RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: *Occurrence and molecular epidemiology of Methicillin Resistant Staphylococcus aureus (MRSA) on-farm, at slaughter and pork.* **- NPB project #09-171 Revised**

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Industry summary:

This study was conducted on ten conventional swine farms in Ohio to investigate the occurrence and prevalence of specific bacteria known to be resistant to methicillin called methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs on-farm (before transportation), at lairage (after transportation), carcass swabs and retail pork. We also studied the relatedness of the MRSA isolates recovered across the pork production chain (farm-to-retail) using phenotypic and genotypic methods. We collected paired nasal and peri-anal swabs from finisher pigs on-farm (n=24/farm) and followed the same batch of pigs and sampled on arrival at lairage. Swab samples were collected from both anterior nares and the peri-anal region of corresponding pigs. Matching carcass swabs (24/farm) were collected at the post evisceration stage before chilling from those batches of pigs sampled on-farm and at lairage. Pork samples from the same batch of pigs were collected at retail market. Samples were examined for the presence of MRSA following conventional methods. Antimicrobial susceptibilities of the isolates were also determined at the USDA-BEAR using Sensititre™. Polymerase chain reaction (PCR) was performed for the detection of *Staphylococcus aureus* -specific gene (*nuc*), methicillin resistance marker gene (*mecA*) and staphylococcal cassette chromosome (SCC*mec*) typing. The relatedness of isolates was determined using a DNA finger printing method called pulsed-field gel electrophoresis (PFGE) and we also used the multi-locus sequence typing (MLST) to determine the sequence types of MRSA isolates across the sampling points. One or more MRSA positive pigs were detected in five of

the ten herds (50%). The prevalence of MRSA in pigs was higher at lairage and ranged from 0% to 54.2% per farm compared to the same batch of pigs sampled on-farm (0% to 12.5%). MRSA was detected in 1.7% (4/235) of the carcass swab and 3.7% (5/135) of the retail pork samples. The MRSA isolates were multidrug resistant (resistance to three or more antimicrobial agents) and besides resistance to β-lactam antimicrobials (such as ampicillin, oxacillin, penicillin and gentamicin), resistance to tetracycline (76.4%), clindamycin (72.7%) and erythromycin (62%) was detected. MRSA isolates belonged to SCC*mec*-type II, III, V and others not belonging to any of the known types. Related isolates were detected across all stages of the pork production chain. Genotyping using MLST of selected isolates revealed that MRSA sequence type ST398 was detected from pigs on-farm, at lairage and retail pork samples. The present findings show that MRSA can be detected through out the pork production chain and suggests the need for further detailed studies in commercial swine farms in the US.

Keywords:

Methicillin-resistant *Staphylococcus aureus (MRSA)*, pigs, farm, slaughterhouse, retail pork

Scientific Abstract:

The present serial cross-sectional study targeting ten cohorts of commercial swine farms was conducted to investigate the occurrence and prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in finishing pigs on-farm, at lairage and assess the potential of carriage at slaughter and retail levels. Paired nasal and peri-anal swab samples were collected from market-age pigs on-farm and the same batch of pigs were followed and sampled at the lairage before slaughter and carcass swabs at post evisceration stage before chilling. Pork samples from the same batch of pigs were collected at retail market. Samples were examined following conventional cultural methods using selective enrichment method. Isolates were tested for antimicrobial resistance by the broth microdilution method. PCR was used to detect the presence of species-specific gene (*nuc*) and methicillin resistance marker gene (*mecA*). The genotypic relatedness of isolates was determined using the pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). One or more MRSA positive pigs were detected in five of the ten herds (50%). The prevalence of MRSA in pigs was higher at lairage and ranged from 0% to 54.2% per farm compared to the same batch of pigs sampled on-farm (0% to 12.5%). MRSA was detected in 1.7% (4/235) of the carcass swab and 3.7% (5/135%) of the retail pork samples. MRSA isolates recovered from various stages of sampling were multidrug resistant (MDR) and besides β-lactams, resistance to tetracycline (76.4%), clindamycin (72.7%) and erythromycin (62%)

was detected. Genotypically related isolates were recovered across all stages of the pork production chain based on cfr9I PFGE. Genotyping using MLST of selected isolates revealed that ST398 was detected from pigs on-farm and at lairage and retail pork samples and in addition ST5, ST9, ST39 and ST72 were detected at different points of sampling. Results of the present study show that MRSA can be detected at all sampling points through out the pork production chain and suggests the need for further detailed epidemiological studies involving more representative farms and slaughterhouses and associated environments across major pig producing states in the US.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as an important pathogen as a cause of hospital associated infections (HA-MRSA) and also among individuals in the community (CA-MRSA) (Huijsdens et al., 2006; Khanna et al., 2008; Lewis et al., 2008). MRSA strains are resistant to methicillin and other antimicrobials and resistance is encoded by the *mecA* gene, located on a staphylococcal chromosomal cassette (SCC*mec*) element (Khanna et al., 2008). It is well known that MRSA primarily causes human disease and animals, particularly food animals, had not been considered as important sources of infections until recently. Among food animals, pigs have recently been implicated as one source of potential infections to humans who may be in frequent contact with MRSA colonized pigs mainly through occupational exposure (Voss et al., 2005; Huijsdens et al., 2006).

In recent years, a unique strain of MRSA (referred to as ST398) has been reported in pigs, pig farmers and their families, veterinarians and abattoir workers who are at risk because of their direct contact with animals. Transmission of this MRSA strain from pigs to human has been reported in The Netherlands (Voss et al., 2005; Huijsdens et al., 2006). The Netherlands study indicated that pig farmers were 760 times more likely to be colonized with MRSA than the general population (Voss et al., 2005). Since then, numerous reports have shown that pigs in several European countries have been reported to be colonized with MRSA (de Neeling et al., 2007; van Duijkeren et al., 2007).

Very limited investigations have been done in North America. A recent study in the United States by Smith et al. (2009) examined 299 pigs and 20 farm workers from two production systems in Iowa and Illinois and reported 49% and 45% prevalence of MRSA in pigs and farm workers respectively. MRSA was detected in one of the production systems included in the study and the authors suggested that the ST398 may have been brought into the U.S. via live swine or pork products from Canada. Another published report of MRSA from North America is the work of Khanna and colleagues (2008) from Ontario, Canada. The overall prevalence of MRSA colonization in pigs was

25% (71/285); 20% (17/85) in suckling pigs; 28% (27/95) in weanlings; and 26% (27/105) in grower finishers (Khanna et al., 2008). A study conducted in Louisiana on 120 retail meat samples also identified that while more than 45% of pork and 20% of beef were positive for *S. aureus*, MRSA was identified from five pork and one beef meat samples (Pu et al., 2009). Other than the limited studies described above, data on MRSA prevalence and occurrence in pigs on-farm and at slaughter and carcass and retail meat contaminations in the U.S. is very limited. In addition, there is paucity of data on the genotypic relatedness of isolates from pigs on-farm, at slaughter and retail meat products worldwide.

Objectives:

Objective 1: Determine and compare the prevalence of MRSA among swine herds using non-selective media with that of highly-selective media specific to MRSA.

Objective 2: Compare the phenotypic and genotypic relatedness of MRSA isolated on-farm, at slaughter and from retail pork specimens using molecular epidemiologic approaches: pulsed-field gel electrophoresis (PFGE) and staphylococcal chromosomal cassette (SCC*mec*) typing.

Objective 3: Determine the clonal relatedness of MRSA isolated from pigs and pork with that previously reported in humans in the United States (US100-US1100) using PFGE and with isolates reported from humans and unique swine-adapted types (ST398) identified in other continents using multi-locus sequence typing (MLST).

Materials and Methods:

Study design and sample collections: A serial cross-sectional sampling design was used on 10 batches of market-age pigs in Ohio. A total of ten farms were identified and one main factor for recruitment was farms that slaughter pigs in plants and products that are sold within a known retail outlet where tracking products to retail would be reasonably feasible. We collected paired nasal and peri-anal swabs from randomly selected pigs (24 per farm) from a total of 10 farms (n=480). Paired nasal swabs from anterior nares and peri-anal swabs were collected from each pig on-farm and at lairage prior to stunning. Matching carcass swab samples (24/farm) were then collected from the same batch of slaughtered pigs at post-evisceration stage and before chilling (n=235). We followed the same batch of carcasses and a total of 135 retail pork samples (n=12-15 per batch) were collected from grocery stores in the same locality on the day of arrival at the store. For details please refer to Table 1.

Isolation and identification: Isolation of staphylococci was conducted using two approaches for each of the samples: one a non-discriminatory approach to isolate any *S. aureus* (using mannitol salt agar) and a second one selective for methicillin resistant staphylococci (using MRSA selective agar media). Nasal and peri-anal swab samples collected aseptically using sterile swabs were inserted into liquid Stuart's medium (Becton, Dickson) and kept at 4ºC during transportation and processed immediately upon arrival at the laboratory. Swab samples were inoculated into 5mL Mueller-Hinton (MH) broth (B&D) with 6.5% NaCl. Carcass swabs were cultured in 90ml of MH broth with 6.5% NaCl and retail pork samples (25g) were added into 225 ml of MH broth and incubated overnight at 37°C. On the second day a loopful of the broth was inoculated onto Oxacillin Resistance Screening Agar (ORSA, Oxoid) and mannitol salt agar (Becton, Dickinson, Sparks, MD) plates. Plates were incubated for 24 to 48 hours at 37ºC. Presumptive colonies were further confirmed by performing the catalase test, the tube coagulase test and *S. aureus* latex agglutination assay (Pastorex^{TM} Staph-Plus, Bio Rad). Presumptive *S. aureus* isolates were stored at -80°C for further phenotypic and molecular testing.

Speciation of *Staphylococcus* species*:* Identification of the *Staphylococcus* species was performed at the USDA-ARS, Bacterial Epidemiology and Antimicrobial Resistance Research (BEAR) Laboratory, Athens, Georgia. *S*pecies identification was performed using the Vitek 2 system (bioMerieux, Durham, NC) and the Vitek 2 Gram-positive identification cards according to manufacturer's directions.

Antimicrobial susceptibility testing: The antimicrobial susceptibility of all *Staphylococcus aureus* isolates was tested at the USDA-ARS (BEAR) Laboratory. Minimum inhibitory concentrations (MIC) for staphylococci were determined by the broth microdilution panels using the Sensititre^{TM} semi-automated antimicrobial susceptibility system (Trek Diagnostic Systems, Inc., Cleveland, OH) and the Sensititre^{TM} Gram-Positive Plate GPN3F according to the manufacturer's directions. Results were interpreted according to CLSI (Clinical and Laboratory Standards Institute) guidelines when defined. The antimicrobials tested include: ampicillin, ceftriaxone, ciprofloxacin, clindamycin, daptomycin, erythromycin, gatifloxacin, gentamicin, levofloxacin, linezolid, oxacillin, penicillin G, quinupristin/dalfopristin, rifampicin, streptomycin-high, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin.

Molecular characterization and genotyping: *S. aureus* isolates were identified as MRSA by detecting *mecA* (methicillin-resistant staphylococci specific) and *nuc* (*S. aureus* specific) genes. Multiplex PCR (Kondo et al., 2007) was used to determine and characterize staphylococcal cassette chromosome

mec (SCC*mec*) types and *mecA* gene carriage on *Staphylococcus aureus* isolates recovered from the various samples during the study period.

Pulsed-field gel electrophoresis (PFGE)*:* PFGE was conducted on selected MRSA isolates (n=40) following a modified protocol as has been described elsewhere (Mulvin et al., 2001). As ST398 strains are non-typable by PFGE using the *SmaI* macrorestriction enzyme, genotypic relatedness of the MRSA strains was analyzed by PFGE using the cfr9I macrorestriciton enzyme (Argudin et al., 2009; Bosch et al., 2010). The isolates were selected randomly based on origin, sample type, stage of sampling and antimicrobial resistance profiles. In brief, isolates to be tested were grown overnight at 37°C on Mueller-Hinton (MH) agar plates (Becton Dickinson, MD). Bacterial cell suspensions were prepared in tubes containing 2ml of cell suspension buffer (CSB). Each of the adjusted cell suspension (optical density at 610 nm of 1.3 to 1.4) was transferred to a labeled microcentrifuge tube containing 2µl lysostaphine (2mg/ml) and mixed gently. Agarose-embedded cells were then lysed with cell lysis buffer (CLB; 10mM Tris-HCl pH 7.2, 50 mM EDTA , 50mM NaCl, 0.2% deoxycholate, 0.5% N-laurylsarcosine, 1% sarcosyl [Sigma, St. Louis, MO], 20 mg/ml proteinase K). Slices of the plugs were digested with 40U of the cfr9I restriction enzyme (Fermentas Inc. Glen Burnie, MD) for overnight at 37°C. The DNA fragments were then separated using the CHEF-DR III pulsed-field gel electrophoresis system (Bio-Rad Laboratories, Hercules, CA) with the following conditions and reagents: 1% SeaKem Gold agarose (FMC BioProducts, Rockland, ME) in 0.5% Tris-borate EDTA buffer, temperature at 14°C, voltage at 6 v/cm, run time of 19 hours with initial switch time of 5.3 and final switch time of 34.9 seconds. We used the PulseNet "universal" standard marker strain, *Salmonella enteric* serovar Braenderup H9812, as a molecular reference marker. The PFGE images (stained with ethidium bromide) were then analyzed with BioNumerics software version 4.6 (Applied Maths NV, Belgium). Analysis of cluster was done using the unweighted-pair group method using average linkages (UPGMA), with 2.0% band position tolerances and 1.5% optimization values. Those isolates with PFGE banding patterns having similarity indexes of 85% were grouped within the same cluster.

Multi-locus sequence typing (MLST): Out of the 40 PFGE typed MRSA isolates, we selected 21 isolates based on origin, sample type, stage of sampling and antimicrobial resistance profiles and were selected and genotyped using MLST. Briefly, seven house keeping genes including *arcC, aroE, glpF, gmk, pta, tpi* and *yqiL* and specific regions of 400-450 bp from each of these genes were amplified and sequenced using an eight capillary electrophoresis, CEQ-8000 genetic analysis system (Beckman Coulter, Palo Alto, CA). Allelic profiles and sequence types were generated once specific sequences were submitted to the global database at http://saureus.mlst.net.

Results:

Objective 1: *Determine and compare the prevalence of MRSA among swine herds using non-selective media with that of highly-selective media specific to MRSA.*

MRSA in pigs on-farm and at lairage*:* Table 1 shows the occurrence and prevalence of MRSA at various stages of sampling along the pork production chain. Of the total ten herds included in this study, one or more pigs tested MRSA positive in 5 (50%) of the herds, Table 1. Out of the total pigs we examined on-farm (before transportation), 2.9% (7/240) tested MRSA positive and the prevalence per farm ranged from 0% to 12.5%. Swab samples (nasal and/or peri-anal) collected from pigs on-farm indicated that herd #1, 3 and 6 had one or more MRSA positive pigs, Table 1. We followed the same batch of pigs and sampled on arrival at lairage before stunning and 11.3% (27/240) of the pigs from five herds (herd #1, 2, 3, 6 and 9) tested MRSA positive, Table 1. We noticed that the prevalence of MRSA was significantly higher in pigs after transportation (11.3%) compared to on-farm prevalence (before transportation, 2.9%). The prevalence of MRSA among pigs per farm ranged 0% to 54.2% in lairage. In addition, the proportion of MRSA positive samples was relatively higher in nasal swabs (2.5%; 6/240) compared to peri-anal swabs (0.8%; 2/240) collected from same batch of pigs on-farm and at lairage (nasal swabs: 8.3%; 20/240 compared to peri-anal samples: 5%; 12/240).

Table 1: Prevalence of MRSA in pigs on-farm and at lairage, carcass swabs and retail pork

Herd #	Number of pigs examined		Carcass swabs (n=240)	Retail pork (n=131)
	on-farm (n=240)	lairage (n=240)		
1	3/24 (12.5%)	13/24 (54.2%)	0/24	1/15 (6.7%)
2	0/24	1/24 (4.2%)	0/24	0/15
3	3/24 (12.5%)	11/24 (45.8%)	3/24 (12.5%)	2/15 (13.3%)
4	0/24	0/24	0/23	0/12
5	0/24	0/24	1/24 (4.2%)	0/14
6	1/24 (4.2%)	1/24 (4.2%)	0/24	0/14
7	0/24	0/24	0/23	0/12
8	0/24	0/24	0/23	0/12
9	0/24	1/24 (4.2%)	0/23	2/12 (13.3%)
10	0/24	0/24	0/23	0/12
Total	**7/240 (2.9%)**	**27/240 (11.3%)**	**4/235 (1.7%)**	**5/131 (3.8%)**

MRSA in carcass swabs: We followed the same batch of pigs which we sampled before stunning and carcass swabs were taken at post-evisceration and before chilling. Of the 235 carcass swabs we examined, 4/235 (1.7%) tested MRSA positive, Table 1. In three of the positive carcass swabs, the carcasses originated from pigs belonging to herd #3, which had MRSA positive pigs while testing pigs on-farm and at lairage. The fourth MRSA positive carcass swab originated from pigs belonging to herd #5, however, pigs in this particular herd tested MRSA negative while testing on-farm and at lairage. On the other hand in three of the herds (herd #1, 6 and 9) in which MRSA positive pigs were detected on-farm and at lairage, MRSA was not detected in any of the carcasses we examined.

MRSA in retail pork samples*:* We followed the same batch of carcasses to retail level and a total of 135 pork samples were examined of which 5 (3.7%) tested MRSA positive and originated from herd #1, 3 and 9 (Table 1). In herd #1 one or more MRSA positive samples were detected in pigs on-farm, at lairage and retail pork, however, all carcass swabs (n=24), which originated from this herd tested MRSA negative. On the other hand in herd #3 MRSA was detected across all sampling stages (on-farm, at lairage, carcass swabs and retail pork). The third group of retail pork samples in which MRSA was detected originated from herd #9 in which one of the pigs belonging to herd #9 tested MRSA positive at lairage. However, MRSA was not detected among the carcass swabs from herd #9.

The table below shows the number of *S. aureus* isolates from individual pigs per farm isolated using a selective and non selective (on-farm samples).

Table 2: Distribution of *S. aureus* isolates recovered from pigs on-farm, slaughterhouse and retail pork

Herd #	**Media Type**	**On -farm**		**Slaughterhouse**			**Retail Pork**
		Nasal swabs	Perianal swabs	Nasal swabs	Perianal swabs	Carcass swab	
I	ORSA[1]	4/24	1/24[3]	7/24	8/24	1/24	2/15
	MSA[2]	19/24	2/24	22/24	0/24	8/24	0/15
II	ORSA	0/24	0/24	1/24	0/24	0/24	0/15
	MSA[4]	11/24	1/24	1/24	0/24	0/24	0/15
III	ORSA	2/24	0/24	8/24	5/24	2/24	2/15
	MSA	7/24	0/24	6/24	0/24	1/24	2/15
IV	ORSA	0/24	0/24	0/24	0/24	0/24	0/15
	MSA	8/24	1/24	0/24	0/24	0/24	0/15
V	ORSA	0/24	0/24	0/24	0/24	1/24	0/14
	MSA	0/24	1/24	0/24	0/24	1/24	0/14

[1]ORSA= Oxacillin Resistant Screening Agar

[2]MSA= Mannitol Salt Agar

[3]Number of S. aureus pigs/total number of pigs sampled per farm (from each positive sample, three colonies per plate were picked and stored for further phenotypic and genotypic characterization)

[4]For all samples collected from farm, we used ORSA and MSA for comparison purposes for the first five farms. In the remaining sampling stages (slaughterhouse, carcass and retail pork) we used ORSA and MSA supplemented with antibiotics (except for Farm I which we used MSA without antibiotics for all stages of sampling)

Results of comparison between using selective (ORSA) and non-selective agar (MSA) media showed that no MRSA was detected in any of the mannitol salt agar plates we used.

Objective 2: Compare the phenotypic and genotypic relatedness of MRSA isolated on-farm, at slaughter and from retail pork specimens using molecular epidemiologic approaches: pulsed-field gel electrophoresis (PFGE) and staphylococcal chromosomal cassette (SCC*mec*) typing.

Antimicrobial resistance profiles: MRSA isolates recovered from various stages of sampling were multidrug resistant (MDR), resistance ranging from three to up to 11 antimicrobials (Table 2). The isolates were resistant to penicillin (96.3%), ampicillin (93.6 %), oxacillin (90%), tetracycline (76.4%), clindamycin (72.7%), erythromycin (62%), gentamicin (52%) and resistance <3% was detected to gatifloxacin, lavofloxacin, synercid, streptomycin and timethoprim/sulfamethoxazole. All MRSA isolates tested were susceptible to the antimicrobial effects of ciprofloxacin, daptomycin, linezolid, rifampin and vancomycin. MRSA isolates (n=11) which tested susceptible to oxacillin using the broth microdilution method were re-tested for oxacillin and cifoxitin using the disk diffusion method and three of them showed resistance to both antimicrobials and one of them only to oxacillin.

Table 2: Antimicrobial resistance patters of MRSA isolates recovered from pigs, carcass swabs and retail pork

Herd #	**Antimicrobial resistance pattern (# isolates)**	**Origin (# of isolates)**
3	AmCeCaErGmOxPnStSyTeSXT* (1)	NS-L[1]
3	AmCaErGfGmLfOxPnSyTeSXT (1)	PA-S-L[2]
3	AmCaErGmOxPnStSyTeSXT (1)	NS-L

3	AmCeCaErGmOxPnTe (1)	NS-L
1, 2, 3	AmCaErGmOxPnTe (47)	NS-F[3] (5), NS-L (25), PA-L- (11), CS[4] (2), RP[5] (4)
9	AmCeCaErGmOxPn (2)	RP (2)
3, 9 3 3, 9	AmCaErGmOxPn (4) AmCaErGmPnTe (2) AmCaErOxPnTe (2)	CS (1), RP (3) NS-L (2) NS-F (1), NS-L (1)
6	AmCaErOxPn (2)	NS-F (2)
1, 5	AmCaOxPnTe (17)	NS-F (1), NS-L (8), PA-S-L (7), CS (1)
3	AmGmOxPnTe (1)	NS-L (1)
3, 5	AmErOxPn (6)	CS (6)
5	AmOxPnTe (2)	CS (2)
1	AmCaPnTe (1)	PA-S-L (1)
3	CaErPnTe (1)	RP (1)
6	AmOxPn (4)	NS-F (1), NS-L (3)
1	AmPnTe (6)	NS-F[5] (1), PA-S-F (1), NS-L (2), RP (2)
1	AmPn (1)	PA-S-F (1)
3	Pansusceptible(1)	PA-S-L (1)

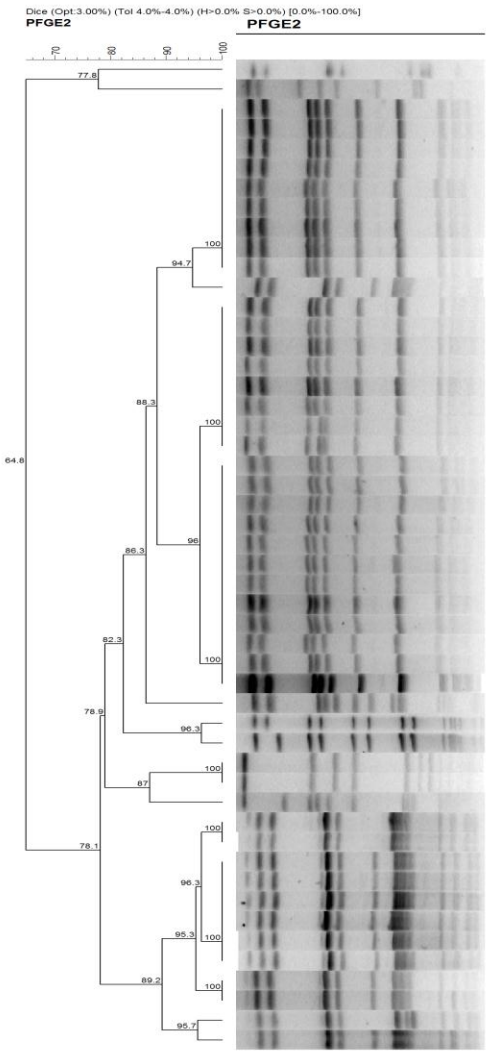
*Am=ampicillin; Ce: ceftriaxone, Ca: clindamycin; Er: erythromycin;Gf: gatifloxacin; Gm: gentamicin, Lf: levofloxacin; Ox: oxacillin; Pn: penicillin G; St: streptomycin; Sy: Synercid: Te: tetracycline; SXT: trimethoprim/sulfamethoxazole

[1]NS-L: Nasal swabs at lairage; [2]PA-S-L: peri-anal swabs at lairage; [3]NS-F: nasal swabs on-farm; [4]CS: carcass swabs; [5]RP: retail pork; PA-S-F: peri-anal swabs on-farm

Objective 3: Determine the clonal relatedness of MRSA isolated from pigs and pork with that previously reported in humans in the United States (US100-US1100) using PFGE and with isolates reported from humans and unique swine-adapted types (ST398) identified in other continents using multi-locus sequence typing (MLST).

Genotyping of MRSA isolates: Of the total MRSA isolates tested for SCCmec types, type II (7%), IV (5.5%), V (16.2%), and non-typeable (4%) were detected. However, the majority of the MRSA isolates (66.7%) did not belong to the known types (I-X). In these groups of isolates the mec gene complex and/or ccr gene complex have been amplified but did not match to the known groups. The pulsed-field

gel electrophoresis (PFGE) genotyping using cfr9I restriction enzyme on selected 40 MRSA isolates indicated the presence of genotypic relatedness among isolates recovered from pigs as well carcass and retail pork. Analysis of the PFGE dendogram (Figure 1) showed that among the various clusters, one of them consisted of isolates recovered from pigs at lairage (isolate# 2513, 2516, 2517 and 2519), carcass (isolate# 2523) and retail meat (isolate# 2535) and originated from same farm (farm# 3). All isolates belonging to this cluster had similar R-type (AmCaErGmOxPnTe) except one isolate from pigs at lairage which was pansusceptible (using the broth microdilution method) and resistant to oxacillin using the disk diffusion method.



ID	Farm	Sample	Stage	Resistance	SCCmec Type
2194	1	Nasal	Farm	AmCaOxPnTe	None/A*
2360	1	Peri-anal	Lairage	AmCaErGmOxPnTe	not I - IX
2482	3	Nasal	Lairage	AmCaErGmOxPnTe	None/A*
2485	3	Nasal	Lairage	AmCaErGmOxPnTe	None/A*
2488	3	Nasal	Lairage	AmCaErGmOxPnTe	None/A*
2491	3	Nasal	Lairage	ErPn	None/A*
2494	3	Nasal	Lairage	AmCaErGmOxPnStSyTeSXT	None/A*
2495	3	Nasal	Lairage	AmCaErGmOxPnTe	None/A*
2508	3	Peri-anal	Lairage	AmCaErGfGmLfOxPnSyTeSXT	None/A*
2509	3	Peri-anal	Lairage	AmCaErGmOxPnTe	None/A*
2511	3	Peri-anal	Lairage	AmCaErGmOxPnTe	C/A*
2959	9	Nasal	Lairage	AmCaErOxPnTe	V
2513	3	Peri-anal	Lairage	AmCaErGmOxPnTe	None/A*
2516	3	Peri-anal	Lairage	AmCaErGmOxPnTe	C/A*
2517	3	Peri-anal	Lairage	Pansusceptable	None/A*
2519	3	Peri-anal	Lairage	AmCaErGmOxPnTe	None/A*
2523	3	Carcass	Pre-Chill	AmCaErGmOxPnTe	None/A*
2535	3	Meat	Retail	AmCaErGmOxPnTe	C/A*
2714	6	Nasal	Farm	AmCaErOxPn	None/A*
2720	6	Nasal	Lairage	AmOxPn	None/A*
2445	3	Nasal	Farm	AmCaErGmOxPnTe	None/A*
2446	3	Nasal	Farm	AmCaErGmOxPnTe	C/A*
2450	3	Nasal	Farm	AmCaErOxPnTe	None/A*
2451	3	Nasal	Farm	AmCaErGmOxPnTe	None/A*
2465	3	Nasal	Lairage	AmCaErGmOxPnTe	None/A*
2467	3	Nasal	Lairage	AmCaErGmPnTe	None/A*
2470	3	Nasal	Lairage	AmCaErGmOxPnTe	None/A*
2473	3	Nasal	Lairage	AmCaErGmOxPnTe	None/A*
2476	3	Nasal	Lairage	AmCaErGmOxPnTe	None/A*
2477	3	Nasal	Lairage	AmCeCaErGmOxPnStSyTeSXT	None/A*
2479	3	Nasal	Lairage	AmCeCaErGmOxPnTe	None/A*
2525	3	Carcass	Pre-Chill	AmCaErGmOxPn	II
2532	3	Meat	Retail	CaErPnTe	None/A*
2202	1	Peri-anal	Farm	AmPn	C/None*
2285	1	Meat	Retail	AmPnTe	not I - IX
2960	9	Meat	Retail	AmCaErGmOxPn	II
2963	9	Meat	Retail	AmCaErGmOxPn	II
2520	3	Carcass	Pre-Chill	AmErOxPn	A2B2/None*
2358	1	Peri-anal	Lairage	AmCaOxPnTe	III + V
2359	1	Peri-anal	Lairage	AmCaOxPnTe	V
2291	1	Nasal	Lairage	AmCaOxPnTe	C/None*
2294	1	Nasal	Lairage	AmPnTe	V
2300	1	Nasal	Lairage	AmCaOxPnTe	V
2355	1	Peri-anal	Lairage	AmCaOxPnTe	V
2361	1	Peri-anal	Lairage	AmCaPnTe	V
2362	1	Peri-anal	Lairage	AmCaOxPnTe	V
2289	1	Nasal	Lairage	AmCaOxPnTe	C/None*
2357	1	Peri-anal	Lairage	AmCaOxPnTe	not I - IX
2200	1	Peri-anal	Farm	AmPnTe	C/None*
2297	1	Nasal	Lairage	AmCaOxPnTe	V

Figure 1: Dendrogram of cfr9I PFGE profiles of representative *MRSA* isolates recovered from pigs on-farm and at lairage, carcass swabs and retail pork samples and association with sources (farm number [1-10], sample type [nasal, per-anal, carcass swab, retail meat], stage of sampling [where sampling was done: on-farm, at lairage, carcass-pre-chilling and retail store]), antimicrobial resistance patterns and

SCCmec types. For list of abbreviation of antimicrobials, refer to Table 2. In the SCCmec type definition we used a combination of the *ccr* type and *mec* class, and the term ”None” refers to absence of amplifications of the *ccr* gene complex/ or the *mec* gene complex by the multiplex PCR used.

Selected isolates in this cluster genotyped with MLST belonged to ST5. The other cluster consisted of two genotypically related isolates from pigs on-farm (isolate# 2202) and retail pork (isolate# 2285) and originated from farm #1 and shared a similar antimicrobial resistance pattern (R-type AmPn/Te) and belonged to ST9.

Genotyping using MLST: Among selected isolates (n=19) genotyped using MLST (Table 3), ST398 (n=5) was detected among MRSA isolates from three herds (herd #1, 3 and 9) and isolates were recovered from pigs on-farm (herd #1), at lairage (herd #1 and 9) and retail pork (herd #3). In addition, ST5 (n=9) was detected from two herds (herd #3 and 6) and isolates were recovered from pigs on-farm (n=2), at lairage (n=5), carcass (n=1) and retail pork (n=1). Other sequence types identified include ST9 (n=2) from pigs on-farm and retail pork (herd #1), ST39 (n=2) from retail meat and ST72 (n=1) from carcass.

Table 3: Multi-locus sequence types (MLST) and associated antimicrobial resistance patterns of selected isolates (n=19)

Herd #	Origin	Isolate ID	Antimicrobial resistance pattern (R- Type)	Sequence Type
1	Peri-anal (Farm)	ST02200	AmPnTe*	ST-398
1	Nasal (Lairage)	ST02300	AmCaOxPnTe	ST-398
1	Peri-anal (Lairage)	ST02355	AmCaOxPnTe	ST-398
3	Retail Pork	ST02532	CaErPnTe	ST-398
9	Nasal (Lairage)	ST02959	AmCaErOxPnTe	ST-398
3	Nasal (Farm)	ST02446	AmCaErGmOxPnTe	ST-5
3	Nasal (Lairage)	ST02479	AmCeCaErGmOxPnTe	ST-5
3	Nasal (Lairage)	ST02494	AmCaErGmOxPnStSyTeSXT	ST-5
3	Peri-anal (Lairage)	ST02508	AmCaErGfGmLfOxPnSyTeSXT	ST-5
3	Peri-anal (Lairage)	ST02519	AmCaErGmOxPnTe	ST-5
3	Carcass	ST02523	AmCaErGmOxPnTe	ST-5
3	Retail Pork	ST02535	AmCaErGmOxPnTe	ST-5
6	Nasal (Farm)	ST02714	AmCaErOxPn	ST-5
6	Nasal (Lairage)	ST02720	AmOxPn	ST-5
1	Peri-anal (Farm)	ST02202	AmPn	ST-9
1	Retail Pork	ST02285	AmPnTe	ST-9
9	Retail Pork	ST02960	AmCaErGmOxPn	ST-39
9	Retail Pork	ST02963	AmCaErGmOxPn	ST-39
3	Carcass	ST02520	AmErOxPn	ST-72

*For list of abbreviations refer to Table 2.

Discussion:

Results of the present study showed that more than half of the cohorts (6 of 10) were positive for MRSA carriage at some point during the farm to retail continuum. Among the ten herds included in this study, at least one MRSA colonized pig was detected in three of the herds (30%) sampled on-farm and five of the herds (50%) at lairage showing the presence of other risk factors contributing to an increased proportion of colonized pigs with MRSA during transportation and holding at lairage. A recent study in The Netherlands (Broens et al., 2010) reported that pigs can become colonized with MRSA in a short period of time during transportation from the farm to the slaughterhouse. The difference in the prevalence of MRSA among finisher pigs sampled on-farm and at lairage could partly be associated with the occurrence of MRSA in the environment. MRSA has already been detected in pig transportation trucks, at lairage, in slaughter areas, and from farm and slaughterhouse personnel (de Neeling et al., 2007; Broens et al., 2010; van Cleef et al., 2010; Beneke et al., 2011).

In this study in two of the herds (herd #2 and #9), none of the samples taken from pigs on-farm were MRSA positive whereas MRSA was detected in pigs belonging to the same herds sampled at lairage supporting the findings of previous studies (Broens et al., 2010; van Cleef et al., 2010; Beneke et al., 2011) on the potential role of the environment as a source of MRSA to pigs. Since in our study we did not sample the environment or personnel associated with transportation and slaughtering of pigs, it is difficult to draw any conclusion on the role of the environment as sources of colonization of pigs with MRSA during transportation and holding at lairage or during carcass and retail meat processing. We also noted that even though the proportion of MRSA positive samples was relatively higher in nasal swabs collected from same batch of pigs on-farm (2.5%) and at lairage (8.3%), a considerable number of paired peri-anal samples collected from the same batch of pigs tested MRSA positive both on-farm (0.8%) and at lairage (5%) suggesting the frequent occurrence of MRSA in the peri-anal region of finisher pigs and its potential implications in contaminating carcasses during the slaughtering process.

We followed the same batch of finisher pigs from farm to slaughter and carcass swabs were collected at post-evisceration stage and before chilling and indicated that 1.7% of the carcasses tested MRSA positive. Previous study undertaken elsewhere (Beneke et al., 2011) reported a 6% prevalence of MRSA in pig carcasses. Both studies confirm the occurrence of contamination of pig carcasses with MRSA during the slaughtering process. The positive carcass samples in our study originated from two herds (herd #3 and #5), Table 1. In herd #3, MRSA was also detected in pigs on-farm (12.5%) and at lairage (45.8%) and three of the 24 (12.5%) carcasses from same herd tested MRSA positive depicting

the persistent occurrence of MRSA at all stages of the pork production chain in this particular herd. In herd #5, none of the samples collected from pigs on-farm and at lairage were MRSA positive and one of the carcasses from this herd tested MRSA positive suggesting the presence of other sources of MRSA contamination in the slaughtering process. It is also important to note that in four of the five herds (herd #1, 2, 6 and 9) in which MRSA was detected in pigs at lairage, none of the carcass swab samples tested MRSA positive which could partly be attributed to hygienic measures during the slaughtering process minimizing contamination of carcasses from MRSA colonized pigs.

Of the retail meat samples we examined, 3.7% (5/135) were contaminated with MRSA and our findings were in agreement with previous reports on MRSA in retail pork (Pu et al., 2009; Weese et al., 2010). All five MRSA positive retail meat samples originated from pig herds (herd #1, 3 and 9) in which MRSA was detected either in pigs on-farm and/or at lairage or carcass swabs. In one of the herds (herd #3), we detected MRSA across all sampling points (pigs on-farm [12.5%], at lairage [45.8%], carcass swabs [12.5%] and retail meat [13.3%]). The majority of the isolates which originated from this herd showed similar phenotypic (antimicrobial resistance patterns: AmCaErGmOxPn) and genotypic characteristics (PFGE and MLST) and representative isolates recovered from pigs on-farm, at lairage, carcass swab and retail meat (herd #3) and genotyped using MLST indicated that tested isolates from this herd belonged to ST5. The third group of retail pork samples in which MRSA was detected originated from herd #9 in which one of the pigs belonging to herd #9 tested MRSA positive at lairage. However, MRSA was not detected among the carcass swabs from herd #9. Analysis of the cfr9I PFGE dendogram indicated that isolates from pig (isolate# 2959) and meat (isolate# 2960, 2963) from herd #9 were not genotypically related and belonged to different clusters and sequence types (ST398 and ST39). Such a diverse genotypic occurrence could be due to environmental contamination.

We identified MRSA from pigs on-farm and at lairage and subsequently from carcass swabs and retail meat. However, detection of MRSA isolates from different sampling stages by itself does not indicate that pigs are the only sources for the meat contamination. Therefore attempts were made to determine how much of the retail meat contamination could be attributed to strains identified from pigs on-farm and at lairage or carcass swabs at slaughter through comparisons of phenotypic and genotypic relatedness of isolates recovered from various sampling points. The results indicated the presence of phenotypic and genotypic relatedness among isolates recovered from pigs, carcasses and retail pork as depicted in the cfr9I PFGE dendogram, Figure 1. As expected many of the isolates recovered from pigs on-farm and at lairage from the same herd were more clonal and of the same sequence type compared to isolates recovered from different herds. One of the most interesting clusters was the one

which consisted of phenotypically and genotypically related six isolates recovered from pigs at lairage (isolate# 2513, 2516, 2517, 1519), carcass swabs (isolate# 2523) and retail pork (isolate# 2535). All of the isolates originated from same herd (herd #3) and exhibited similar antimicrobial resistance patterns (AmCaErGmOxPnTe), except isolate #2517 (pansusceptible using the broth microdilution method and resistant to oxacillin using the disk diffusion method).

Based on MLST, five different sequence types (STs) were detected out of 19 isolates including ST5, ST398, ST72, ST39 and ST9). The tested strains belonged to ST5 suggesting the circulation of the same MRSA clones at all stages of the pork production chain. The other cluster consisted of two isolates from pigs on-farm (isolate# 2202) and retail pork (isolate# 2285) which were genotypically related and originated from herd #1, shared similar antimicrobial resistance pattern (R-type AmPn/Te) and both belonged to ST9. Even though few MRSA isolates were genotyped with MLST (n=19) in our study, sequence type 398 (ST398) was identified in three of the five MRSA positive herds from pigs on-farm and at lairage (herd #1), pigs at lairage (herd #9) and retail meat (herd #3) indicating the occurrence of MRSA ST398 from farm to retail levels in three of the four study herds.

Three of the five ST398 strains (isolate# 2959, 2300 and 2355) carried SCCmec type V which is often associated with this clone. A previous study (Smith et al., 2009) detected ST398 in pigs on-farm and farm workers in Iowa and Illinois. In our study we report the occurrence of MRSA ST398 not only in pigs on-farm and at lairage but also in the US retail pork. A recent study (Bhargava, et al., 2011) from Detroit, Michigan failed to identify ST398 in the retail meat samples, however, in this particular study retail pork samples were purposively excluded from the study. Even though ST398 has been the predominant strain identified among pigs elsewhere, we also identified ST5 and ST9 among US pigs on-farm and at lairage, carcass and/or retail pork. ST5 has been reported recently from US retail pork (Waters et al., 2011). Khanna et al. (2008) from Canada reported both ST398 and ST5 in pigs on-farm and farm workers. ST9 had been previously reported from pigs elsewhere (Wagenaar et al., 2009). While these strains are reported from swine, they are known to also occur in other species including humans. Other less common MLST sequence types in pigs identified included ST72 from carcass and ST39 from retail pork. ST72 MRSA clone has been previously reported from pork and beef (Lim et al., 2010) and from both community surveillance and hospital-acquired outbreaks (Lee et al., 2011).

In summary, this study showed that MRSA can be detected at different stages of the pork production chain and indicates the potential carriage from farm-to-retail levels. The proportion of colonized pigs with MRSA was significantly higher after transportation compared to the same batch of pigs sampled on-farm suggesting the presence of other sources of MRSA contamination from farm to

slaughter. Even though we have not typed all MRSA isolates recovered in this study, the identification of MRSA ST398 clones colonizing US pigs on-farm and at lairage and contamination of retail meat and other clones including ST5, ST9, ST39 and ST72 provide evidence for the presence of diverse MRSA clones across the pork production chain. Comparisons of phenotypic and genotypic relatedness of isolates recovered from various sampling points across the pork production chain suggested the presence of clonal isolates recovered from pigs, carcass and retail pork originating from the same herd and belonging to the same sequence type. In the present study we examined limited number of batches of pigs, carcass swabs and retail pork samples in one state and in addition we did not sample the farm-slaughterhouse environments as sources of MRSA contamination and therefore the findings should not be generalized. However, the preliminary results of the present study and others conducted in US pigs and retail pork suggest the need for a detailed epidemiological studies and mechanisms of transmission of MRSA involving representative number of commercial pig farms, abattoirs and associated environments across major pig producing states in the US..

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