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CLINICAL PREVALENCE OF METHICILLIN RESISTANCE STAPHYLOCOCCI IN A PIG FARM FROM ARAD COUNTY – PRELIMINARY STUDY

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Abstract

Colonization with Staphylococcus aureus resistant to methicillin (MRSA) has recently been identified in pigs and that the people working in these sectors and increasing concern about the role of pigs as reservoirs of MRSA infections in humans were reported more frequently in the literature. The research had as purpose to determine the frequency of staphylococcal strains isolated from pig herds, phenotypic characterization of isolates and identification of methicillin-resistant strains and types of resistance. Samples were taken from clinically healthy pigs from a swine farm in Arad, from October to December 2013. Samples required bacteriological examinations were collected from a total of 87 clinically healthy pigs. After growth, staphylococcal isolates were identified according to their characteristics as outlined in Bergey's Manual of Determinative Bacteriology and the Manual of Clinical Microbiology. From pig farm were isolated 28 strains of staphylococci, including 20 coagulase positive strains (CoP, represented by S. hyicus and S. aureus) and 8 coagulase negative strains (CON, represented by S. haemolyticus, S. epidermidis respectively S. sciuri), isolated from clinically healthy pigs in different anatomical areas. All strains of staphylococci isolated from pigs showed sensitivity of 100% for novobiocine, rifampicine, pristinamycin, ciprofloxacin, vancomycin, ceftriaxone, cefoxitin, cefaclor and ampicillin/sulbactan, considered the drug of choice for these bacteria. As against used β-lactams (methicillin, ceftriaxone, cefoxitin, cefaclor, ampicillin with sulbactan) antibiotic sensitivity was highest, except methicillins, which were isolated 4 resistant strains, two S. hycus methicillin-resistant strains and two S. aureus strains. After testing staphylococci strains isolated from pigs, against 17 antibiotics were identified methicillin-resistant strains and several types of resistance to β-lactams, tetracyclines, macrolides and polymyxin B.

Key words*: staphylococci, methicillin resistance, pigs*

INTRODUCTION

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 MRSA strains (Guardabassi et al., 2006). Since then, MRSA has become a major human pathogen, responsible for considerable mortality, morbidity and healthcare expenditure in both nosocomial and community settings (Anon, 2009; Smith et al., 2013).

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 2 years (Armand-Lefevreet et al., 2005; Huijsdens et al., 2006; Voss et al., 2005).

Methicillin-resistant Staphylococcus aureus (MRSA) has become a major nosocomial pathogen, highly prevalent in many European countries and throughout the world (Anon, 2009).

S. aureus are normal inhabitants of pigs, and occur in all herds (Armand-Lefevre et al. 2005).The prevalence of MRSA in pig herds varies widely (0 to 50%) among European countries (Anon, 2009). The pig herd prevalence of MRSA in North America is uncertain, but appears lower than in many European countries (Smith et al. 2013; Weese et al. 2011). MRSA prevalence is high (>50%) in pigs in positive herds, but has minimal effect on swine health (Smith et al., 2013).

The capacity of *S. aureus*/MRSA strains of livestock origin to colonize, spread, and cause disease in humans remains uncertain (Smith et al., 2013).

MATERIALS AND METHODS

The research was conducted in the Laboratory of Bacteriology, Department of Infectious Diseases and Preventive Medicine (Faculty of Veterinary Medicine Timisoara). Samples were taken from clinically healthy pigs from a swine farm in a village in the county of Arad during October-December 2013.

Samples required bacteriological examinations were collected from a total of 87 clinically healthy pigs (sows with piglets in the maternity, pregnant sows - breeding sector, weaners and fat pigs), are represented by samples from different anatomical areas: ear, skin, perianal and genital. Samples were collected using sterile cotton wool pads secured to the plastic rod and placed in sterile tubes (standard product). After sampling, the samples were refrigerated until bacteriological examination performance. Pathological materials were plated on agar with 5% defibrinated sheep blood poured into Petri dishes. *Staphylococcus* strains isolated and purified were tested on biochemical and pathogenic characters.

Mannitol fermentation tested on Chapman Agar (*Staphylococcus* Selective Agar, Liofilchem, Italy). Testing fermentation about other sugars, including maltose, was made using Api Staph multi test systems.

Isolates were identified as species using Api Staph system.

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-related, used was Prolex STAPH Latex rapid kit produced by Pro-Lab Diagnostics. Free diffusible coagulase was highlighted from technique in tubes using four types of citrated plasma. To test the sensitivity to antibiotics, staphylococci strains isolated was used Kirby-Bauer disc diffusion method, using the following ingredients: Mueller-Hinton broth and agar, Petri dishes and bio discs impregnated with antibiotics (Oxoid). The following antibiotics were used: methicillin, ampicillin with sulbactan, amoxicillin with clavulanic acid, tetracycline, doxycycline, gentamicin, kanamycin, erythromycin, vancomycin, ciprofloxacin, polymyxin B, novobiocin, rifampicin, pristinamycin, lincomycin, ceftrioxone, cefoxitine and cefaclor. This has been tested staph sensitivity, aiming types of resistant strains and methicillin resistance frequency.

RESULTS AND DISCUSSION

In this study were isolated 28 strains of staphylococci, including 20 coagulase positive strains (CoP, represented by *S. hyicus* and *S. aureus*) and eight coagulase negative strains (CON, represented by *S. haemolyticus*, *S. sciuri* respectively *S. epidermidis*) isolated from pigs in different anatomical areas. Bacteriological examination results are shown in Table 1.

Table 1.

The distribution of strains of staphylococci isolated from pigs

Age category/anatomical area	Number of samples	Positive samples	Strains of staphylococci isolated				
			S. aureus	***S. hycus***	***S. epidermidis***	***S. sciuri***	***S. haemolyticus***
Sows with piglets							
skin-nipples	18	5 (27,77%)	2	2	-	-	1
Peri vulvar	10	3 (30,0%)	1	1	-	1	-
Pregnant sows							
skin-peri vulvar	11	2 (18,18%)	-	1	-	-	1
nipples	9	3 (33,33%)	2	1	-	-	-
Weaned piglets							
Skin-ears	18	7 (38,88%)	3	2	-	1	1
Fat pigs							
skin	21	8 (38,09%)	1	4	1	1	1
TOTAL	**87**	**28 (32,18%)**	**9**	**11**	**1**	**3**	**4**

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 resistance phenomenon.

The results of antibiotic susceptibility testing of *Staphylococcus*strains isolated from pigs are shown in Table 2.

Table 2.

The results of the sensitivity to antibiotics of *Staphylococcus* strains isolated from pigs

Name of antimicrobial substance (initials-MIC)	Number of susceptible isolates				
	S. hyicus (n=11)	*S. aureus* (n=9)	*S. haemolyticus* (n=4)	*S. sciuri* (n=3)	*S. epidermidis* (n=1)
Methicillin - ME - 30µg	9	7	4	3	1
Gentamicin – CN – 10µg	5	6	4	3	1
Tetracycline – TE – 30µg	4	5	2	3	1
Ciprofloxacin – CIP – 30 µg	11	9	4	3	1
Kanamycin – K – 30 µg	8	7	4	3	1
Novobiocin – NV – 30 µg	11	9	4	3	1
Doxycycline – DO – 30 µg	7	7	1	1	1
Erytromycin – E – 15 µg	6	6	1	1	1
Vancomycin – VA – 30 µg	11	9	4	3	1
Ceftriaxone – CRO – 30 µg	11	9	4	3	1
Cefoxitine – FOX - 10µg	11	9	4	3	1
Polymyxin – PB – 50UI	0	0	1	1	0
Rifampicin – RA – 30 µg	11	9	4	3	1
Lincomycin – L – 30 µg	5	4	2	3	1
Cefaclor – CEC – 30 µg	11	9	4	3	1
Pristamycin – PT – 15 µg	11	9	4	3	1
Ampicillin/sulbactan – SAM – 30 µg	11	9	4	3	1

Were monitored during the study the phenomenon of multiple resistances, namely resistance type frequency methicillin-resistant strains of a number of 28 strains of staphylococci isolated from pigs using 17 different classes of antibiotics (Table 3).

Table 3

The resistance type of the species of staphylococci isolated from pigs

Species of stafilcoci	Resistant strains	The antimicrobial resistances models (no. of resistant strains)
Staphylococcus hycus	6/11	TE, K, DO, E, **ME**, PB (1), K, PB, TE, L (1), TE, PB, L, DO (1), PB, DO, E, L, **ME** (1), PB, TE, DO, E, L, CN (2)
Staphylococcus aureus	4/9	TE, CN, L, K, **ME** (1), CN, DO, E, PB (1), TE, CN, L, K, PB (1), CN, PB, E, L, **ME** (1)
Staphylococcus haemolyticus	2/4	CN, TE, E, L, K, PB (1), CN, PB, E, DO (1)
Staphylococcus sciuri	1/3	TE, CN, E, PB, L (1)
Staphylococcus epidermidis	1/1	PB (1)

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

In the case of antibiotics: novobiocin, rifampicin, pristinamycin, ciprofloxacin, vancomycin, ceftriaxone, cefoxitin, cefaclor and ampicillin/sulbactan, considered the drug of choice for staphylococci, the number of sensitive strains were 100%, all isolates were sensitive (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 is kit from a staphylococcal infections or typically used in humans, in the treatment of this infections in animals, respectively.

β-lactams (methicillin, ceftriaxone, cefoxitin, cefaclor and ampicillin with sulbactan), sensitivity was highest, except *Staphylococcus aureus*, which were isolated 4 methicillin resistant strains. Of these two methicillin resistant strains of *S. hycus* and two strains of *S. aureus* (Table 3). The strains tested were mostly sensitive to β-lactams other as 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 (King et al. 2006; Kluytmans et al. 2006; Vandenesch et al. 2003; Weese et al. 2011; Wulf et al. 2006).

Aminoglycosides (gentamicin, kanamycin) and macrolides (erythromycin and vancomycin), sensitivity was different, the maximum to vancomycin (Table 2). In the case of gentamicin, were isolated 9 resistant strains, 5 strains resistant to kanamycin and 9 strains resistant to erythromycin (Table 3).

Most of the strains were resistant to polymyxin B (13 strains), due to an excessive use of this antibiotic in the past (Table 3).

Sensitivity to tetracyclines (tetracycline, doxycycline) was reduced, 15 strains were resistant to this group of antibiotics, to the phenomenon of resistance is plasmid and chromosomal type (9 strains to tetracycline and 6 strains to doxycycline) (Tables 2, 3).

All strains tested were sensitive to ciprofloxacin.

The development of staphylococci resistance to different antibiotics is a consequence of wasteful use in the treatment of diseases in pigs. Antibiotics used irrationally creates a selection pressure, are 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 (Gibbs et al. 2006; Guardabassi et al. 2006; Smith et al. 2013).

After testing staphylococci strains isolated from pigs against 17 antibiotics identified methicillin-resistant strains and various types of resistance against β-lactams, tetracyclines, macrolides and polymyxin B.

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 (Gibbs et al. 2006; Guardabassi et al. 2006; Smith et al. 2013).

S. aureus is an epiphyte, a normal microorganism in pigs, and occurs in all herds (Armand Lefevre et al. 2005). The prevalence of MRSA strains in pig herds varies widely (0-50%) among European countries (Anon, 2009). Actual prevalence of MRSA in pigs in North America is uncertain, but appears to be lower than in many European countries (Smith et al. 2013; Wertheim et al. 2004). 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 (Gibbs et al. 2006), and healthy people working in these farms and shelters, often are carriers of *S. aureus* from pig nasal mucosa (Armand Lefevre et al. 2005; Khanna et al. 2008; Smith et al. 2013; Voss et al. 2006; Wan Mintao et al. 2013). 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) (Bode et al. 2011; Gorwitz et al. 2008).

The risk of exposure to MRSA from animals is largely restricted to persons who have direct contact with animals and their families, respectively (Bisdorff et al. 2012; Cuny et al. 2009; Van Cleef et al. 2010).

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 for months or years without developing infections (Frana et al. 2013; Graveland et al. 2011; Sun et al. 2013; Van Cleef et al. 2011; Verkade et al. 2013). In Dutch hospitals was identified ST398 line spread between people 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

In one swine farm were isolated 28 *Staphylococcus* strains including 20 coagulase positive strains (CoP, represented by *S. hyicus* and *S. aureus*) and eight coagulase negative strains (CON, represented by *S. haemolyticus*, *S. epidermidis*, respectively *S. sciuri*) from clinically healthy pigs in different anatomical areas.

All *Staphylococcus* strains isolated from pigs showed sensitivity of 100% for antibiotics: novobiocin, rifampicin, pristinamycin, ciprofloxacin, vancomycin, ceftriaxone, cefoxitin, cefaclor and ampicillin/sulbactan, considered the drug of choice for these bacteria.

β-lactams (methicillin, ceftriaxone, cefoxitin, cefaclor, and ampicillin with sulbactan) sensitivity was highest, except *Staphylococcus aureus*, which were isolated four methicillin-resistant strains, two of *S. hycus* and two of *S . aureus* strains.

After testing staphylococci strains isolated from pigs, against 17 antibiotics, were identified methicillin-resistant strains and more type of resistances, against β-lactams, tetracyclines, macrolides and polymyxin B.

REFERENCES

Anon. 2009. 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 Journal, 7(11), 1376.

Armand-Lefevre L., Ruimy R., Andremont A., 2005. Clonal comparison of Staphylococcus aureus isolates from healthy pig farmers, human controls, and pigs. Emerg. Infect. Dis., 11(5), 711-714.

Bisdorff B., Scholhölter J.L., Claussen K., Pulz M., Nowak D., Radon K., 2012. MRSA-ST398 in livestock farmers and neighbouring residents in a rural area in Germany. Epidemiol. Infect., 140(10), 1800-8.

Bode L.G., Wertheim H.F., Kluytmans J.A., Bogaers-Hofman D., Vandenbroucke-Grauls C.M., 2011. Sustained low prevalence of meticillin-resistant Staphylococcus aureus upon admission to hospital in The Netherlands. J. Hosp. Infect., 79, 198-201.

Cuny C., Nathaus R., Layer F., Strommenger B., Altmann D., Witte W., 2009. Nasal colonization of humans with methicillin-resistant Staphylococcus aureus (MRSA) CC398 with and without exposure to pigs. PLoS ONE. 4(8): e6800.

Frana T.S., Beahm A.R., Hanson B.M., Kinyon J.M., Layman L.L., Karriker L.A., Ramirez A., Smith T.C., 2013. Isolation and characterization of methicillin-resistant Staphylococcus aureus from pork farms and visiting veterinary students. PLoS One. 8(1), e53738.

Gibbs S.G., Green C.F., Tarwater P.M., Mota L.C., Mena K.D., Scarpino P.V., 2006. Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation. Environ. Health Perspect., 114, 1032-1037.

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., 2008. Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001-2004. J. Infect. Dis., 197(9), 1226-1234.

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

Guardabassi L., Courvalin P., 2006. Modes of antimicrobial action and mechanisms of bacterial resistance, În Antimicrobial resistance in bacteria of animal origin. Ed. ASM Press, Washington, D.C., p. 1-18.

Khanna T., Friendship R., Dewey C., Weese J.S., 2008. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. Vet. Microbiol., 128, 298-303.

King M.D., Humphrey B.J., Wang Y.F., Kourbatova E.V., Ray S.M., Blumberg H.M., 2006. Emergence of community-acquired methicillin-resistant Staphylococcus aureus USA 300 clone as the predominant cause of skin and soft-tissue infections, Ann. Intern. Med., 144, 309–317.

Kluytmans-Vandenbergh, M.F., Kluytmans J. A., 2006. Communityacquired methicillin-resistant Staphylococcus aureus: current perspectives, Clin. Microbiol. Infect., 12 (suppl 1), 9–15.

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., 2013. Methicillin-resistant Staphylococcus aureus in pigs and farm workers on conventional and antibiotic-free swine farms in the USA. PLoS ONE, 8(5), e63704.

Sun J., Linhares L., Yang M., Sreevatsan S., Davies P., 2013. 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.

Van Cleef B.A., Graveland H., Haenen A.P., Van De Giessen A.W., Heederik D., Wagenaar J.A., Kluytmans J.A., 2011. Persistence of livestock-

associated methicillin-resistant Staphylococcus aureus in field workers after short-term occupational exposure to pigs and veal calves. J. Clin. Microbiol., 49, 1030-1033.

Van Cleef B.A., Verkade E.J., Wulf M.W., Buiting A.G., Voss A., Huijsdens X.W., 2010. Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands. PLoS ONE, 5,e9385.

Vandenesch F., Naimi T., Enright M.C., 2003. Community acquired methicillin-resistant Staphylococcus aureus carrrying the Panton–Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis., 9, 978–984.

Verkade E., Van Benthem B., Den Bergh M.K., Van Cleef B., Van Rijen M., Bosch T., Kluytmans A., 2013. Dynamics and determinants of Staphylococcus aureus carriage in livestock veterinarians: a prospective cohort study. J.Clin. Infect. Dis., 57(2), e11-7.

Voss A., Loeffen F., Bakker J., Wulf M.W., 2006. Methicillin-resistant Staphylococcus aureus in pig farming. Vet. Microbiol., 14, 65-68.

Voss A., Loeffen F., Bakker J., Wulf M., Klaassen C., 2005. Methicillin-resistant Staphylococcus aureus in pig farming. Emerg. Infect. Dis., 11, 1965–1966.

Wan Mintao, Lauderdale T.L., Chou Chincheng, 2013. Characteristics and vrirulence factors of livestock associated ST9 methicillin-resistant Staphylococcus aureus with a novel recombinant staphylocoagulase type. Veterinary Microbiology, 162, (24), 779-784.

Weese J.S., Rousseau J., Deckert A., Gow S., Reid-Smith R.J., 2011. Clostridium difficile and methicillin-resistant Staphylococcus aureus shedding by slaughter-age pigs. BMC Vet. Res., 7, 41.

Wertheim H.F., Vos M.C., Boelens H.A., 2004. 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., 56, 321–325.

Wulf M., Van Nes A, Eikelenboom-Boskamp A., 2006. Prevalence of methicillin-resistant Staphylococcus aureus in veterinary doctors and students, The Netherlands. Emerg. Infect. Dis., 12, 1939–1941.