Prevalence of methicillin resistant staphylococci (MRS+) in pigs and farm workers

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Abstract

This study aims to determine the prevalence of MRSA and other methicillin resistant staphylococci in swine and swine farm workers. We collected swab and fecal samples from 96 pigs of 6-9 weeks of age from four farms in Ohio. Swab samples were collected from both anterior nares and fecal samples directly from the rectum of corresponding pigs. Nasal and oropharyngeal samples were collected from consenting farm workers. Samples were processed following conventional cultural methods and we used methicillin resistant Staphylococcus aureus selective agar (MRSA Chromagar®). PCR was performed for the detection of mecA gene and staphylococcal cassette chromosome mec (SCCmec) typing was also done. MRSA was detected in one of the 96 pigs, and methicillin resistant coagulase negative Staphylococcus species (MRCoNS) were detected in 15.6% (15/96) of the pigs. MRSA was also detected in one of the 17 farm workers. All the 39 MRCoNS isolates from farm workers and swine carried mecA gene. SCCmec type 1 was most commonly detected (83%) among the isolates. From the corresponding 96 fecal samples collected from pigs, one had mecA gene. Staphylococci isolates were further identified to species level and S. hominis was found to be the most common (n=10) followed by S. saprophyticus (n=9), S. sciuri (n=6), S. warneri (n=5), S. aureus (n=4), S. haemolyticus (n=3), S. cohnii, S. arlettae, S. kloosii and S. epidermidis (n=2). All the staphylococcal isolates tested for antimicrobial susceptibility were resistant to one or more of the antimicrobials tested, particularly to β-lactams and tetracycline in addition to methicillin. The high rate of MRCoNS may be a concern to pork safety as these species have been known to be important sources of the methicillin resistance gene to susceptible S. aureus.

Introduction

MRSA is an important pathogen in hospital settings and is now emerging in community settings among humans. MRSA associated with pigs has been identified as a reservoir for human MRSA infections (1, 2). The prevalence of MRSA in swine has been reported in a number of European countries and Canada (1-4). Even though MRSA has been identified in other farm animals like horses in the United States (5), currently there are limited number of studies of MRSA in swine (6). The objectives of this study were to determine the prevalence of MRSA in swine and swine farm workers on conventional swine farms in Ohio and to characterize MRSA and other methicillin resistant staphylococci (MRS+) isolates from Ohio swine farms using phenotypic and genotypic methods.

Methods

Study design and sample collection: A cross-sectional study was conducted from August 2008 to March 2009 on four conventional swine farms in Ohio to determine the occurrence and prevalence of MRSA among swine and pig farm workers. Nasal swabs from both anterior nares and fecal samples directly from the rectum were collected from 24 pigs per farm within the age group of 6-9 weeks from 4 farms in Ohio. At the same time nasal and oropharyngeal samples were collected from consenting farm works that are in close contact with pigs. A brief questionnaire was administered pertaining to risk factors for MRSA to the participating individuals.

Isolation and identification of staphylococci: All nasal swine samples and human nasal and oropharyngeal samples were transferred directly into liquid stuart's medium. Samples were cultured following conventional cultural methods and we used MRSA CHROMagar™ (BBL, Becton, Dickinson) as a selective plating agar medium. Each presumptive staphylococci isolate was confirmed as Staphylococcus aureus or coagulase negative staphylococci by a series of staphylococcal confirmation tests including
catalase test, tube coagulase test, *S. aureus* latex agglutination test (Bio-Rad Laboratories). *Staphylococcus aureus* isolates were tested for MRSA using PBP2'α latex agglutination test (Oxoid).

*Polymerase chain reaction:* DNA extractions (DNeasy® blood and tissue kits) were performed on the staphylococci isolates from swine and humans according to manufacturers directions. All staphylococcal swine and human isolates were tested for the presence of the chromosomal genes *mecA* following

*Staphylococcal cassette chromosome mec (SCCmec) typing:* Multiplex PCR was used to identify SCCmec types. All *mecA* positive isolates were tested for SCCmec types (I to IV).

*Speciation of staphylococci isolates:* A presumptive staphylococci colony was identified to staphylococcal genus and species using the Vitek 2 system (bioMerieux, Durham, NC) and the Vitek 2 Gram-positive identification cards according to manufacturer’s directions at USDA Bacterial Epidemiology and Antimicrobial Resistance Research Unit (USDA-ARS).

*Antimicrobial susceptibility testing:* Minimum inhibitory concentrations (MIC, µg/ml) for staphylococci were determined at USDA-ARS by broth microdilution using the Sensititre™ semi-automated antimicrobial susceptibility system (Trek Diagnostic Systems, Inc., Cleveland, OH) and the Sensititre™ 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 following antimicrobials were used: ampicillin, ceftriaxone, ciprofloxacin, clindamycin, daptomycin, erythromycin, gatifloxacin, gentamicin, levofloxacin, linezolid, oxacillin, penicillin G, quinupristin/dalfopristin, rifampicin, streptomycin-high, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin.

**Results and discussion**

*Prevalence:* MRSA was detected from one of the 96 pigs examined during the study period. Out of the total 17 farm workers included in this study, one was colonized with MRSA. The prevalence of methicillin resistant coagulase negative staphylococci (MRCNs) was 15.6% (15/96) in pigs and among the 17 farm workers one was positive for MRCNs, (Table 1).

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<td>MRCNs a</td>
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<td>50% (12/24)</td>
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*Methicillin Resistant Coagulase Negative Staphylococci (MRCN)*; b *Methicillin Resistant Staphylococcus aureus (MRSA)*

Table 1. Prevalence of MRCNs and MRSA per farm from pigs and humans

*Staphylococci species:* The 43 isolates from humans and swine were identified to species level. Among the 43 isolates the *Staphylococcus* species include *S. hominis* (n=11), *S. saprophyticus* (n=9), *S. sciuri* (n=6), *S. warneri* (n=5), *S. aureus* (n=4), *S. haemolyticus* (n=3), *S. epidermidis* (n=2), *S. cohnii* (n=2), *S. arlettae* (n=1), *S. kloosii* (n=1). *S. aureus* was the only coagulase positive species among all species identified in the swine and human isolates. The remain staphylococci species were coagulase negative: *S. hominis, S. saprophyticus, S. sciuri, S. warneri, S. haemolyticus, S. epidermidis, S. cohnii, S. arlettae, S. kloosii.*
Antimicrobial resistance profiles: Out of the total 43 isolates (n=31) were tested for antimicrobial susceptibility. All 31 staphylococcal isolates (100%) were resistant to one or more of the antimicrobials tested. The antimicrobials that were most commonly resistant were ampicillin, ceftriaxone, oxacillin, penicillin, and tetracycline (Figure 1). All 31 isolates were resistant to penicillin. All resistant *Staphylococcus* isolates (n=31) were multidrug resistant (≥ 3 antimicrobials). The most common antimicrobial resistance pattern (76%; 16/21) among the swine isolates was to ampicillin, ceftriaxone, oxacillin, penicillin and tetracycline. Among the human MRSA and MRCoNS isolates MDR was exhibited to ampicillin, ceftriaxone, oxacillin, penicillin and tetracycline 100% (5 of 5).

![Graph showing antimicrobial resistance](image)

Fig 1. Frequency of antimicrobial resistance of staphylococci isolates from swine and farm workers

**meca gene and SCCmec types:** PCR results indicated that all the 39 MRCoNS isolates (100%) from human and swine had the meca gene. The four *S. aureus* isolates (three isolates from human and one isolate from swine) also had the meca gene. Out of the total swine and human isolates, only one swine isolate was not methicillin resistant, but had the meca gene. The meca gene was only detected in <1% (1 of 96) of the DNA extracts from fecal samples.

All the 39 MRCoNS swine and human isolates and four MRSA isolates from humans positive for the meca gene were SCCmec- typed. The prevalence of SCCmec- types among the 43 meca positive isolates was as follows: 83% (36 of 43) had SCCmec-type I, 12% (5 of 43) had SCCmec- type IV, 5% (2 of 43) had SCCmec -type I and II. All 43 meca positive isolates were typeable. The three human MRSA isolates were SCCmec- type I and three pig isolates (100%) identified from the same farm were also SCCmec- type I. The two MRCoNS human isolates were SCCmec- type I and 93% (27 of 29) of the pig isolates identified from the same farm had SCCmec -type I.
Summary

The high prevalence of MRCoNS may be a concern to pork safety and public health as MRCoNS species having been known to be important sources of the methicillin resistance gene transfer to susceptible *S. aureus*. The identification of MRSA in farm workers may also be a concern as farm workers could eventually be a source of MRSA to pigs. In addition the high level of multidrug resistance among the swine isolates could be associated with the high use of antimicrobials in swine production units for various purposes. To provide a better picture of MRSA in swine and farm workers in the population, further sampling need to be conducted from different swine production systems including those antimicrobial free production units to determine if antimicrobial use in swine is serving as a selective pressure in the colonization of MRSA.

References