Methicillin Resistant Staphylococcus aureus (MRSA) in market age pigs on-farm, at slaughter and retail pork

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Abstract

This study was conducted to determine the occurrence and prevalence of methicillin resistant Staphylococcus aureus (MRSA) in finishing pigs on-farm, at lairage and assess the likelihood of carriage at slaughter and retail levels. A cross-sectional study targeting ten cohorts of commercial swine farms was conducted for carriage of MRSA. Paired nasal and peri-anal swab samples (n=24/farm) 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. We assessed phenotypic and genotypic relatedness from the various sources. Conventional cultural methods using oxacillin resistance screening agar was used. Antimicrobial resistance was tested to a panel of 21 antimicrobials. 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 that same batch of pigs on-farm (0% to 12.5%). The proportion of MRSA positive isolates recovered from nasal swab samples was relatively higher (4.8%) compared to peri-anal samples (2.7%). We detected MRSA in 1.6% (4/240) of the carcass swab and 3.7% (5/135%) of the retail pork samples. Genotypically similar isolates were detected from farm to the retail chain based on PFGE. Using MLST, ST398 was detected from farm, lairage and retail pork. In addition ST5, ST9, ST39 and ST72 were detected at different points of sampling.

Introduction

In recent years, occurrence of livestock-associated methicillin-resistant Staphylococcus aureus (MRSA) referred to as multi-locus sequence type (ST) 398 has been reported among pigs and pig farmers in the Netherlands (Voss et al., 2005; Huijsdens et al., 2006), Canada (Khanna et al, 2008) and in the United States (Smith et al., 2009). When MRSA-positive food animals such as pigs are slaughtered, carcasses could easily be contaminated with MRSA and consequently meat from these animals might get contaminated. A study conducted by de Boer and colleagues (2009) on the prevalence of MRSA in raw retail meat products including pork, beef, veal, lamb and poultry indicated that 11.9% (264/2217) of the samples were contaminated with MRSA. Another study conducted in Louisiana on 120 retail meat samples also reported that while more than 45% of pork and 20% of beef were positive for Staphylococcus aureus, MRSA was identified from five pork and one beef meat samples (Pu et al., 2009). In general data on MRSA prevalence and occurrence in pig production units, slaughtered pigs and retail meat in the U.S. is very limited.

Materials and methods

Study design and sample collections: A serial cross-section sampling design was used on 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 conducive. We collected paired nasal and peri-anal swabs from randomly selected 24 pigs per farm from 10 farms (n=480). Nasal swabs from anterior nares and peri-anal swabs were collected from each identified pig on-farm and at lairage from animals prior to stunning. A matching 24 carcass swab samples were then collected from the same batch of slaughtered pigs at post-evisceration stage (n=240). In addition, a total of 131 retail pork samples [n=12-15 per batch] were collected from grocery stores in the same locality.

Isolation and identification: For the isolation and identification of Staphylococcus aureus, we followed selective culture methods using oxacillin resistance screening agar. Identification of the Staphylococcus species was performed at the USDA-ARS, Bacterial Epidemiology and Antimicrobial Resistance Research (BEAR) Laboratory, Athens, Georgia 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 including MRSA was tested at the USDA-ARS (BEAR) Laboratory. Minimum inhibitory concentrations (MIC) for staphylococci were determined by broth microdilution panels using the SensititreTM semi-automated antimicrobial susceptibility system (Trek Diagnostic Systems, Inc., Cleveland, OH) and the SensititreTM 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, lavofloxacin, linezolid, oxacillin, penicillin G, streptomycin, synercid, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin.

Molecular characterization and genotyping: PCR was used to detect the presence of species-specific gene(nuc) and methicillin resistance marker gene (mecA). Multiplex PCR (Kondo et al., 2007) was used to determine and characterize staphylococcal cassette chromosome mec (SCCmec) types and mecA gene carriage on Staphylococcus aureus isolates recovered from the various samples during the study period.
Pulsed-field gel electrophoresis (PFGE) was conducted in selected MRSA isolates (n=40) as described (Mulvin et al., 2001) using the cfr91 macrorestriction enzyme. The isolates were selected randomly based on origin, sample type, stage of sampling and antimicrobial resistance profiles. Out of the 40 PFGE typed MRSA isolates we randomly selected 21 isolates based on origin, sample type, stage of sampling and antimicrobial resistance profiles for multi-locus sequence typing (MLST).

Results and Discussion

Occurrence and prevalence of MRSA in pigs on-farm, at lairage, carcass and retail pork: Of the total ten herds included in this study, one or more MRSA positive pigs were detected in five of herds, Table 1. We detected that the prevalence of MRSA was relatively higher in pigs after transportation (11.3%) compared to on-farm prevalence (2.9%), before transportation. The proportion of MRSA positive samples was relatively higher in nasal swabs (4.8%) compared to peri-anal swabs (2.7%) collected from batch of pigs on-farm and at lairage.

The same batch of pigs sampled on farm and before stunning were followed and carcass swabs taken before chilling and of the 240 carcass swabs we examined, 4 (1.6%) tested MRSA positive, Table 1. The same batch of carcass were followed to retail level and a total of 131 retail pork samples were included in this study of which 5 (3.8%) were MRSA positive and the pork samples originated from herd # I, III and IX (Table 1).

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

Batch #	Number of pigs		Carcass swabs (n=240)	Retail pork (n=131)
	on-farm (n=240)	lairage (n=240)		
I	3/24 (12.5%)	13/24 (54.2%)	0/24	1/15 (6.7%)
II	0/24	1/24 (4.2%)	0/24	0/15
III	3/24 (12.5%)	11/24 (45.8%)	3/24 (12.5%)	2/15 (13.3%)
IV	0/24	0/24	0/24	0/12
V	0/24	0/24	1/24 (4.2%)	0/14
VI	1/24 (4.2%)	1/24 (4.2%)	0/24	0/14
VII	0/24	0/24	0/24	0/12
VIII	0/24	0/24	0/24	0/12
IX	0/24	1/24 (4.2%)	0/24	2/12 (13.3%)
X	0/24	0/24	0/24	0/12
Total	7/240 (2.9%)	27/240 (11.3%)	4/240 (1.7%)	5/131 (3.8%)

Antimicrobial resistance profiles: MRSA isolates recovered from various stages of sampling were highly 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 <3% resistance was detected to gatifloxacin, lavofloxacin, synercid, streptomycin and trimethoprim/sulfamethoxazole. All MRSA isolates tested were 100% susceptible to the antimicrobial effects of ciprofloxacin, daptomycin, linezolid, rifampin and vancomycin.

Table 2: Antimicrobial resistance patters of MRSA isolates recovered from various sampling stages

Batch #	Antimicrobial resistance pattern (# isolates	Origin (# of isolates)
III	AmCeCaErGmOxPnStSyTeSXT* (1)	NS-L[1]
III	AmCaErGfGmLfOxPnSyTeSXT (1)	PAS-L[2]
III	AmCaErGmOxPnStSyTeSXT (1)	NS-L
III	AmCeCaErGmOxPnTe (1)	NS-L
I, II, III	AmCaErGmOxPnTe (47)	NS-F[3] (5), NS-L (25), PAS-L (11), CS[4] (2), RP[5] (4)
IX	AmCeCaErGmOxPn (2)	RP (2)
III,IX	AmCaErGmOxPn (4)	CS (1), RP (3)
III	AmCaErGmPnTe (2)	NS-L (2)
III, IX	AmCaErOxPnTe (2)	NS-F (1), NS-L (1)
VI	AmCaErOxPn (2)	NS-F (2)
I, V	AmCaOxPnTe (17)	NS-F (1), NS-L (8), PAS-L (7), CS (1)
III	AmGmOxPnTe (1)	NS-L (1)
III, V	AmErOxPn (6)	CS (6)
V	AmOxPnTe (2)	CS (2)
I	AmCaPnTe (1)	PAS-L (1)
III	CaErPnTe (1)	RP (1)
VI	AmOxPn (4)	NS-F (1), NS-L (3)
I	AmPnTe (6)	NS-F (1), PAS-F[6] (1), NS-L (2), RP (2)
I	AmPn (1)	PAS-F (1)
III	Pansusceptible (1)	PAS-L (1)

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

[1]NS-L: Nasal swabs at lairage; 2PAS-L: peri-anal swabs at lairage; 3NS-F: nasal swabs on-farm; 4CS: carcass swabs; 5RP: retail pork; 6PAS-F: peri-anal swabs on-farm

Molecular characterization and genotyping: Of the total MRSA isolates tested for SCCmec types, type II (7%), IV (5.5%), V (16.2%), and non typable (4%) were detected. However, the majority of the MRSA isolates (66.7%) did not belong to the known types. In these groups of isolates their mec gene complex and/or ccr gene complex have been amplified but did not match to the known groups and suggest the need for further study. The pulsed-field gel electrophoresis (PFGE) genotyping on selected MRSA isolates indicated the presence of genotypic relatedness among few isolates recovered on-farm and lairage as well carcass and retail pork. Among selected isolated genotyped using MLST, ST398 was detected on those isolates recovered from farm, lairage and retail pork. In addition ST5, ST9, ST39 and ST72 were detected at different points of sampling. Finding MRSA in carcass and retail pork may have some food safety implication. Co-occurrence of enterotoxin genes is underway to assess the likelihood of foodborne intoxication.

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Acknowledgements

This study was funded by the National Pork Board to The Ohio State University (PI: Gebreyes WA, Grant# 09-171).