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RESEARCH OF METHICILLIN RESISTANCE STAPHYLOCOCCI IN A PIG'S FARM

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Summary

In pigs, staphylococci are important opportunistic pathogens often found in the microflora of skin and mucosal surfaces of the upper respiratory tract. Since the introduction of antibiotics into human clinical use staphylococci have shown rapid acquisition of resistance to almost all major classes of antibiotics, particularly in those strains associated with nosocomial infections in humans. Little is known about the development and spread of antimicrobial resistance in staphylococci in pigs. In this study, investigated a total 187 samples from healthy pigs, different skin areas (nipples, per vulvar, ear and abdominal) were examined for the presence of Staphylococcus through standard methods. The antibiotic susceptibility of the isolated strains was tested using the Vitek 2 system. The microorganism was found in 48 pigs (25.67%), colonized in the nipples skin (9/38; 23.68%) and per vulvar skin (3/25; 12.00%) from sows with piglets; nipples skin (3/23; 13.04%) and per vulvar skin (2/22; 9.09%) from pregnant sows; ear skin (13/38; 34.21%) from weaned piglets and abdominal skin (18/41; 43.90%) from fat pigs. Antimicrobial susceptibility testing revealed a remarkably susceptible population, all of isolates, to nine drugs tested, and resistant to benzyl penicillin (50.00%; 24/48), tetracycline (37.5%; 18/48), gentamicin (35.41%; 17/48), erythromycin (25.00%; 12/48), ampicillin (22.91%; 11/48), and kanamycin (20.83%; 10/48). Eighth methicillin resistant isolates (oxacillin, respectively cefoxitin) were identified. Although 12.5% (6/48) of isolates were chloramphenicol resistant, 10.41% (5/48) trimethoprim/sulfamethoxazole resistant, and 4.1% (2/48) nitrofurantoin resistant. No inducible clindamycin resistance was found. Correct identification of staphylococcal isolates is very important for the accurate management of staphylococcal infections, but it is also essential for a better understanding of the pathophysiological factors affecting the clinical outcome and for epidemiological surveillance and the distribution these bacteria in pigs and people. Our results showed the presence of non-host-specific staphylococcal species with multidrug resistance, including that to methicillin (oxacillin and cefoxitin).

Keywords: Staphylococcus, swine, methicillin, resistance

Staphylococci are important opportunistic pathogens often found in the microflora of skin and mucosal surfaces of the upper respiratory tract of man and animals.

One year after the introduction of methicillin in clinical practice (1961), have been described methicillin resistant *Staphylococcus aureus* (MRSA) strains (10). Since then, MRSA has become a major human pathogen, responsible for considerable mortality, morbidity and healthcare expenditure in both nosocomial and community settings (1, 15).

Although rarely reported in the past, the prevalence of MRSA in pigs, along with cases of possible pig-to-human transmission and vice versa, have been the subject of considerable and increasing interest over the past few years (2, 21).

MRSA has become a major nosocomial pathogen, highly prevalent in many European countries and throughout the world (1).

S. aureus are normal inhabitants of pigs, and occur in all herds (2). Prevalence of MRSA in pig herds varies widely (0 to 50%) among European countries (1). The pig herd prevalence of MRSA in North America is uncertain, but appears lower than in many European countries (15, 24). MRSA prevalence is high (>50%) in pigs in positive herds, but has minimal effect on swine health (15).

The capacity of *S. aureus*/MRSA strains of livestock origin to colonize spread, and cause disease in humans remains uncertain (15).

Since the introduction of antibiotics into human clinical use staphylococci have shown rapid acquisition of resistance to almost all the major classes of antibiotics, particularly in those strains associated with nosocomial infections in humans.

Little is known about the development and spread of antimicrobial resistance in staphylococci in swine.

Thus, the current study was undertaken to investigate the occurrence of staphylococcal flora from pig's farm in healthy animals and to provide new data about their antibiotic resistance.

Materials and methods

This research was conducted in the Laboratory of Bacterial infectious diseases, Department of Infectious Diseases and Preventive Medicine, of the Faculty of Veterinary Medicine Timisoara, Western Romania. Samples were taken from clinically healthy pigs from a swine farm in a village in the Arad County, in Western Romania, during July-December 2018.

Samples required bacteriological examinations and were collected from a total of 187 clinically healthy pigs (sows with piglets, pregnant sows, weaners and fat pigs), representing samples from different anatomical areas (skin): nipples, per vulvar, ear and abdomen. Samples were collected using sterile cotton wool pads secured to the plastic rod and placed in sterile tubes (standard product), which were further processed for bacterial isolation.

Pathological materials were plated on 5% sheep blood agar (Biomedics, Madrid, Spain). Staphylococci strains isolated and purified were tested on biochemical and pathogenic characters.

Strains were sub-cultured twice, and then grown for 18–24 h at 37°C on 5% sheep blood agar (Biomedics, Madrid, Spain) in air. Suspensions of these cultures were made in 0.45% saline, adjusted to the turbidity of a 0.6 McFarland Standard, and used to load the test cards for VITEK 2 Systems, which was used in accordance with the manufacturer's directions.

Isolates were further differentiated to species level on the basis of their biochemical properties using the Vitek 2 Systems (bioMérieux, Marcy-l'Etoile, France), and Vitek GP ID card (bioMérieux, France). All characterized isolates have shown very good and excellent (consistence) confidence level (96-99%).

Pathogenic factors controlled were haemolysins and the presence of the two types of coagulase. Highlighting coagulase was made by the two techniques used for this purpose. To highlight coagulase Prolex STAPH Latex rapid kit (Pro-Lab Diagnostics, United Kingdom) was used. Free diffusible coagulase was highlighted from technique in tubes using rabbit plasma with EDTA (Bactident Coagulase, Merk, Canada).

Subsequently, bacterial resistance of all isolated Staphylococcus strains were tested for susceptibility to nineteen commonly used antibiotics through Vitek 2 AST GP69 card (bioMérieux, France).

The following classes of antibiotics were used: beta lactams (benzyl penicillin, ampicillin, oxacillin, and imipenem), cephalosporin (cefoxitin), monobactam (ampicillin/sulbactan), aminoglycoside (gentamicin, kanamycin), fluoroquinolone (enrofloxacin, marbofloxacin), macrolide (erythromycin), lincosamide (clindamycin), glycopeptide (vancomycin), tetracycline (tetracycline), other antimicrobials (fusidic acid), monoxycarbolic acid (mupirocin), amphenicol (chloramphenicol), rifamycin (rifampicin) and sulphonamides (trimethoprim/sulfamethoxazole, nitrofurantoin). Tests for inducible clindamycin resistance were used. Oxacillin susceptibility test was used to predict mecA-mediated resistance in *S. aureus*. The mecA gene responsible for methicillin resistance was detected by PCR.

All strains with high resistance to oxacillin and cefoxitin tested for the presence of *S. aureus* specific DNA element, such as the mecA gene (533 bp), in accordance with the methods of Reischl et al. (14). Amplification products were analyzed on 1.5% agarose gel stained with ethidium bromide and a UV transilluminator.

Statistical analyses were performed using the online version of VassarStats software (26).

Results and discussions

Overall, 48 (25.7%; 95% confidence interval [CI] 19.7-32.66%) of the 187 collected samples were found to be *Staphylococcus* positive through standard examination methods. All oxacillin and cefoxitin resistant isolated bacterial strains were successfully amplified targeting the *mecA* gene, confirming the results of the antibiotics sensitivity test. The obtained PCR products showed typical profiles for methicillin resistance in the 1.5% agarose gel.

In this study 48 strains of staphylococci were isolated, including 30 coagulase positive strains, represented by *S. hyicus* and *S. aureus*, respectively 18 of coagulase negative strains represented by *S. haemolyticus*, *S. sciuri* and *S.*

epidermidis, isolated from pigs in different anatomical areas. The VITEK 2 system correctly identified to the species level of the 48 strains (Table 1), 19 *S. aureus* (99% accurate), 11 of *S. hycus* (98,5% accurate), 7 of *S. sciuri* (96.9% accurate), 7 of *S. epidermidis* (92.7% accurate), and 5 of *S. haemolyticus* (96.5% accurate).

Table 1

Distribution of staphylococci strains of isolated from healthy pigs according to skin areas

Age category/skin areas	Number of processed samples	No. of *Staphylococcus* positive samples	95% CI	Strains of staphylococci isolated				
				S. aureus	*S. hycus*	*S. epidermidis*	*S. sciuri*	*S. haemolyticus*
Sows with piglets								
nipples skin	38	9 (23.7%)	12.02-40.61	3	2	2	1	1
per vulvar skin	25	3 (12.0%)	3.15-32.34	1	1	-	1	-
Pregnant sows								
per vulvar skin	22	2 (9.0%)	1.59-30.62	-	1	-	1	1
nipples skin	23	3 (13.0%)	3.43-34.66	2	1	-	-	-
Weaned piglets								
ears skin	38	13 (34.2%)	20.14-51.42	5	2	3	2	1
Fat pigs								
abdominal skin	41	18 (43.9%)	28.82-60.11	8	4	2	2	2
Total	187	48 (25.7%)	19.7-32.66	19	11	7	7	5

Strains of staphylococci unexposed to the pressure of antibiotics are sensitive to these substances; however, isolates from pigs with various conditions under pressure due to antibiotic therapy may show multiple resistances' phenomenon.

The results of antibiotic susceptibility testing of staphylococci strains isolated from pigs, using Vitek 2 ASTGP69 card are presented in Table 2.

The used Vitek 2 system has proved an accurate, rapid (18h), and relatively easy to use determination of antimicrobial susceptibility of the bacterial strains.During the study the phenomenon of multiple resistances and the resistance type from oxacillin and cefoxitin (methicillin) were monitored, for all staphylococci strains isolated from pigs (Table 3).

Table 2

Results of the sensitivity to antibiotics of *Staphylococcus* strains isolated from healthy pigs

Name of antimicrobial substance	Number of susceptible staphylococcal strains				
	S. aureus (n=19)	*S. hyicus* (n=11)	*S. epidermidis* (n=7)	*S. sciuri* (n=7)	*S. haemolyticus* (n=5)
Benzyl penicillin (P)	9	7	4	3	3
Ampicillin (AM)	14	9	6	5	4
ampicillin/sulbactan (SAM)	19	11	7	7	5
Oxacillin (Ox)	15	7	7	7	5
Imipenem (IPM)	19	11	7	7	5
Gentamicin (GM)	11	10	5	4	3
Kanamycin (K)	15	8	6	6	2
Enrofloxacin (ENR)	19	11	7	7	5
Marbofloxacin (MAR)	19	11	7	7	5
Erythromycin (E)	15	9	4	6	4
Clindamycin (CM)	19	11	7	7	5
Vancomycin (VA)	19	11	7	7	5
Tetracycline (TE)	16	6	3	3	2
Fusidic acid (FA)	19	11	7	7	5
Mupirocin (MUP)	19	11	7	7	5
Chloramphenicol (C)	17	9	5	7	5
Rifampicin (RA)	19	11	7	7	5
Trimethoprim/sulfamethoxazole (SXT)	16	10	6	7	5
Nitrofurantoin (NIF)	17	9	7	7	5
Cefoxitin (FOX)	15	7	7	7	5

Table 3

Resistance type of the species of staphylococci isolated from healthy pigs

Antimicrobials /*Staphylococcus spp.* (n=48)	*S. aureus*	*S. hycus*	*S. epidermidis*	*S. sciuri*	*S. haemolyticus*	Total resistant strains (n/%)	95% CI
Benzyl penicillin (P)	10	4	3	4	3	24 (50.0)	35.43-64.57
Ampicillin (AM)	5	2	1	2	1	11 (22.9)	12.52-37.67
Oxacillin (Ox)	4	4	0	0	0	8 (16.7)	7.97-30.77
Gentamicin (GM)	8	2	2	3	2	17 (35.4)	22.55-50.61
Kanamycin (K)	4	1	1	1	3	10 (20.8)	10.95-35.4
Erythromycin (E)	4	3	3	1	1	12 (25.0)	14.11-39.89
Tetracycline (TE)	3	4	4	4	3	18 (37.5)	24.32-52.67
Chloramphenicol (C)	2	2	2	0	0	6 (12.5)	5.19-25.94
Trimethoprim /sulfamethoxazole (SXT)	3	1	1	0	0	5 (10.4)	3.9-23.45
Nitrofurantoin (NIF)	2	0	0	0	0	2 (4.2)	0.73-15.43
Cefoxitin (FOX)	4	4	0	0	0	8 (16.7)	7.97-30.77

Antimicrobial susceptibility testing revealed a remarkably susceptible population, all of isolates, to nine drugs tested, and resistant to benzyl penicillin (50.0%; 24/48; CI 35.43-64.57), tetracycline (37.5%; 18/48; CI 24.32-52.67), gentamicin (35.4%; 17/48; CI 22.55-50.61), erythromycin (25.0%; 12/48; CI 14.11-39.89), ampicillin (22.9%; 11/48; CI 12.52-37.67), and kanamycin (20.8%; 10/48; CI 10.95-35.4). Eighth methicillin resistant isolates (oxacillin, respectively cefoxitin) were identified. Although 12.5% (6/48; CI 5.19-25.94) of isolates were chloramphenicol resistant, 10.4% (5/48; CI 3.9-23.45) trimethoprim/sulfamethoxazole resistant, 4.1% (2/48; CI 0.73-15.43) nitrofurantoin resistant, no inducible clindamycin resistance was found (Table 2).

Sensitivity to tetracycline was reduced, 18 strains were resistant to this group of antibiotics (Table 2).

All strains tested were susceptible to enrofloxacin and marbofloxacin, even if fluoroquinolones are used in the therapy of swine.

Analysing results from the table it can be seen that the sensitivity to antibiotics was variable depending on the group and classes of antibiotics.

In the case of antibiotics, ampicillin/sulbactan, imipenem, enrofloxacin, marbofloxacin, clindamycin, vancomycin, fusidic acid, mupirocin and rifampicin, considered the drug of choice for staphylococci, the number of sensitive strains was 100% (Table 2). This suggests that isolates tested came from pigs to which these antibiotics were not used. Also, it can be said that all of these antibiotics are part of the kit for staphylococcal infections, typically used in humans and in the treatment of these infections in animals, respectively.

When compared β-lactams (benzyl penicillin, ampicillin, oxacillin, and imipenem) and monobactam (ampicillin/sulbactan), sensitivity was highest, except Staphylococcus aureus and S. hyicus, for which eight oxacillin and cefoxitin resistant strains were isolated. Of these, three strains of S. hyicus and five strains of S. aureus (Table 3). The strains tested were mostly sensitive to β-lactams, a result of previous treatments done correctly.

The phenomenon of antibiotic resistance in the case of β-lactam is based on the type of genetic determinants of plasmid and chromosomal governing the synthesis of β-lactamase, broad spectrum, which provides the resistance of staphylococci. Resistance to methicillin is transmitted by plasmids (R factor) having a pattern common to other β-lactams. For this reason, methicillin-resistant staphylococcal strains are considered zoonotic risk strains of staphylococci, particularly with a complex circuit human- animal - human, respectively (12, 13, 19, 24, 26).

The development of staphylococci resistance to different antibiotics, it is a consequence of wasteful use in the treatment of diseases in pigs. An antibiotic used irrationally creates a selection pressure, that is, selected and transmitted genetic determinants of plasmid and chromosomal type. Consequently, the phenomenon of multiple resistance that is transmitted intra and interspecific. It is important particularly because the resistance to methicillin can be associated with

resistance to β-lactams and other groups of antibiotics (7, 10, 15).

After testing staphylococci strains isolated from pigs, against different classes of antibiotics, oxacillin and cefoxitin resistant strains, and more type of resistances, against β-lactams, tetracyclines were identified.

The data on methicillin resistance and type of resistances identified are similar to the results communicated by other authors on the phenomenon of resistance to antibiotics (7, 10, 15).

S. aureus is an epiphyte, a normal microorganism in pigs, and occurs in all herds (2). The prevalence of MRSA strains in pig herds varies widely (0-50%) among European countries (1). Actual prevalence of MRSA in pigs in North America is uncertain, but appears to be lower than in many European countries (15, 25). The prevalence of MRSA is high (> 50%) in pigs from herds positive but has little effect on the health of pigs.

S. aureus is found in dust and air in the pigs farms (7), and healthy people working in these farms and shelters, often are carriers of *S. aureus* from pig nasal mucosa (2, 11, 15, 22, 23). MRSA can be detected in the case of 20-80% of clinically healthy workers operating in MRSA positive pig herds, much more than other categories of people (1.5% in the US; <0.11% in Netherlands) (4, 8).

The risk of exposure to MRSA from animals is largely restricted to persons who have direct contact with animals and their families respectively (3, 5, 18).

The ability of *S. aureus* strains/animal MRSA to colonize, to spread and cause disease in humans remains uncertain. It seems that the line ST398 persists only for a short time (hours or days) to most people, but some can colonize humans months or years without developing infections (6, 9, 16, 17, 20). In Dutch hospitals ST398 line spread between people was identified, and was four times more common than MRSA strains of human origin. There have been described outbreaks of infection of MRSA ST398 line data so far. Other lines of MRSA may also occur in pigs (eg., ST9 in Asia, North America ST5), but public health implications are unknown.

Conclusions

Massive growth of Gram positive haemolytic, catalase positive and negative cocci was observed in samples from clinically healthy pigs. The strains was identified as *Staphylococcus aureus*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, *Staphylococcus sciuri* and *Staphylococcus haemolyticus* by Vitek GP ID card biochemical tests (bioMérieux® SA, France) and this result was confirmed by latex agglutination test and free diffusible coagulase test (Prolex STAPH Latex rapid kit (Pro-Lab Diagnostics, United Kingdom and Bactident Coagulase, Merk, Canada). From pig farm 48 strains of staphylococci were isolated, including 30 coagulase positive strains (*S. hyicus* and *S. aureus*) and 18 of coagulase negative strains (*S. haemolyticus*, *S. epidermidis*, respectively *S. sciuri*), from clinically healthy pigs in different anatomical areas.

All strains of staphylococci isolated from pigs showed sensitivity of 100% for antibiotics: ampicillin/sulbactan, imipenem, enrofloxacine, marbofloxacin, clindamycin, vancomycin, fusidic acid, rifampicin, and mupirocin, considered the drug of choice for these bacteria.

When compared β-lactams (benzyl penicillin, ampicillin, oxacillin, and imipenem) and monobactam (ampicillin/sulbactan) sensitivity was highest, except *Staphylococcus aureus* and *S. hycus*, which were isolated with eight oxacillin and cefoxitin resistant strains. Most staphylococcal strains isolate from healthy pigs have developed multidrug resistance. According to the concentration gradient (E-Test) methods (Vitek 2 AST GP69 card**,** bioMérieux, France), the isolated oxacillin and cefoxitin resistant *S. aureus* and *S. hyicus*, confirmed by isolation of plasmid and amplification of *mec*A gene, is responsible for methicillin resistance, by PCR. Phenotypically, all staphylococcal isolates were resistant to 11 antibiotics of the 20 tested, but sensitive to β-lactams (imipenem), monobactam (ampicillin/sulbactan), fluroquinolone (enrofloxacine, marbofloxacin), lincosamide (clindamycin), glico peptide (vancomycin), acid monocarboxylic (Mupirocin), rapamycin (rifampicin), and other antimicrobial (fusidic acid).

In conclusion, correct identification of staphylococcal isolates (coagulase positive and coagulase negative) is very important for the accurate management of staphylococcal infections, but it is also essential for a better understanding of the pathophysiological factors affecting the clinical outcome and for epidemiological surveillance and the distribution these bacteria in pigs and people.

Our results showed the presence of non-host-specific staphylococcal species with multidrug resistance, including that to methicillin (oxacillin and cefoxitin).

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References

1. **Anon**, Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008 [1] - Part A: MRSA prevalence estimates EFSA J, 2009, 7(11), 1376.
2. **Armand-Lefevre, L., Ruimy, R., Andremont, A.,** Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs, Emerg. Infect. Dis., 2005, 11(5), 711-714.

3. **Bisdorff, B., Scholhölter, J.L., Claussen, K., Pulz, M., Nowak, D., Radon, K**., MRSA-ST398 in livestock farmers and neighboring residents in a rural area in Germany, Epidemiol Infect, 2012, 140(10), 1800-8.
4. **Bode, L.G., Wertheim, H.F., Kluytmans, J.A., Bogaers-Hofman, D., Vandenbroucke-Grauls, C.M.,** Sustained low prevalence of methicillin-resistant *Staphylococcus aureus* upon admission to hospital in The Netherlands, J. Hosp. Infect., 2011, 79, 198-201.
5. **Cuny, C., Nathaus, R., Layer, F., Strommenger, B., Altmann, D., Witte, W.,** Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs, PLoS ONE, 2009, 4(8), e6800.
6. **Frana, T.S., Beahm, A.R., Hanson, B.M., Kinyon, J.M., Layman, L.L., Karriker, L.A., Ramirez, A., Smith, T.C.,** Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from pork farms and visiting veterinary students, PLoS One, 2013, 8(1), e53738.
7. **Gibbs, S.G., Green, C.F., Tarwater, P.M., Mota, L.C., Mena, K.D., Scarpino, P.V.,** Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation, Environ Health Perspect, 2006, 114, 1032-1037.
8. **Gorwitz, R.J., Kruszon-Moran, D., McAllister, S.K., McQuillan, G., McDougal, L.K., Fosheim, G.E., Jensen, B.J., Killgore, G., Tenover, F.C., Kuehnert, M.J.,** Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004, J. Infect. Dis., 2008, 197(9), 1226-1234.
9. **Graveland, H., Wagenaar, J.A., Bergs, K., Heesterbeek, H., Heederik, D.,** Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact, PLoS One, 2011, 6, e16830.
10. **Guardabassi, L., Courvalin, P.,** Modes of antimicrobial action and mechanisms of bacterial resistance, In: Antimicrobial resistance in bacteria of animal origin Ed ASM Press Washington, D.C., 2006.
11. **Khanna, T., Friendship, R., Dewey, C., Weese, J.S.,** Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers, Vet. Microbiol., 2008,128, 298-303.
12. **King, M.D., Humphrey, B.J., Wang, Y.F., Kourbatova, E.V., Ray, S.M., Blumberg, H.M.,** Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections, Ann. Inter. Med., 2006, 144, 309–317.
13. **Kluytmans-Vandenbergh, M.F., Kluytmans, J.A.,** Community acquired methicillin-resistant *Staphylococcus aureus*: current perspectives, Clin. Microbiol. Infect., 2006, 12(1), 9–15.
14. **Reischl, U., Linde, H.J., Metz, M., Leppmeier, B., Lehn, N.,** Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous

species confirmation using real-time fluorescence PCR, J. Clin. Microbiol., 2008, 38, 2429–33.
15. **Smith, T.A., Gebreyes, W.A., Abley, M.J., Bayleyegn, M., Harper-Maples, A., Forshey, B.M., Male, M.J., Martin, H.W., Sreevatsan, S., Thakur, S., Thiruvengadam, M., Davies, P.R.,** Methicillin-resistant *Staphylococcus aureus* in pigs and farm workers on conventional and antibiotic-free swine farms in the USA, PLoS One, 2013, 8(5), e63704.
16. **Sun, J., Linhares, L., Yang, M., Sreevatsan, S., Davies, P.,** Longitudinal study of Staphylococcus aureus and MRSA colonization of US swine veterinarians Proceedings, 10th International Conference on the Epidemiology and Control of Foodborne Pathogens in Pork (SafePork), Portland ME Sept 10-12, 2013.
17. **Van Cleef, B.A., Graveland, H., Haenen, A.P., Van De Giessen, A.W., Heederik, D., Wagenaar, J.A., Kluytmans, J.A.,** Persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* in field workers after short-term occupational exposure to pigs and veal calves, J. Clin. Microbiol., 2011, 49, 1030-1033.
18. **Van Cleef, B.A., Verkade, E.J., Wulf, M., Buiting, A.G., Voss, A., Huijsdens, X.W.,** Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands, PLoS ONE, 2010, 5, e9385.
19. **Vandenesch, F., Naimi, T., Enright, M.C.,** Community acquired methicillin-resistant *Staphylococcus aureus* carrying the Panton–Valentine leucocidin genes: worldwide emergence, Emerg. Infect. Dis., 2003, 9, 978–984.
20. **Verkade, E., Van Benthem, B., Den Bergh, M.K., Van Cleef, B., Van Rijen, M., Bosch, T., Kluytmans, A.,** Dynamics and determinants of *Staphylococcus aureus* carriage in livestock veterinarians: a prospective cohort study, J. Clin. Infect. Dis., 2013, 57(2), e11-7.
21. **Voss, A., Loeffen, F., Bakker, J., Wulf, M., Klaassen, C.,** Methicillin-resistant *Staphylococcus aureus* in pig farming, Emerg. Infect. Dis., 2005, 11, 1965–1966.
22. **Voss, A., Loeffen, F., Bakker, J., Wulf, M.W.,** Methicillin-resistant *Staphylococcus aureus* in pig farming, Vet. Microbiol., 2006, 14, 65-68.
23. **Wan M.T., Lauderdale, T.L., Chou C.C.,** Characteristics and virulence factors of livestock associated ST9 methicillin-resistant *Staphylococcus aureus* with a novel recombinant staphylocoagulase type, Vet. Microbiol., 2013, 162(24), 779-784.
24. **Weese, J.S., Rousseau, J., Deckert, A., Gow, S., Reid-Smith, R.J**., Clostridium difficile and methicillin-resistant *Staphylococcus aureus* shedding by slaughter-age pigs, BMC Vet. Res., 2011, 7, 41.
25. **Wertheim, H.F., Vos, M.C., Boelens, H.A.,** Low prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) at hospital admission in the Netherlands: the value of search and destroy and restrictive antibiotic use, J. Hosp. Infect., 2004, 56, 321–325.
26. ***http://vassarstats.net/prop1.html