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Short Communication

Epidemiology of *Staphylococcus aureus* in Pigs and Farmers in the Largest Farm in Dakar, Senegal

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Abstract

Between December 2009 and November 2011, we collected 57 (12.3%) *Staphylococcus aureus* isolates from 464 pigs and 16 (30.8%) isolates from 52 farmers in the largest farm in Dakar. Fifty-one isolates (70%) belonged to four major multilocus sequence typing clonal complexes (CCs): CC152 (26.0%), CC15 (19.2%), CC5 (13.7%), and CC97 (10.9%). The CC variability among the pigs was similar to that observed among the farmers. Six isolates that were recovered only among pigs were resistant to methicillin (10.5%). They were assigned to the ST5-staphylococcal cassette chromosome *mec* type (SCC*mec*) IV ($n = 5$) and ST88-SCC*mec* IV ($n = 1$) clones. The *luk*-PV genes encoding Panton-Valentine leukocidin (PVL), present in 43 (58.9%) isolates overall, including all major CCs and the MRSA ST5-SCC*mec* IV clone, were highly prevalent compared to data from industrialized countries. This finding is of major concern with regard to the potential virulence of these strains.

Introduction

Worldwide spread of methicillin-resistant *Staphylococcus aureus* (MRSA) strains has become a serious challenge for human infection control and antibiotic therapy. Livestock-associated MRSA strains (LA-MRSA) have recently emerged in pig farming and are now prevalent in farms in industrialized countries (24.9–80%). MRSA sequence type (ST) 398 is the predominant clone in pigs from Europe and North America, whereas MRSA ST9 is predominant in Asia. Direct contact with MRSA-positive animals has been reported to be a risk factor for MRSA carriage (5.6–37.8% among farmers). LA-MRSA strains are now becoming increasingly responsible for human infection (Otter and French, 2012) and have entered the hospital setting (Graveland *et al.*, 2012). No relevant data are available in Africa. Yet, knowledge of MRSA prevalence among pigs and farmers, as well as the genetic backgrounds of methicillin-susceptible *Staphylococcus aureus* (MSSA) and MRSA strains and associated virulence factors, is important to understand the dynamics of the emergence of LA-MRSA, their adaptation to humans, and their potential impact on human health. Here, we report the molecular characterization of *S. aureus* isolates from pigs and farmers in Dakar, Senegal.

Materials and Methods

Study population

Approximately 300 fattening female and male pigs were present in the farm, with a turnover of approximately 150 pigs per month. About 50 pigs were randomly investigated every 3 months between December 2009 and November 2011. Nasal swabs were collected from pigs and farmers. For humans, a standardized specific questionnaire was completed to collect demographic data, medical history over the previous 12 months, and information about contact with pigs.

Microbiological analysis, DNA extraction, mecA detection, toxin gene profiling, and genotyping

The swabs were immediately placed at +4°C and analyzed within 4 h. Briefly, swabs were inoculated in a pre-enrichment medium consisting of brain-heart broth containing 7.5% NaCl. After overnight aerobic incubation at 37°C, selective enrichment was performed in brain-heart broth (bioMérieux) supplemented with colistin (4 mg/L). Twenty-five microliters of the selective enrichment broth was inoculated on specific agar plates (BBL CHROMagar™ Staph aureus and BBL CHROMagar™ MRSA; Becton Deckinson, Franklin Lakes, NJ).

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Table 1. Characterization of *Staphylococcus aureus* Isolates from Pigs and Pig Farmers in Dakar (Senegal)

	MLST			Swine						Human					
spa-CC	CC	ST	Total (n)	*spa*-types (n)	Resistance phenotype[b] (n)	Toxin genes[c] (n)	luk-PV %	*MRSA* (n)	*MSSA* (n)	*spa*-types (n)	Resistance phenotype[b] (n)	Toxin genes[d] (n)	luk-PV %	*MSSA* (n)	
355	152	152 377	19	t355 (6), t1172 (1), t4235 (7), t4690 (3)	PEN (17), SXT (9), TET (7)	*luk-PV*(17), *sed* (3), *sea* (1), *sem* (1), *ser* (3)	100	0	17	t335 (1), t1172 (1)	PEN (2), TET (1)	*luk-PV* (2), *selp* (2)	100	2	
084	15	15	14	t084 (8)	PEN (10), SXT (8), TET (4)	*luk-PV*(2), *eta* (2), *seh* (1), *sec* (1),	20	0	10	t084 (4)	PEN (4), SXT (3)	*luk-PV* (2), *sea* (2)	50	4	
311	5	5	10	t311[a] (7)	PEN (7), OXA (5), FOX (5), TET (5), SXT (4)	*luk-PV* (5), *sem* (7)	71	5	2	t311 (3)	PEN (3), ERY (2), SXT (1)	*sem* (2), etb (2)	0	3	
267	97	97 747	8	t267 (5)	PEN (5), RIF (1)		0	0	5	t267 (2), t359 (1)	PEN (3), TET (1)	*luk-PV* (1)	33	3	
314	121	121	5	t314 (3), t645 (1)	PEN (4), STX (4),	*luk-PV* (2), *sem* (1),	50	0	4	t314 (1)	PEN (1)	*luk-PV* (1), *sem* (1)	100	1	
008	8	8 836	4	t1476 (3), t1617 (1)	PEN (4), SXT (3)	*sea* (2), *tst* (1), *selp* (1) *ser* (1)	0	0	4				0	0	
127	1	852	4	t127 (2)	PEN (2), AMI (1), SXT (1)	*seh* (2), *luk-PV* (1), *sea* (1)	100	0	2	t127 (1)	PEN (1)	*luk-PV* (1), *sea* (1), *seh* (1)	100	1	
148	72	72	2	t148 (2)	PEN (2), SXT (1), TET (1)	*seh* (1), *sec* (1)	0	0	2				0	0	
Singleton *spa* types			7	t1510, t2700, t3489[a], t8481, t8482	PEN (5), OXA (1), FOX (1), SXT (1), TET (1)	*luk-PV* (1), *seh* (1), *sem* (1), *eta* (1)	20	1	4	t094, t8480	PEN (2)	*seh* (1), *tst* (1), *eta* (1)	0	2	
Singleton *spa* type shorter than five repeats			1	t2915	PEN (1)	*sea* (1)	0	0	1				0	0	
Total			73		PEN (57), SXT (31), TET (18), OXA (6), FOX (6), RIF (1)	*luk-PV* (28), *sem* (13), *seh* (5), *sea* (5), *ser* (4), *eta* (3), *sed* (3), *sec* (2), *tst* (1), *sep* (1)	49	6	51		PEN (16), SXT (4), TET (2), ERY (2)	*luk-PV* (7), *sea* (3), *sem* (3), *sep* (2), *seh* (2), *etb* (2), *eta* (1) *tst* (1)	44	16	

[a]*spa* types associated with MRSA isolates (t311 correspond to ST5 staphylococcal cassette chromosome mec [SCC*mec*] IV and t3489 to ST88 SC*mec* IV).
[b]All strains were susceptible to kanamycin, tobramycin, gentamicin, lincomycin, pristinamycin, fosfomycin, fusidic acid, pefloxacin, and vancomycin. Furthermore, all strains were susceptible to oxacillin and cefoxitin, except for six strains isolated from pigs.
[c]*etb, hlb,* and *lukM* were not detected in any of the isolates.
[d]*hlb, lukM*, *sec*, *sed,* and *ser* were not detected in any of the isolates.
spa CC, staphylococcal protein A clonal complexe; MLST CC, multilocus sequence typing clonal complex; MLST ST, multilocus sequence typing sequence type; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; *eta*, exfoliatin A; *etb*, exfoliatin B; *luk*-PV, Panton–Valentine leukocidin; *se*, staphylococcal enterotoxin; *tst*, toxic shock syndrome toxin; ERY, erythromycin; FOX, cefoxitin; OXA, oxacillin; RIF, rifampicin; PEN, penicillin; SXT, co-trimoxazole; TET, tetracycline.

Table 2. Risk Factors for *Staphylococcus aureus* Carriage Among 55 Farmers in Dakar (Senegal)

Variable	S. aureus *carriage*		p-*value*
	Presence	*Absence*	
Demographic data			
Age (year), mean	44.4	44.2	0.59
Male, *n* (%)	5 (31.2)	22 (56.4)	0.16
Medical history			
Antibiotic(s) in the last 3 months, *n* (%)	2 (12.5)	5 (12.8)	0.68
Contact(s) with health-care center in the last 12 months, *n* (%)	10 (62.5)	21 (53.8)	0.77
Skin and soft tissue infection(s) in the last 12 months, *n* (%)	6 (37.5)	8 (20.5)	0.33
Contact with pigs			
Length of employment on farm (year), mean	10.6	11.7	0.61
Direct contact(s) with pig per day (number), mean	2.4	4.0	0.63
Cleaning on farm, *n* (%)	10 (62.5)	32 (82.1)	0.23
Cleaning of pigs, *n* (%)	0	3 (7.7)	0.63
Slaughtering of pigs, *n* (%)	3 (18.7)	7 (18.0)	0.75
Breeding of pigs, *n* (%)	11 (68.8)	34 (87.2)	0.23

Significance was assumed at $p < 0.05$.

S. aureus identification, antimicrobial susceptibility, genomic extraction, screening in all S. *aureus* isolates for the *mecA*, *sea*, *sec*, *sed*, *seh*, *sem*, *sep*, *ser*, *tst*, *eta*, *etb*, *luk-PV*, and *lukM* genes, genotyping by MLST and *spa* typing, and SCC*mec* typing for *mecA*-positive strains were performed as previously described (Breurec *et al.*, 2011a).

Statistical analysis

The chi-square test and Wilcoxon's test were used to compare categorical and continuous variables, respectively, using R software. Significance was assumed at $p < 0.05$.

Results

S. aureus was recovered from 57 (12.3%) of the 464 pigs screened and from 16 (30.8%) of the 52 farmers (male/female ratio 1.08; median age, 45 years). Six isolates found only among pigs were resistant to methicillin (10.5%). In addition, strains were characterized by a high level susceptibility to most of the antibiotics tested, except for penicillin (100.0% of resistance), co-trimoxazole (47.9%), and tetracycline (27.4%; Table 1). Univariate analysis identified no risk factors for human *S. aureus* carriage (Table 2).

The 73 isolates belonged to 22 *spa* types that were separated by the Based Upon Repeat Pattern (BURP) algorithm into eight *spa* clonal complexes (CCs) and seven singletons (groups represented by a single *spa* type). Each *spa* CC corresponded to a single multilocus sequence typing (MLST) CC. Seventy percent belonged to four major MLST CCs: CC152 (26.0%), CC15 (19.2%), CC5 (13.7%), and CC97 (10.9%). The CC variability among the pigs was similar to that observed among the farmers (Table 1). MRSA isolates ($n = 6$) found only among the pigs were assigned to the ST5-staphylococcal cassette chromosome *mec* type (SCC*mec*) IV ($n = 5$, *spa* type t311) and ST88-SCC*mec* IV ($n = 1$, *spa* type t3489) clones.

The *luk-PV* genes encoding Panton–Valentine leukocidin (PVL) were present in 49.1% ($n = 36$) and 43.8% ($n = 7$) of porcine and human isolates, respectively (10 different *spa*-types, regardless of the host, including all major CCs and the MRSA ST5-SCC*mec* IV clone).

Discussion

MRSA prevalence among pigs was far lower than that reported in industrialized countries. This may reflect the lesser use of antibiotics for animal growth and therapy in Senegal (unpublished data), as well as the absence of evidence of the ST398 MRSA clone common in pigs in developed countries (Espinosa-Gongora *et al.*, 2012; Morcillo *et al.*, 2012; van der Wolf *et al.*, 2012). To the best of our knowledge, no other data are available on the prevalence of MRSA carriage among pigs and pig farmers in Africa.

The MRSA clones found among the pigs, ST5-SCC*mec* IV and ST88-SCC*mec* IV, have already been described as major MRSA lineages responsible for human infections in Dakar (Breurec *et al.*, 2011a). As no MRSA clones were recovered from the farmers, longitudinal epidemiological studies among pigs, farmers and family members are needed to investigate the interspecies transmission of these clones. The presence of *luk*-PV genes encoding PVL in ST5 MRSA is potentially of particular concern as the presence of this toxin has been linked to deep abscesses, severe necrotizing pneumonia, and severe bone and joint infections in human epidemiological studies (Breurec *et al.*, 2011a).

Apart from CC97, the major MSSA CCs (CC15 and CC152) found among the pigs and the farmers have already been described as major MSSA lineages responsible for human infections in Dakar. Lineage CC97 is a pandemic bovine *S. aureus* lineage frequently responsible for mastitis but rarely detected in swine (Aires-de-Sousa *et al.*, 2007).

Our data, together with those of a previous study on human infections in Dakar (Breurec *et al.*, 2011b), indicate that Senegal has a far higher prevalence of PVL-positive *S. aureus* than industrialized countries (0–2% of human or swine nasal carriage isolates) (O'Hara *et al.*, 2008), whatever the origin of the isolates. The reasons for such a high prevalence are currently unclear, but the increased virulence of these strains, combined with factors that increase the risk of interindividual transmission (e.g., poor hygienic and sanitary conditions, and overcrowding), enhances the transmission potential of *S. aureus* (Massey *et al.*, 2006).

Conclusion

These data indicate a low prevalence of MRSA strains among pigs and pig farmers. Ongoing MRSA surveillance in animals, farmers, and family members is needed to study the transmission between species and detect changes in epidemiology.

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Disclosure Statement

No competing financial interests exist.

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