showed amazingly a higher proportion of MRSA strains, 80% (201/205) of the total strains [10]. Another study in Cameroon resulted with 21.3% of MRSA [11]. Very few studies have been conducted on this issue (zoonosis) which causes major health problems in Cameroon despite the fact that the country has the largest pig population in Central Africa, with an average herd of 2,858,548 heads [12]. Given the paucity of information available on these microorganisms in Cameroon pig breeding farms, it is crucial to detect the prevalence of *Staphylococcus aureus* resistant to methicillin present in pigs and breeders' co-transmission and co-infections so as to evaluate its impact on public health.

2. Material and Method

2.1. Study Design

This was a cross-sectional and analytical study conducted in Littoral, Adamawa and Far North regions of Cameroon from September 2019 to February 2020. The study included pig breeders whose names were found on the list provided by MINEPIA (Ministry of Livestock, Fisheries and Animal Industries) delegates, pig breeders of village farms present in the chosen sites and breeders who had consented to the study and possessed a herd of pigs.

2.2. Farm Selection and Enrollment

To ensure the representativeness of our study population all subdivisions or county from the three regions were involved in the study. In each subdivision we approached the sub divisional Delegates of the Ministry of Livestock, Fisheries and Animal Industries (MINEPIA) in order to have the list of the modern pigs' breeders. Later an exhaustive recruitment of all the breeders (modern pig breeders by searching their names on the lists provided by the MINEPIA and the village pig breeders by self-field investigation) was carried out. Farms were selected based on the willingness of breeders to participate.

2.3. Data Collection

The study was carried out in 2 stages:

1) Data was collected from the livestock breeders using self-administered questionnaires;

2) Laboratory analysis was carried on Nasal samples were collected from the animals. And pig breeders.

Data from participants were collected using self-administered questionnaires. Direct contact was made with each participant to ensure the latter understood each question in the questionnaire and to create an atmosphere of confidence. The participants provided their baseline data and protective measures used when working on their pigs and these via questionnaires which samples are as follows: 1) Names of antibiotics frequently administered? 2) Where do you get supplied in antibiotics? 3) Who advise you in the choice of antibiotics? 4) What antibiotic do you take when you feel ill? 5) What do you do when the antibiotic seems not working? 6) Do personal protective equipments used include boots? 7) Do personal protective equipments used include gloves?

2.4. Sample Collection

We collected samples from weaned pigs. Using sterile swabs, nasal samples were collected from both nares precisely at the nasal septum of weaned pigs and the procedure was same for pig breeders. Nasal swabs were collected from 152 pig breeders of 119 herds of pig sand inoculated in the hearth brain broth, placed in a cooler containing cold accumulator for storage and transport to their respective laboratories for microbiological analyses to be done.

2.5. Bacteriology

On arrival at the laboratory, the inoculated samples in the enrichment broth (constituents of the broth) were incubated. After 24 hours of incubation at 37˚C, a loopful of broth was inoculated onto Mannitol Salt Agar medium, using the quadrant technique respecting the methods of exhaustion and isolation. These plates were incubated 18 - 24 hours at 35˚C and examined for MRSA. The isolated colonies were confirmed to be *Staphylococcus aureus* by macroscopic examination, appearance on Gram stain, by catalase test, tube coagulase test, Deoxy ribonuclease (DNase) Test. Methicillin resistant strains were identified by antimicrobial susceptibility testing and E-test.

2.6. Antimicrobial Susceptibility Testing

The antibiotic susceptibility of all *S. aureus* positive isolates was tested using the disc diffusion method according to the laboratory standards. The isolates were tested with a panel of 13 antibiotics: β-lactamines, Penicillin G, Oxacillin, Cefoxitin, Cefotaxime, Amoxicillin, Ticarcilline, Macrolides-Lincosamines-Streptrogramines, Aminosides, Glycopeptides, fluoroquinolones, Fusidic acid, and Chloramphenicol. Plates were incubated at 37˚C for 24 hours and examined under transmitted light. The zone of growth inhibition was interpreted as sensitive or susceptible, intermediate and resistant according to the recommendations of the Committee of the Antibiogram of the French Society of Microbiology (CASFM) September 2020. The minimal inhibition concentrations (MIC) of Vancomycin and Oxacillin were determined by the Epsilometer-test or E-test (AB BioMérieux, Sweden) [13]. Mueller Hinton agar with 2% NaCl was used as culture medium.

2.7. Statistical Analysis

The results obtained were recorded in the Excel software and analyzed using the Startview 5.1 software. Descriptive (mean, frequency and standard deviation) analyses were conducted to describe the demographic characteristics. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated in uni- and multivariate analysis for possible presumptive variables. Variables with $p \leq 0.05$ were statistically significant.

2.8. Ethical Consideration

This study was approved by the Institutional Review Committee of the "Universite des Montagnes" and local Authorization obtained from the Regional Delegation of the ministry of Livestock, Fisheries and Animal husbandries of the three regions.

3. Results

3.1. Description of Study Population

This study was conducted on a population of 152 pig farmers belonging to 119 herds distributed in 03 regions of Cameroon including Adamawa 43 (28.29%), Far North 31 (20.40%) and the Littoral 78 (51.32%). The average age of the breeders was 35.72 ± 16.8 years old with a minimum of 8 years and a maximum of 81 years with a sex ratio M/F of 3.2.

3.2. Distribution of MRSA

Less than a third, 33 (21.71%) breeders were carriers of *S. aureus* including 32 MRSA and 1 MSSA. The prevalence of MRSA nasal carriage was 21.05% ± 6.48. A total 25 pigs out of 275 were carriers of MRSA hence the prevalence of MRSA nasal carriage was (9.09%).

The pig breeders who properly used body protection as recommended, had a prevalence of 8.70% compared to 26.42% of those who did not protect themselves. This difference was statistically significant ($p = 0.02$) (**Table 1**).

The highest frequency of MRSA carriage was found in the Littoral region 12 (9.52%) pigs and 13 (16.67%) breeders (**Table 2**).

3.3. Determinants of MRSA Nasal Carriage, Multivariate Logistic Regression

There was a significant negative association between body protection and nasal carriage of MRSA (OR = 0.29, 95% CI [0.093; 0.93]; p = 0. 04). Therefore, body protection as recommended is a protective factor against nasal MRSA carriage (**Table 3**).

3.4. Resistance Profile of MRSA among Pigs and Pig Breeders

There was a very high resistance in the Far North region with a prevalence of 100% for penicillin G, oxacillin, cefoxitin, amoxicillin and ticarcillin. In the Adamawa and Littoral regions, the prevalence was higher for penicillin G (100% and 69% respectively), oxacillin (100% and 77% respectively), and cefoxitin (83.33% and 77% respectively). Methicillin-resistant strains of Staphylococcus aureus exhibit co-resistance for other families of antibiotics with prevalence ranging from 16% to 85.71%. The highest prevalence was from those of the M-L-S family with 69% for the Littoral, 50% in the Adamawa and 85.71% in the Far North (Table 4).

Table 1. Distribution of MRSA by pig farmers' baseline data, their regions and their bodily (protective measures).

Independent variables	Total n (%) or mean	nasal carriage of MRSA n (%) or mean	Rough OR	CI 95% OR	p-value *
Age					
Mean ± SD	35.72 ± 16.8	36.88 ± 19	1.01	[0.98; 1.03]	0.66
Sex					
Female	116 (76.32)	21 (18.10)	Ref		
Male	36 (23.68)	11 (30.56)	0.50	[0.21; 1.18]	0.11
Region					
Adamawa	43 (28.29)	8 (18.61)	Ref		
Far North	31 (20.39)	11 (35.49)	2.41	[0.83; 6.98]	0.11
Littoral	78 (51.32)	13 (16.67)	0.88	[0.33; 2.31]	0.78
Body protection					
No	106 (69.74)	28 (26.42)	Ref		
Yes	46 (30.26)	4 (8.70)	0.27	[0.09; 0.81]	**0.02**

$p \leq 0.05$.

SD. Standard Deviation.

Table 2. Prevalence of MRSA by pigs' distribution in the three regions.

Region	Total Number of pigs	Pig nasal carriage n (%)	Total Number of breeder	Breeder nasal carriage n (%)
Adamawa	80	06 (7.50%)	43	8 (18.61%)
Far North	69	07 (5.00%)	31	11 (35.49%)
Littoral	126	12 (9.52%)	78	13 (16.67%)
Total	275	25 (9.09%)	152	32 (21.05%)

Table 3. Determinants of Nasal Carriage of MRSA, multivariate logistic regression.

Independent variables	Adjusted OR	CI 95%OR	p-value*
Body protection			
No	Ref		
Yes	**0.29**	[0.093; 0.93]	**0.04**
Region			
Adamawa	Ref		
Far North	1.77	[0.58; 5.41]	0.32
Littoral	0.91	[0.33; 2.54]	0.86
Sex			
Female	Ref		
Male	0.50	[0.20; 1.26]	0.14
$p \leq 0.05$			

Table 4. Resistance profile of MRSA to β-lactams and others families.

Antibiotic family	Littoral n (%)		Adamawa n (%)		Far North n (%)	
	Breeders	Pigs	Breeders	Pigs	Breeders	Pigs
β-Lactams						
Penicillin G	22 (69)	21 (83)	32 (100)	4 (16.66)	32 (100)	25 (100)
Oxacillin	24 (75)	25 (100)	32 (100)	21 (83.33)	32 (100)	25 (100)
Cefoxitin	24 (75)	25 (100)	27 (83.33)	21 (33.3)	32 (100)	25 (100)
Cefotaxim	17 (54)	17 (67)	0 (0)	4 (16.67)	16 (50)	4 (14.28)
Amoxicillin	10 (31)	4 (17)	11 (33.33)	0 (0)	32 (100)	14 (57.14)
Ticarcillin	17 (54)	4 (17)	16 (50)	4 (16.67)	32 (100)	21 (85.71)
Other antibiotics' families						
M-L-S	22 (69)	6 (25)	16 (50)	0 (0)	27 (85.71)	4 (14.28)
Aminosides	12 (38)	8 (33)	21 (66.66)	4 (16.66)	14 (42.86)	7 (28.57)
Glycopeptides	12 (38)	14 (58)	11 (33.33)	12 (50)	18 (57.14)	14 (57.14)
Fusidic Acid	17 (54)	14 (58)	21 (16.16)	4 (66.66)	18 (57.14)	25 (100)
Fluoroquinolones	10 (31)	4 (17)	11 (33.33)	0 (0)	23 (71.83)	11 (42.86)
Chloramphenicol	14 (46)	4 (17)	0 (0)	0 (0)	17 (54)	14 (57.14)

M-L-S. Macrolides-Lyncosamdes-Streptrogramines.

4. Discussion

The presence of livestock associated MRSA in major food animal species especially pigs and the little awareness of the public in our country especially those in close contact with these animals raise numerous questions and concerns for public health and livestock activities (breeding, selling and industrial transformation) where the demand for processed meat products increases enormously. This has been demonstrated in the current study that showed that transmission of MRSA from pigs to pig breeders or vice-versa can be an important transmission route of MRSA which could lead to its dissemination [14].

The mean age of pig breeders in the current study was 35.72 ± 16.8 years ranging from 8 to 81 years with the male gender being predominant. This shows that pig breeding is mostly practiced by young adult males in Adamawa, Far-North and Littoral regions of Cameroon.

The study showed that the MRSA prevalence was 9.09% among pigs; this highlight on one hand antibiotic misuse in livestock industries, particularly in pig breeding in these three regions and the danger it represents for the community which directly or indirectly enters into contact with this livestock on the other hand. The prevalence of MRSA nasal carriage among breeders was 21.05%. This finding is similar (21%) to one study carried out in the Netherlands [15] but lower (26.5%) than two other studies on pig farmers in the Netherland [16], as well as in Germany (45%) [17].

Going forward, this study reveals a statistical association between body protection and pig breeders' nasal MRSA carriage ($p = 0.04$), and in agreement with a study carried out in the USA by Maya *et al.* [18]. This similarity is due to the fact that proper use of personal protective equipment is observed which prevents interspecies transmission of MRSA. There is an indication therefore that pig breeders' motivation to the use protective equipments and their education to the same may constitute an asset to tackle MRSA.

Data similar to our study were present in Latvian study [19] concerning antibiotic resistance profiles, where most MRSA isolates were multidrug-resistant, including resistance to penicillin G, oxacillin, cefoxitin, fusidic acid and ticarcillin.

Work was carried out in 3 regions of Cameroon to search for methicillin-resistant *S. aureus* (MRSA). According to data from the literature, MRSA is mainly found in farm animals, particularly pigs and workers exposed to these animals [20]. In our study, we took pig farmers and pigs to elucidate the zoonotic aspect of the transmission of MRSA from pigs to breeders. In this momentum, several families of antibiotics were tested and the zones of inhibition of the different strains were interpreted.

A strain of *S. aureus* resistant to methicillin was found in both pigs and breeders in the Littoral region. This had the same profile of resistance to antibiotics tested belonging to the β-lactam family and other families of antibiotics. It is therefore necessary to recognize and deplore the reality of the existence of the transmission of MRSA strains of animal origin to humans. The example of porcine MRSA in the Netherlands is undoubtedly one of the most emblematic cases of this zoonotic risk for its transmission from breeders to humans, at least the one with more scientific and political benefits [21]. This study demonstrates that pigs represent a reservoir of MRSA that can be transmitted to humans.

Similarly, in the Adamawa region, a strain of MRSA found in pigs and breeders showed the same patterns of resistance to the same antibiotics. Indeed, the same diameters of inhibition on agar have been observed. This could testify to the transmission of this strain from pigs to the breeder possessing the same resistant gene. This observation is highlighted by Leite-Martins [22] that showed in their study that isolated bacteria in animals and humans share the same mechanisms of resistance. In addition, Graveland [23] concluded that occupational exposure (pig farmers) is the main, if not the only, risk factor for zoonotic transmission of multidrug-resistant MRSA. The same observation was also made in the Far North region concerning the similarity of the inhibition diameters of a strain of MRSA isolated in pigs and breeders. This assumes that this strain has genes with the same phenotype of β-lactam resistance and co-resistance to other families of antibiotics. According to Grace and Fletsch [24], the presence of accessory genes carried by integrated plasmids in the SCCmec cassette allows the MRSA strains to acquire a multi-resistance phenotype.

Beyond this finding at the identical inhibition diameters of MRSA strains, the increase in zoonotic transmission is also more increased and exponential depending on the health aspect of the sampling areas. This is the case of the department of Djérem where we obtained 40% of nasal MRSA carriage in pig farmers against 25% in the Department of Mayo Banyo, 22.22% in the Mbéré and 20.83% in the Vina as regards the region of Adamawa. Regarding the Moungo in the Littoral region, it also appears that the breeders were the most colonized by MRSA with 25% than those of other departments; and lastly, in the Far North region, we found that the breeders of the Gazawa district were the most colonized by MRSA with 60% compared to other departments. The non-compliance with the rules of hygiene by the breeders and the misuse of the antibiotics either by the breeders or by food supplement with the pigscan be linked to these high levels of prevalence. This observation is similar to that of Aissani [25], who noted that excessive and inappropriate use of antibiotics in livestock breeds selection pressure on bacteria such as *Staphylococcus aureus*, causing them to become resistant to escape to the effect of these antibiotics. Nevertheless, according to another study in Madagascar [26], there is no significant difference between the prevalence of nasal carriage of MRSA among breeders with a notion of antibiotic therapy and those who do not.

The principal limit of this study is the lack of molecular biology techniques which are more reliable methods, but this absence could be explained by limited resources available in the laboratory during this study.

5. Conclusion

MRSA has a low prevalence in the pig farming community (pigs and pig breeders) in the three regions of Cameroon. Nevertheless, zoonotic transmission is present and warrants further research as zoonoses are a public health concern.

This research provides information on the presence of MRSA in pigs and pig breeders and the presence of zoonotic transmission from pigs to humans. There is therefore a necessity for continuous trainings on barriers methods on the prevention and/transmission of MRSA from pigs to pig farmers. A follow up study using molecular characterization of the MRSA strains and the role of the food chain (carcass or meat samples) in consumers exposed to MRSA, will bring more fundamental insight knowledge into zoonotic diseases.

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Consent for Publication

All authors consented for publication.

Availability of Data and Material

All data are available upon request.

Authors' Contributions

CID conceived the project and designed the study. CID searched relevant literature, scrutinized all relevant information and draft the manuscript. CID conducted and coordinated the field study. CID, DEN, OP, MJG, ETK, AST, CKM, VMN, CSN and VTN collected and processed the samples and data. CID and VT analyzed the data. All authors provided additional information. CID further analyzed the data. CID and VTN participated in the conception and revised the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

Authors declare no competing interests.

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