Vet Res Commun
DOI 10.1007/s11259-016-9661-x

ORIGINAL ARTICLE

SCC *mec* typing and antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) from pigs of Northeast India

S. Rajkhowa[1] · **D. K. Sarma**[1] · **S. R. Pegu**[1]

Received: 19 April 2016 / Accepted: 18 July 2016

Abstract *Staphylococcus aureus* is one of the most important pathogens of both humans and animal. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important human pathogen that causes serious infections both in hospitals and communities due to its multidrug resistance tendency. This study was undertaken to characterize the MRSA isolates from pigs and to determine the antimicrobial resistance of these isolates. Forty nine MRSA strains (one strain per positive pig) isolated from pigs of Northeast India were characterized by SCC*mec* typing and antimicrobial resistance. The overall prevalence of MRSA was 7.02 % with the highest prevalence recorded in pigs aged 1–3 months ($P = 0.001$) and in nasal samples ($P = 0.005$). Two SCC *mec* types (type III and V) were found in Indian pigs with predominance of type V. All isolates were resistant to penicillin. Seventeen resistance groups were observed where 87.75 % isolates showed multidrug resistance (showed resistance to three or more classes of antimicrobials). The most predominant resistance pattern observed was Oxytetracycline + Penicillin + Sulfadiazine + Tetracycline accounting 12.24 % of the isolates. The present study contributes to the understanding of characteristics and antimicrobial resistance of porcine MRSA isolates which in turn will help in devising strategy for the control of this pathogen. Findings of the study also throw light on multidrug resistance MRSA and emphasize the need for judicious use of antimicrobials in animal practice.

Keywords Antimicrobial resistance. *mecA* gene. MRSA. Pig. SCC*mec* typing

✉ S. Rajkhowa
swaraj.rajkhowa@gmail.com

[1] ICAR-National Research Centre on Pig, Indian Council of Agricultural Research, Rani, Guwahati, Assam 781 131, India

Introduction

Staphylococcus aureus is a common bacterium found on the skin and nasal passages of healthy people. It is commonly associated with skin and soft tissue infections and sometimes it causes pneumonia, bacteremia, meningitis, sepsis and pericarditis. *S. aureus* bacteria harboring the *mecA* gene are resistant to methicillin and other β-lactam antimicrobials and are referred to as methicillin-resistant *S. aureus* (MRSA). MRSA has become a pathogen of increasing importance in hospitals (healthcare-associated MRSA), the community (community-associated MRSA) and livestock operations (livestock-associated MRSA) (Graveland et al. 2011b). To date, livestock-associated MRSA (LA-MRSA) has been found worldwide, particularly among people who are involved with livestock farming (Frana et al. 2013). These bacteria can be transmitted to humans in close contact with MRSA-colonized animals (Smith and Pearson 2011). Livestock, especially pigs can serve as reservoirs for LA-MRSA (Lewis et al. 2008). Studies on the prevalence and characterization of MRSA from pigs have been carried out by several authors from different parts of the world. Dierikx et al. (2016) reported that 39 % of pigs and 81 % of the slaughter batches at Dutch slaughterhouses were MRSA positive. Verhegghe et al. (2016) studied the prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in Belgian pork and also characterized the MRSA isolates by SCC*mec* typing, multiple-locus variable number tandem repeat analysis and antimicrobial susceptibility testing, a selection of isolates were subjected to pulsed-field gel electrophoresis and spa typing. Narvaez-Bravo et al. (2016) investigated the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA), their spa-types and antimicrobial resistance profiles at various steps during commercial pork production from three plants and they found a higher prevalence of MRSA in the

Published online: 23 July 2016

nasal cavity of incoming pigs at all three plants, but a notable reduction in MRSA along the pork processing steps. Sun et al. (2015) studied the prevalence as well as characterized *Staphylococcus aureus* from growing pigs in the USA and observed a relatively low herd prevalence of MRSA in the US swine industry, but confirmed that methicillin susceptible variants of the most common MRSA genotypes found in swine globally are endemic in the US.

A thorough understanding of the molecular epidemiology and evolution of MRSA is required for detection, tracking, control as well as prevention of human diseases caused by this organism. Full characterization of MRSA requires definition of not only the putative bacterial genetic background but also of the complex and heterologous SCC*mec* elements. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing is essential for understanding the molecular epidemiology of MRSA. To date, at least 11 types (I-XI) of SCC*mec* elements have been identified (Ito et al. 2014). The new MRSA nomenclature scheme set by the International Union of Microbiology Societies incorporates SCC*mec* typing information in conjunction with that provided by multilocus sequence typing (Robinson and Enright 2004).

The widespread use of antibiotics has led to the emergence of multidrug-resistant strains, making their eradication more difficult. Practices like preventive therapy (mainly of digestive and respiratory disorders), deviations from approved posology (prolonged treatment duration or underdosing) are common in animal production facilities (Timmerman et al. 2006). The use of antimicrobial drugs in food-producing animals is considered to contribute to the emergence of antimicrobial resistance (Gyssens 2001). Although contact with animals seems to be the most important risk factor for human, meat products may also be a source of the bacteria (Van Duijkeren et al. 2007).

Although MRSA is endemic in India and its incidence varies from 25 % in western part of India to 50 % in South India (Gopalakrishnan and Sureshkumar 2010; Patel et al. 2010), these studies are confined only to isolates from humans and available literature revealed that no reports are available either on prevalence or characterization of MRSA isolates from animals (including pigs) in India. The present study was undertaken to characterize the MRSA isolates from pigs as well as to determine the antimicrobial resistance of the isolates.

Materials and methods

Sample collection, isolation and identification of MRSA

Six hundred and ninety eight (698) pigs in the age group of 2–18 weeks belonging to the North Eastern States of India were used for the study. The total number of pig farms included in the study was six (6) representing the major pig producing states of Northeast India. The distance between the farm ranged from 120 Km to 600Km. The average number of pigs per studied farm was 267. Nasal ($n = 258$), skin ($n = 230$) and rectal ($n = 210$) swab samples were collected from these pigs during the period of 2012–15. Nasal swabs were taken from both anterior nares using dry cotton swabs, skin swab samples were taken by swabbing three to four times the skin surface behind one ear using cotton swabs humidified by PBS and rectal swabs were collected directly from the rectum. All swab samples were incubated for 48 h at 37 °C in pre-enrichment media containing tryptic soy broth (Himedia, Mumbai, India) with 10 mL of 10 % NaCl. Then the samples were streaked onto mannitol salt agar (Himedia, Mumbai, India) with 6 mg/L of oxacillin (Himedia, Mumbai, India) and incubated at 37 °C for 24 h. The colonies suspected to be *S. aureus* derived from each sample (one strain per positive sample) were selected and identified by Gram staining with Gram-positive cocci and catalase activity. The colonies were re-streaked onto tryptic soy agar plates (Himedia, Mumbai, India) and incubated at 37 °C for 24 h. A coagulase test was carried out, and the presumptive positive samples were further screened for methicillin resistance by disc diffusion with 1 μg oxacillin (diameter of the inhibition zone for MRSA must be less than 10 mm.) (CLSI 2009). Several authors have reported detection of MRSA by cefoxitin (30 μg) disc diffusion method in accordance with the recommendations of Clinical and Laboratory Standards Institute (Fayyaz et al. 2013; Uzun et al. 2013; Çıkman et al. 2015). When *mecA* gene analysis was considered as the reference method and several methods were compared for detection of MRSA, the highest specificity and positive predictive values were obtained by the cefoxitin discdiffusion (CDD) method (Uzun et al. 2013). Similarly Mallick and Basak (2011) opined that CDD method could be a good choice for detecting methicillin resistance in *S aureus* strains in day to day practice where *mecA* PCR cannot be performed. Identification of MRSA isolates was further confirmed by multiplex PCR which simultaneously detects 16S rRNA, *mecA* and *nuc* genes (Louie et al. 2002). All MRSA isolates were kept in brain-heart infusion broth (Himedia, Mumbai, India) with 15 % glycerol at −80 °C for SCC*mec* typing.

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing

Isolated bacteria were subcultured twice onto 5 % sheep blood Columbia agar plates (Himedia, Mumbai, India) prior to DNA extraction. Genomic DNA was extracted by using DNeasy Blood and Tissue Kit (Qiagen, CA, USA). In order to classify the SCC*mec* type and subtype, monoplex PCR assays using the sequence of primers and conditions described by Zhang et al. (2005) was used.

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2009). The isolates were interpreted as sensitive, intermediately sensitive and resistant according to the inhibition zone diameter using CLSI recommendations.

Data analysis

Analyses were performed with SPSS 13.0 software for Windows with a probability (P) value <0.05 as statistically significant. The main purpose of the statistical analysis was to find out the influence (if any and if present statistically significant or not) of age and location (anatomical location) on the prevalence of MRSA in pigs. Analysis was also carried out to find out the statistical difference (if any) in the temporal distribution of MRSA isolates in the studied area.

Results

Methicillin resistant gene (*mecA*) could be detected in 49 pigs (out of 698 pigs examined) giving the overall prevalence of MRSA as 7.02 % in the present study. Positive animals were present in all the herds included in the study and the prevalence in the studied farms ranged from 4.70 % to 8.16 %. Highest prevalence was observed in nasal (10.85 %) and skin (6.08 %) samples and lowest in faecal (3.33 %) samples ($P = 0.005$, Table 1). The prevalence of MRSA was found to be highest (11.28 %) in pigs aged 1–3 months whereas prevalence was lowest (2.33 %) in pigs above 3 months of age ($P = 0.001$, Table 1). Studies on temporal distribution of positive samples revealed non-significant difference in prevalence (Table 1). SCC *mec* typing of MRSA isolates revealed the presence of types III and V of which 40.81 % (20) isolates belonged to type III and 59.18 % (29) belonged to type V. High frequency of resistance was observed for antimicrobials such as penicillin (100 %), oxytetracycline (83.67 %) and tetracycline (81.63 %). Antimicrobial resistance patterns of the MRSA isolates are shown in Table 2. Seventeen resistance groups (R1 to R17) were observed in the present study where 87.75 % isolates showed resistance to three or more classes of antimicrobials. The most predominant resistance pattern observed was O + P + SZ + TE which accounted for 12.24 % of the isolates.

Discussion

Considerable works have been carried out to determine the prevalence of MRSA in the pig population and its association with human infection because of the potential public health risk associated with this organism (Voss 2005). The study reports the prevalence of MRSA in pig population of North east India and we could detect methicillin resistant gene (*mecA*) in 7.02 % pigs in the present study. The prevalence recorded in the study was found to be much lower than those reported by countries such as 22.7 % in Korea (Lim et al. 2012), 26 % in Canada (Khanna et al. 2008), 36 % in the USA (Smith et al. 2009), 39 % in the Netherlands (de Neeling et al. 2007) and 49 % in Germany (Tenhagen et al. 2009). The difference in prevalence rate as observed in the present study could be due to several factors such as geographical location of the study area, sampling methods, laboratory testing methods and age of the pigs tested (Broens et al. 2011). We have recorded highest prevalence of MRSA in nasal samples in comparison to samples collected from other locations of the body which is consistent with the findings of Dewaele et al. (2011) who reported that MRSA was predominantly detected in the nares, followed by the perineum and skin and to a lesser degree in the rectum. The distribution of MRSA along the production chain was studied in a slaughterhouse with a connected processing unit producing fresh pork (Beneke et al. 2011) and results indicated that MRSA were frequently found in the noses of pigs (65 %), but less frequently on carcasses (16 %). Although the present study was not designed to assess the influence of factors on the prevalence of MRSA in pigs, a strong association was observed between presence of young pigs (1–3 months of age) and prevalence of MRSA which also corroborated with the findings of several authors (Smith et al. 2009; Weese et al. 2011) who reported highest prevalence of MRSA in piglets between 6 and 12 weeks of age. Molecular characterization using PCR assay revealed the presence of only two SCC*mec* types of which 40.81 % of the MRSA isolates belonged to SCC*mec* III and 59.18 % belonged to SCC*mec* V. The present study is consistent with the findings of Tulinski et al. (2012) who observed that majority (86.67 %) of MRSA isolates from pigs belonged to SCC*mec* type V whereas only 13.33 % isolates belonged to SCC*mec* type IVa. Similarly Park et al. (2013) found that of the 30 MRSA isolates identified from nasal samples of the pigs, 29 isolates were SCC*mec* type V. On the contrary, Tenhagen et al. (2009) observed that most of the MRSA isolates from pigs belonged to SCC*mec* type III which might be due to geographic variations of MRSA strains as reported by Stefani, and Varaldo, (2003).

Similar SCC *mec* types have also been reported from human from Northeast India as well as from other parts of India apart from reporting other (other than types III and V) SCC *mec* types of MRSA (D'Souza et al. 2010; Bhutia et al. 2015). SCC *mec* typing of MRSA isolates from human belonging to the state of Sikkim, Northeast India revealed the presence of types I (1.88 %), II (1.88 %), III (3.77 %), IVa (1.88 %) and V (11.32 %) (Bhutia et al. 2015). Similarly molecular characterization of MRSA from patients from Mumbai, India revealed

Table 1 Prevalence of MRSA in pigs

Characteristics	No. of animals tested	Prevalence (%)	χ^2	d.f.	*P*
Age of pigs					
< 1 month	270	5.92	13.39	2	0.001
1–3 months	257	11.28			
> 3 months	171	2.33			
Type of samples					
Nasal swab	258	10.85	10.43	2	0.005
Skin	230	6.08			
Rectal swab	210	3.33			
Temporal distribution (% of positive samples)					
2012	162	4.93	2.06	3	0.56
2013	165	6.66			
2014	181	8.83			
2015	190	7.36			

the presence of SCC *mec* types III (25 %), IV (34 %) and V (41 %) (D'Souza et al. 2010). Genotyping of MRSA from tertiary care hospitals in Coimbatore, South India showed the presence of SCC *mec* types III and IVa of which type III was found to be predominant with a frequency of 94.8 % (Neetu and Murugan 2016). However, analysis of MRSA strains isolated from healthy human and patients from Bengaluru, Mumbai and Pune, India revealed the presence of only SCC *mec* type IV (Nadig et al. 2010).

MRSA is a human bacterial pathogen that has emerged as a major threat in hospitals (as a nosocomial infection) and the cause of community-acquired infection among high-risk groups such as veterinarians, slaughterhouse workers and those in close contact with animals especially with pigs. The use of antibiotics in livestock production has promoted the development of multi-drug resistance. In the present study, all MRSA isolates were resistant to penicillin which is consistent with the findings of Oppliger et al. (2012) who observed that all MRSA isolates from pigs were resistant to penicillin. Similarly in a study conducted in China revealed that most (90 %) of the isolates from pigs were resistant to penicillin (Zhang et al. 2012). Resistance against penicillin is particularly noteworthy because this antimicrobial is commonly used for treatment of animal diseases in India.

Table 2 Antimicrobial resistance pattern of MRSA isolates from pig

Resistance group	Antimicrobial resistance patterns	SCC *mec* types	Number of isolates
R1	O, P, SZ and TE	V	6
R2	E, O, P, TE and TR	V	5
R3	E, O, P and TE	V	4
R4	P	V	4
R5	EX, E, O, P, SZ, TE and TR	III	4
R6	CIP, GEN, O, P, SZ and TR	V	3
R7	EX, E, O, P and TE	V	3
R8	P and TE	V	2
R9	GEN, O, P, TE and TR	III	2
R10	GEN, O, P, SZ, TE and TR	III	2
R11	CIP, E, GEN,O, P and TE	III	2
R12	CIP, GEN, O, P, SZ, TE and TR	III	2
R13	CIP, EX, GEN, P and TE	III	2
R14	E, GEN, O, P, TE and TR	III	2
R15	CIP, EX, E, GEN, O and P	III	2
R16	CIP, EX, E, O, P, TE and TR	V	2
R17	EX, E, O, P, SZ and TE	III	2

E erythromycin, *O* oxytetracycline, *P* penicillin, *TE* tetracycline, *GEN* gentamicin, *TR* trimethoprim, *CIP* ciprofloxacin, *SZ* sulfadiazine, *EX* enrofloxacin

Acquired resistance to β-lactams is mediated through two main mechanisms, β-lactamase production or altered penicillin binding protein (PBP2a) production. Bacterial β-lactamases hydrolyze the β-lactam ring and in staphylococci typically confer resistance to penicillins. Inhibitors of β-lactamase can inhibit this resistance mechanism and β-lactam/β-lactamase inhibitor combinations are widely used in some species, but not in swine production (Paterson and Bonomo 2005). In contrast, altered PBP2a production encoded by *mecA*, results in low affinity for all β-lactams and confers broad resistance to β-lactams that is not affected by β-lactamase inhibitors. Frana et al. (2013) found that resistance to tetracycline derivatives (chlortetracycline, oxytetracycline) overall was quite high (87 %). Similarly several authors opined that tetracycline resistance is a common feature of most of the MRSA isolates (Smith and Pearson 2011; Graveland et al. 2011a). A Belgian study which tested 643 pig MRSA ST398 isolates reported almost similar resistant rates for tetracycline (Crombe et al. 2012).

In the present study, 43 (78.75 %) isolates showed multidrug resistance where seventeen resistance groups (R1 to R17) were observed. Studies conducted on characterization of MRSA isolates from pigs in Hong Kong revealed that most of the MRSA isolates were multidrug resistant (Ho et al. 2012). The most predominant resistance pattern observed in the present study was resistance to β-lactams + tetracyclines + sulphonamides which is consistent with the findings of Kadlec et al. (2009). Van Duijkeren et al. (2008) reported that the pressure of the antimicrobial selection is one of the probable factors that have facilitated the emergence and dispersion of the veterinary MRSA, the most commonly used being tetracycline and trimethoprim-sulfamethoxazole and to which these microorganisms are more resistant. Similarly Ndi and Barton (2012) opined that antibiotics used in animals for therapeutics, food production and diseases prevention promote antibiotic resistance and this may be the reason for multidrug resistance as observed in most of the isolates in this study. Although multidrug resistance is presently defined as resistance to three or more classes of antibiotics (Magiorakos et al. 2012), it should be noted that any lack of therapeutic effectiveness due to resistance to the administered substance can be devastating. Another important aspect is that colonization of livestock with drug resistant bacteria is often considered a risk factor for meat contamination by these resistant bacteria (Tenhagen et al. 2009).

This is perhaps the first report on SCC *mec* types and antimicrobial resistance pattern of MRSA isolates from pigs in India. The study revealed that most of the MRSA strains isolated in the study are resistant to penicillins and tetracyclines, the widely used antimicrobials in animal practice. The study will also form the basis for devising future strategy for the control of this emerging pathogen.

Compliance with ethical standards

Conflict of interest There is no conflict of interest for this manuscript.

References

Beneke B, Klees S, Stuhrenberg B, Fetsch A, Kraushaar B, Tenhagen BA (2011) Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in a fresh meat pork production chain. J Food Prot 74:126–129

Bhutia KO, Singh TSK, Adhikari L, Biswas S (2015) Molecular characterization of community- & hospital-acquired methicillin-resistant & methicillin-sensitive *Staphylococcus aureus* isolates in Sikkim. Indian J Med Res 142:330–335

Broens EM, Graat EAM, Van Der Wolf PJ, Van De Giessen AW, De Jong MCM (2011) Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Prev Vet Med 102:41–49

Çıkman A, Aydın M, Gülhan B, Parlak M, Gültepe B, Kalaycı Y, Bayındır BF, Solmaz S, Özekinci T (2015) Investigation of antibiotic resistance patterns and reduced vancomycin susceptibilities of methicillin-resistant *Staphylococcus aureus* isolates: a multi-center study. Mikrobiyol Bul 49:240–248

CLSI (2009) Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standards. 10th ed. CLSI document M02-A10. Clinical and Laboratory Standards Institute, Wayne

Crombe F, Willems G, Dispas M, Hallin M, Denis O, Suetens C, Gordts B, Struelens M, Butaye P (2012) Prevalence and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* among pigs in Belgium. Microb Drug Resist 18:125–131

de Neeling AJ, van den Broek MJ, Spalburg EC, van Santen-Verheuvel MG, Dam-Deisz WDC, Boshuizen HC, van de Giessen AW, van Duijkeren E, Huijsdens XW (2007) High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet Microbiol 122:366–372

Dewaele I, Messens W, De Man I, Delputte P, Herman L, Butaye P, Heyndrickx M, Rasschaert G (2011) Sampling, prevalence and characterization of methicillin-resistant *Staphylococcus aureus* on two Belgian pig farms. Vet Sci Dev. doi:10.4081/vsd.2011.e1

Dierikx CM, Hengeveld PD, Veldman KT, de Haan A, van der Voorde S, Dop PY, Bosch T, van Duijkeren E (2016) Ten years later: still a high prevalence of MRSA in slaughter pigs despite a significant reduction in antimicrobial usage in pigs the Netherlands. J Antimicrob Chemother Jun 3. doi:dkw190

D'Souza N, Rodrigues C, Mehta A (2010) Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* with Emergence of Epidemic Clones of Sequence Type (ST) 22 and ST 772 in Mumbai, India. J Clin Microbiol 48:1806–1811

Fayyaz M, Mirza IA, Ahmed Z, Abbasi SA, Hussain A, Ali S (2013) *In vitro* susceptibility of chloramphenicol against methicillin-resistant *Staphylococcus aureus*. J Coll Physicians Surg Pak 23: 637–640

Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, Ramirez A, Smith TC (2013) Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from pork farms and visiting veterinary students. PLoS One 8:e53738

Gopalakrishnan R, Sureshkumar D (2010) Changing trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. J Assoc Physicians India 58:25–31

Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA (2011a) Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. Int J Med Microbiol 301:630–634

Graveland H, Wagenaar JA, Bergs K, Heesterbeek H, Heederik D (2011b) Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PLoS One 6:e16830

Gyssens IC (2001) Quality measures of antimicrobial drug use. Int J Antimicrob Agents 17:9–19

Ho J, O'Donoghue M, Guardabassi L, Moodley A, Boost M (2012) Characterization of methicillin-resistant *Staphylococcus aureus* isolates from pig carcasses in Hong Kong. Zoonoses Pub Hlth 59:416–423

Ito T, Kuwahara-Arai K, Katayama Y, Uehara Y, Han X, Kondo Y, Hiramatsu K (2014) Staphylococcal Cassette Chromosome mec (SCC*mec*) analysis of MRSA. Methods Mol Biol 1085:131–148

Kadlec K, Ehricht R, Monecke S, Steinacker U, Kaspar H, Mankertz J, Schwarz S (2009) Diversity of antimicrobial resistance pheno- and genotypes of methicillin-resistant *Staphylococcus aureus* ST398 from diseased swine. J Antimicrob Chemother 64:1156–1164

Khanna T, Friendship R, Dewey C, Weese JS (2008) Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet Microbiol 128:298–303

Lewis HC, Mølbak K, Reese C, Aarestrup FM, Selchau M, Sørum M, Skov RL (2008) Pigs as source of methicillin-resistant *Staphylococcus aureus* CC398 infections in humans, Denmark. Emerg Infect Dis 14:1383–1389

Lim SK, Nam HM, Jang GC, Lee HS, Jung SC, Kwak HS (2012) The first detection of methicillin-resistant *Staphylococcus aureus* ST398 in pigs in Korea. Vet Microbiol 155:88–92

Louie L, Goodfellow J, Mathieu P, Glatt A, Louie M, Simor AE (2002) Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. J Clin Microbiol 40:2786–2790

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18:268–281

Mallick SK, Basak S (2011) Accurate detection of methicillin-resistant *Staphylococcus aureus* in day to day practice: a great help to clinicians. J Indian Med Assoc 109:892–895

Nadig S, Raju SR, Arakere G (2010) Epidemic meticillin-resistant *Staphylococcus aureus* (EMRSA-15) variants detected in healthy and diseased individuals in India. JMM 59:815–821

Narvaez-Bravo C, Toufeer M, Weese SJ, Diarra MS, Deckert AE, Reid-Smith R, Aslam M (2016) Prevalence of methicillin-resistant *Staphylococcus aureus* in Canadian commercial pork processing plants. J Appl Microbiol 120:770–780

Ndi O, Barton M (2012) The Australian perspective in Antimicrobial resistance in the environment, edited by Keen PL and Montforts, 1st edn. USA, John Wiley and Sons Inc, New Jersey, pp. 265–289

Neetu TJ, Murugan S (2016) Genotyping of methicillin resistant *Staphylococcus aureus* from tertiary care hospitals in Coimbatore, South India. J Global Infect Dis 8:68–74

Oppliger A, Moreillon P, Charrière N, Giddey M, Morisset D, Sakwinskab O (2012) Antimicrobial Resistance of *Staphylococcus aureus* Strains Acquired by Pig Farmers from Pigs. Appl Environ Microbiol 78:8010–8014

Park J, Friendship RM, Weese JS, Poljak Z, Dewey CE (2013) An investigation of resistance to β-lactam antimicrobials among staphylococci isolated from pigs with exudative epidermitis. BMC Vet Res 9:211

Patel AK, Patel KK, Patel KR, Shah S, Dileep P (2010) Time trends in the epidemiology of microbial infections at a tertiary care center in west India over last 5 years. J Assoc Physicians India 58:37–40

Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18:657–686

Robinson DA, Enright MC (2004) Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 10:92–97

Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, Capuano AW, Hermaldt LA, Diekema DJ (2009) Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS One 4:e4258.

Smith TC, Pearson N (2011) The emergence of *Staphylococcus aureus* ST398. Vector Borne Zoon Dis 11:327–339

Stefani S, Varaldo PE (2003) Epidemiology of methicilin-resitant staphylococci in Europe. Clin Microbiol Infect 9:1179–1186

Sun J, Yang M, Sreevatsan S, Davies PR (2015) Prevalence and Characterization of *Staphylococcus aureus* in Growing Pigs in the USA. PLoS One 10:e0143670

Tenhagen BA, Fetsch A, Stührenberg B, Schleuter G, Guerra B, Hammer JA, Hertwig S, Kowall J, Kämpe U, Schroeter A, Bräunig J, Käsbohrer A, Appel B (2009) Prevalence of MRSA types in slaughter pigs in different German abattoirs. Vet Rec 165:589–593

Timmerman T, Dewulf J, Catry B, Feyen B, Opsomer G, de Kruif A, Maes D (2006) Quantification and evaluation of antimicrobial drug use in group treatments for fattening pigs in Belgium. Prev Vet Med 74:251–263

Tulinski P, Fluit AC, Wagenaar JA, Mevius D, Vijver LV, Duima B (2012) Methicillin-Resistant Coagulase-Negative Staphylococci on Pig Farms as a Reservoir of Heterogeneous Staphylococcal Cassette Chromosome *mec* Elements. Appl Environ Microbiol 78:299–304

Uzun B, Karataş Şener AG, Güngör S, Afşar I, Yüksel Ergin O, Demirci M (2013) Comparison of Cefoxitin Disk Diffusion Test, Automated System and Chromogenic Medium for Detection of Methicillin Resistance in *Staphylococcus aureus* Isolates. Mikrobiyol Bul 47:1118

Van Duijkeren E, Ikawaty R, Broekhuizen-Stins MJ, Jansen MD, Spalburg EC, de Neeling AJ, Allaart JG, van Nes A, Wagenaar JA, Fluit AC (2008) Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. Vet Microbiol 126:383–389

Van Duijkeren E, Jansen MD, Flemming SC, de Neeling H, Wagenaar JA, Schoormans AH, van Nes A, Fluit AC (2007) Methicillin-resistant *Staphylococcus aureus* in pigs with exudative epidermitis. Emerg Infect Dis 13:1408–1410

Verhegghe M, Crombé F, Luyckx K, Haesebrouck F, Butaye P, Herman L, Heyndrickx M, Rasschaert G (2016) Prevalence and Genetic Diversity of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* on Belgian Pork. J Food Prot 79:82–89

Voss A (2005) Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis 11:1965–1966

Weese JS, Zwambag A, Rosendal T, Reid-Smith R, Friendship R (2011) Longitudinal Investigation of Methicillin-Resistant *Staphylococcus aureus* in Piglets. Zoonoses Pub Hlth 58:238–243

Zhang C, Song L, Chen H, Liu Y, Qin Y, Ning Y (2012) Antimicrobial susceptibility and molecular subtypes of *Staphylococcus aureus* isolated from pig tonsils and cow's milk in China. Canadian J Vet Res 76:268–274

Zhang K, McClure JA, Elsayed S, Louie T, Conly JM (2005) Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 43:5026–5033