

Title: Phenotypic and Genotypic Characterization of Methicillin Susceptible *Staphylococcus aureus* (MSSA) in Pigs and Farm Workers - **NPB #:11-071** revised

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Industry Summary:

This study was conducted to characterize specific bacteria known as methicillin susceptible *Staphylococcus aureus* (MSSA) isolated from pigs and farm workers. The antimicrobial susceptibilities of the isolates were determined using the broth dilution method and the relatedness of isolates using a DNA finger printing method called pulsed-field gel electrophoresis (PFGE) and staphylococcal protein A (*spa*) typing methods. The MSSA isolates besides showing resistance to β -lactam antimicrobials such as to ampicillin, gentamicin and penicillin, resistance to tetracycline (82.5%) and erythromycin (11.8%) was detected. Among the MSSA isolated and tested for antimicrobial resistance, 93% exhibited multidrug resistance (MDR) pattern (resistance to three or more antimicrobials). All MSSA isolates tested were susceptible to the antimicrobial effects of ciprofloxacin, clindamycin, daptomycin, streptomycin, sulphamethoxazole and vancomycin. Amongst the MSSA isolates tested for *spa* types, t0337 (36.6%), t539 (29%), t02 (10.6%) and t036 (4.8%) *spa* types were the predominant ones. Some of the *spa* types including t02, t021, t034, t036 and t337 detected among MSSA isolates have been previously reported in methicillin resistant *Staphylococcus aureus* (MRSA) elsewhere. Results of the present study indicate that multidrug resistant MSSA isolates with various *spa* types were commonly detected among pigs and farm workers in the study sites suggesting the need for further studies on the significance and association with MRSA in commercial swine production units.

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Methicillin susceptible *Staphylococcus aureus*, pigs, farm workers, characterization

Scientific Abstract

The present study was conducted to characterize methicillin susceptible *Staphylococcus aureus* (MSSA) isolates recovered from pigs and farm workers using phenotypic (antimicrobial susceptibility testing) and genotypic (PFGE and *spa* typing) approaches to determine the antimicrobial resistance profiles and genotypic relatedness of the isolates and clonal lineages. In addition, the phenotypic and genotypic traits of the MSSA with the simultaneously circulating MRSA clones in swine herds and farm workers were compared. The MSSA isolates were recovered from a set of swine conventional and antimicrobial-free farms from all study sites (Iowa, Illinois, Minnesota, North Carolina and Ohio) during a previous study (NPB Project #08-179). The antimicrobial susceptibilities of the isolates were determined by the broth microdilution method using the Sensititre™ and the relatedness of isolates using the pulsed-field gel electrophoresis (PFGE) and staphylococcal protein A (*spa*) typing methods. The MSSA isolates recovered from pigs and farm workers exhibited various resistance patterns ranging from one to eight antimicrobials. The isolates were resistant to penicillin (93%), ampicillin (87.7%), tetracycline (82.5%), erythromycin (11.8%), gentamicin (5.7%), synercid (4.5%) and oxacillin (4.4%). Resistance <4% was detected to ceftriaxone, gatifloxacin, levofloxacin, linezolid and rifampin. About 93% of the isolates exhibited multidrug resistance (resistance to three or more antimicrobials) patterns. All MSSA isolates tested were susceptible to the antimicrobial effects of ciprofloxacin, clindamycin, daptomycin, streptomycin, sulphamethoxazole and vancomycin. The predominant *spa* types identified among the tested MSSA isolated included t0337 (36.6%), t539 (29%), t02 (10.6%) and t036 (4.8%). Some of the *spa* types including t02, t021, t034, t036 and t337 detected among the MSSA isolates in our study have been previously reported in methicillin resistant *Staphylococcus aureus* (MRSA) elsewhere. Results of the present study show that MSSA isolates showing similar multidrug resistance patterns and *spa* types to MRSA were detected among pigs and farm workers in the study sites suggesting the need for further detailed studies on the significance and association with MRSA in commercial swine production units.

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) primarily causes human disease. However, it can also colonize and cause infections in variety of animals and among food animals, pigs have been implicated as one source of colonization of humans associated with frequent contact with MRSA colonized pigs mainly through occupational exposure (Khanna et al., 2008; Lewis et al., 2008; Smith et al., 2009). There are unique strains of MRSA known as multilocus sequence type ST398 reported in pigs and pig farmers and their families, veterinarians and abattoir workers who are at risk because of their direct contact with animals. Due to low host specificity, MRSA ST398 could be transferred among different animals and humans in either direction (Voss et al., 2005; Huijsdens et al., 2006; Meemken et al., 2008).

MRSA isolates of multilocus sequence type ST398 have their main reservoir in swine and ST398 methicillin susceptible *Staphylococcus aureus* (MSSA) isolates have also been reported (Argudín et al., 2010; Tenhagen et al., 2009). Previous study (Argudín et al., 2010) reported that MSSA isolates showed the same PFGE profile, demonstrating the common background between ST398 MRSA and MSSA. Therefore, in addition to tracking MRSA dissemination in food animals, humans and environment, there is a need for knowledge about circulating MSSA strains as well. It is not only MRSA but also MSSA isolates from swine carry various multidrug resistance (MDR) genes which can potentially be spread to humans through exposure to swine. In the recently completed study project (NPB Project #08-179) on the prevalence and characterization of MRSA in pigs and farm workers in five major swine producing states (Iowa/Illinois, Minnesota, North Carolina and Ohio), the number of MRSA isolates particularly from Ohio, North Carolina and Minnesota were very limited and were detected only in farm workers whereas the number of methicillin susceptible *Staphylococcus aureus* (MSSA) was relatively high both from pigs and farm workers. In this study we characterized MSSA isolates recovered from pigs and farm workers using phenotypic (antimicrobial susceptibility testing) and genotypic (*spa* typing and PFGE) approaches.

Objectives

The objective of the study was to characterize methicillin susceptible *Staphylococcus aureus* (MSSA) isolates recovered from pigs and farm workers using phenotypic (antimicrobial susceptibility testing) and genotypic (PFGE and *spa* typing) approaches to determine the antimicrobial resistance profiles and

compare genotypic relatedness of the isolates with the simultaneously circulating MRSA isolates in pigs and farm workers in the study sites.

Materials & Methods

MSSA isolates

The MSSA isolates (n=228) were recovered from previous study (NPB Project #08-179) from pigs and consenting farm workers from four states (Iowa/Illinois, Ohio, Minnesota and North Carolina) and were received from three collaborating laboratories (UIOWA, UMN and OSU) and stored at -80°C for further testing: (i). Ohio and North Carolina: A total of 48 MSSA isolates (7 from farm workers and 41 from pigs), (ii). Iowa and Illinois: A total of 80 MSSA isolates were received (62 from pigs and 18 from humans. (iii). Minnesota: A total of 100 MSSA isolates (96 from pigs and 4 from farm workers).

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MIC, µg/ml) of MSSA isolates were determined at USDA-ARS by the broth microdilution using the Sensititre™ semi-automated antimicrobial susceptibility system (Trek Diagnostic Systems, Inc.). Each isolate was tested for the following antimicrobials: ampicillin, ceftriaxone, ciprofloxacin, clindamycin, Daptomycin, erythromycin, gatifloxacin, gentamicin, levofloxacin, oxacillin, penicillin G, rifampin, streptomycin, synergid, tetracycline, sulfamethoxazole, and vancomycin. The abbreviations and breakpoints for each antimicrobial are shown in table 1. *Streptococcus pneumoniae* ATCC® 49619 was used as a quality control strain.

Genotyping

To determine clonal relatedness of isolates and lineages, *spa* typing and the pulsed-field gel electrophoresis (PFGE) approaches were employed.

***spa* typing:** For the staphylococcal protein A or *spa* typing, DNA of the MSSA isolates were extracted using recommended commercial kit and all isolates were analyzed by *spa* typing following standard protocols (Shopsin et al., 1999). *Spa* types were obtained using the sequence comparison against the Ridom Spa Server (<http://spaserver.ridom.de/>). *spa* typing was performed in one of the collaborating labs (T. Smith).

Pulsed-field gel electrophoresis (PFGE): Selected isolates of MSSA from the four sates were genotyped using PFGE at the Infectious Diseases Molecular Epidemiology Lab (Gebreyes Lab) following standard protocols and the results were analyzed and interpreted accordingly.

Results

Antimicrobial resistance: Methicillin susceptible *Staphylococcus aureus* (MSSA) isolates recovered from pigs and farm workers exhibited various resistance patterns ranging from one to eight antimicrobials (Table 1). The isolates were resistant to penicillin (93%), ampicillin (87.7 %), tetracycline (82.5%), erythromycin (11.8%), gentamicin (5.7%), synergid (4.5%) and oxacillin (4.4%). Resistance <4% was detected to ceftriaxone, gatifloxacin, levofloxacin, linezolid and rifampin. All tested *S. aureus* isolates were susceptible to the antimicrobial effects of ciprofloxacin, clindamycin, daptomycin, streptomycin, sulphamethoxazole and vancomycin. MSSA isolates showed most resistance to beta-lactam antimicrobials followed by tetracycline (82.5%) and erythromycin (11.8%).

Table 1: Antimicrobial resistance profiles of MSSA isolates

Antimicrobial type	Abbreviations (breaking points)	% Resistant (total resistant/total tested)	Swine	Farm workers
Ampicillin	Am (≥ 0.5 $\mu\text{g/ml}$)	87.7% (210/228)	92.1% (185/201)	92.3% (25/27)
Ceftriaxone	Ce (≥ 64 $\mu\text{g/ml}$)	1.3% (3/228)	1% (2/201)	3.7% (1/27)
Erythromycin	Er (≥ 8 $\mu\text{g/ml}$)	11.8% (27/228)	10.5 (21/201)	22.2% (6/27)
Gatifloxacin	Gf (≥ 2 $\mu\text{g/ml}$)	3.1% (7/228)	3.5 (7/201)	-
Gentamycin	Gm ($\geq 16, \geq 500$ $\mu\text{g/ml}$)	5.7% (13/228)	3.9% (8/201)	18.5% (5/27)
Levofloxacin	Le (≥ 4 $\mu\text{g/ml}$),	3.1% (7/228)	3.5 (7/201)	-
Linezolid	Li (range 0.5-8 $\mu\text{g/ml}$)	<1% (1/228)	<1 (1/201)	-
Oxacillin	Ox (≥ 4 $\mu\text{g/ml}$),	4.4% (10/228*)	3.9% (8/201)	7.4 (2/27)
Penicillin G	Pn (≥ 0.25 $\mu\text{g/ml}$)	93% (212/228)	93.1% (187/201)	92.3% (25/27)
Rifampin	Ri (≥ 4 $\mu\text{g/ml}$)	1% (2/228)	-	7.4% (2/27)
Synergid	Sy (≥ 4 $\mu\text{g/ml}$)	4.5% (4/228)	1% (2/201)	7.4% (2/27)
Tetracycline	Te (≥ 16 $\mu\text{g/ml}$)	82.5% (188/228)	87.1% (175/201)	48.1% (13/27)
Ciprofloxacin	Cip (≥ 4 $\mu\text{g/ml}$)	0/228	-	-
Clindamycin	Cl (≥ 4 $\mu\text{g/ml}$)	0/228	-	-
Streptomycin	St (≥ 1000 $\mu\text{g/ml}$)	0/228	-	-
Daptomycine	Da (range 0.25-8 $\mu\text{g/ml}$)	0/228	-	-
Sulphamethoxazole	Su (≥ 4 $\mu\text{g/ml}$)	0/228	-	-
Vancomycin	Va (≥ 32 $\mu\text{g/ml}$)	0/228	-	-

*Those isolates which showed resistance to oxacillin (≥ 4 $\mu\text{g/ml}$) were *mecA* negative indicating that the resistance in these isolates was not encoded by the methicillin-resistant determinant (*mecA*) gene. These could be borderline oxacillin resistant isolates, showing a certain level of resistance to oxacillin but do not carry the *mecA* gene. Work is in progress in testing these isolates for *blaZ* gene, which is responsible for β -lactamase production.

About 93% of the MSSA isolates exhibited multidrug resistance (resistance to three or more antimicrobials) patterns. The major resistance types exhibited include AmPnTe (59.6%), AmErPn/Te (11.4%), AmPn (7%) and AmGmPn/Te (4%), Table 2.

Table 2: Resistance patterns of MSSA isolates recovered from swine and farm workers

Antimicrobial resistance pattern (# isolates)	Origin (# of isolates)	
	Swine	Farm worker
AmCeGmOx ¹ PnRiSyTe ² (1)	-	1
AmCeGmOxPnSyTe (2)	2	-
AmGfLePnTe (3)	3	-
AmGfPnTe (1)	1	-
AmGmOxPnRiSyTe (1)	-	1
AmGmOxPnSyTe (1)	1	-
AmLiPnTe (1)	1	-
AmOxPnTe (2)	2	-
AmOxPn (3)	3	-
AmPn (16)	12	4
AmPnSy	1	-
AmPnSyTe (6)	6	-
AmPnTe (136)	121	15
GfLePnTe (1)	1	-
AmErPn (12)	9	3
AmErPnTe (14)	14	-
AmGfPnTe (1)	1	-
AmGmPn (6)	5	1
AmGmPnTe (3)	3	-

AmLiPnTe (1)	1	-
PnTe (6)	6	-
Er (1)	-	1
Te (7)	7	-
Pansusceptible (2)	1	1

¹Am=ampicillin; Ce: ceftriaxone; Er: erythromycin; Gf: gatifloxacin; Gm: gentamicin, Lf: levofloxacin; Li: linezolid; Ox: oxacillin; Pn: penicillin G; Ri: rifampin; St: streptomycin; Sy: synergid; Te: tetracycline

spa types: MSSA isolates recovered from swine and farm workers were classified into the following *spa* types: t002 (*n*=24), t021 (*n*=3), t034 (*n*=6), t036 (*n*=11), t43 (*n*=1), t084 (*n*=2), t091 (*n*=1), t1071 (*n*=1), t114 (*n*=2), t216 (*n*=1), t334 (*n*=1), t337 (*n*=83), t338 (*n*=1), t539 (*n*=66), t548 (*n*=2), t1419 (*n*=1), t2922 (*n*=6), t3346 (*n*=1), 3446 (*n*=4) and new (*n*=9). Amongst the MSSA isolates tested for *spa* types, t337 (36.6%), t539 (29%), t02 (10.6%) and t036 (4.8%) *spa* types were predominant (Table 3).

Table 3: *spa* types identified among MSSA isolates of swine and farm origin by source

<i>spa</i> type	Origin of Isolates						Total
	Ohio/North Carolina		Minnesota		Iowa/Illinois		
	Swine	Farm worker	Swine	Farm worker	Swine	Farm worker	
t02=24			24				24
t021=3		3					3
t034=6						6	6
t036=11					11		11
t43=1				1			1
t084=2						2	2
t091=1						1	1
t1071=1						1	1
t114=2					2		2
t216=1						1	1
t334=1						1	1
t337=83	35	2	1		42	3	83
t338=1						1	1
t539=66			64	2			66
t548=2		2					2
t1419=1					1		1
t2922=6	6						6
T3346=1					1		1

t3446=4					4		4
New=9			7	2			9
Total	41	7	96	5	61	16	226

PFGE: A total of 163 selected MSSA isolates representing different resistance patterns, origin and *spa* types were genotyped using the pulsed-field gel electrophoresis (PFGE) genotyping approach. Analysis of PFGE results is ongoing.

Discussion:

The present study indicated that MSSA isolates showing similar multidrug resistance patterns and *spa* types to MRSA were recovered from pigs and farm workers. About 93% of the isolates from swine and farm workers were multidrug resistant (MDR) to up to eight antimicrobials. A number of *spa* types were detected among the MSSA isolates and *spa* types t337 (36.6%), t539 (29%), t02 (10.6%) and t036 (4.8%) were predominant. Some of the *spa* types including t02, t021, t034, t036 and t337 which we detected among MSSA isolates were previously reported in methicillin resistant *Staphylococcus aureus* (MRSA) in our previous study on MRSA (NPB project #09-171) and in other studies elsewhere. *spa* type t034 and t337 in particular have been associated with MRSA ST 398. Results of the present study show that multidrug resistant MSSA isolates with various *spa* types including t034 and t337 were detected among pigs and farm workers in the study sites suggesting the need for further detailed studies on the significance and association with MRSA in commercial swine production units.

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