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Antimicrobial Resistance in Isolates From Sow Nares From Comercial Farms in Methicilllin Resistant Staphylococci (Mrs) And Expended Spectrum Beta Lactamate Antibiotics (Esbl) in Enterobacteriacea Models

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ABSTRACT

Spreading of antimicrobial resistance is a great problem in human and animal medicine. Rising in occurrence in methicillin-resistant Staphylococcus spp. (MRS) as well as expended spectrum beta lactamate antibiotics resistance (ESBL) in animals may be potential danger for farm workers in disease spread. Nasal and rectal swabs were taken from 53 sows without clinical symptoms on two farms in Serbia. Nasal swabs were tested on MRS and rectal on ESBL. Laboratory test showed 1 ESBL isolate (1.9%) and 18 (35.85%) MRS of which 3 (15.8%) Staphylococcus aureus and 7 (36.85%) Staphylococcus epidermidis. Seven of nineteen random MRS isolates were put on disc diffusion method. None of them showed resistance to vankomycine (VRSA) and fusidic acid.

KEYWORDS sows, pig, MRSA,

Introduction

Bacterial antimicrobial resistance is a significant concern in human and veterinary medicine. Introduction of antimicrobial drugs and antibiotics in medicine expended life expectancy in mankind. In USA probability for an adult person to die from bacterial infection was 1% during one year period in 1900, and on year 2000 probability for adult person to die from bacterial infection is 1% during 15 years period (Guilfolie 2007).

Occurence of Meticillin resistant *Staphylococcus spp.* (MRS) and meticillin resistant *Staphylococcus aureus* (MRSA) occurrence is growing and ranges from 0 to 45% (Tiemersma, et al. 2004). Many reports of occurring bacterial resistance were made in pig breeding farms, and transfer MRSA from animal host to humans also have been reported (Huijsdens, et al. 2006) (Faires, et al. 2010). In food research, MRSA have also been found (Lozano, Lopez, Gomez-Sanz, Ruiz-Larrea, Torres, & Zarazaga, 2009) which implicates importance of MRS and MRSA testing in live animals.

Production of penicillin binding protein (PBP2a), encoded by *mecA* gene which resides on a mobile genetic element called a staphylococcal chromosomal cassette (SCCmec) makes MRS isolates resistant to beta-lactamate antibiotics (T. Khanna, R. Friendship, C. Deweya & J. Weese, 2007).

Production of β-Lactamase enzymes is another mechanism of bacterial resistance to β-lactam antibiotics. Among genera of the *Enterobacteriaceae* family only *Salmonella* lacks a structural gene for β-Lactamases (Medeiros & Antonione 1997). Number of ESBL isolates is rising and represent risk in human health and as well as zoonotic risk. (DARC & ARHAI, 2011) (Dohmen, et al., 2012).

In Serbia in year 2010 in 84 pig nasal samples, 6 MRSA were found (Velebit, Miriloviβ, Teodoroviβ, Fletsch, & Jovanoviβ, 2010). Transfer from pigs to humas varies. Pig farming associated MRSA isolates rate varies from 0.13% to 11,5% (Faires, et al. 2010).

Aim of this article is to show results of preliminary analysis of antimicrobials resistance in bacterial isolates form sows with no clinical signs. For analysis we took two models: meticillin resistant *Staphylococcus spp.* (MRS) and expended spectrum β lactamate antibiotics (ESBL) in common *Enterobacteriaceae*.

Materials and method

Nares and rectum swabs were taken from sows on 2 commercial pig farms. Both farms use pen boxing system. Both nasal and rectal swabs were taken from same animals. On farm A we took 24 and from farm B, 29 swabs.

Rectal swabs were put during the same day as sampling on MacConkey agar (Becton, Dickinson and Company USA), and incubated aerobically on 37°C. Preliminary diagnostic were done after 24 hours. Bacteria of *Enterobacteriaceae* family (bacillary forms, gram stain negative, catalase positive and oxidise test negative) (Becton, Dickinson and Company - BD USA) were tested to imipenem resistance and ESBL according to CLSI (Clinical and Laboratory Standards Institute 2006) recommendations. Following antibiotics were used ceftazidime (30 µg), cefotaxime (30 µg), ceftazidime with clavulonic acid (30/10 µg), cefotaksime with clavulonic acid (30/10 µg) and imipeneme (10 µg) (BD BBL Sensi-Disc Antimicrobial Susceptibility Test Discs, BD USA)

Nasal swabs were put on MRSA agar (Bio-Merieaux, France) during sampling day and incubated aerobically on 37°C. After 24h period colonies were examined, preliminary identification of *Staphylococcus spp.* were done by Gram stain, coagulasis test in tubes (Veterinarski zavod, Zemun, Serbia), and haemolysis test on Columbia blood agar (COB Bio-Merieaux,

France) after aerobically incubation on 37°C for 24 hours for. Seven colonies of *Staphylococcus spp.* were randomly selected for disc diffusion method with following antibiotics: penicillin (10IU), cefoxtin (30 µg), oxacillin (1 µg), sulfamethoxazole with trimethoprim (SXT) (23, 75/1, 25 µg), neomycin (30 µg), amoxicillin with clavulanic acid (AMC) (20/10 µg), gentamicin (10 µg), erythromycin (15 µg), fusidic acid (10 µg), vancomycin (30 µg), ciprofloxacin (5 µg) and azithromycin (15 µg) according to CLSI (Clinical and Laboratory Standards Institute 2006). All *Staphylococcus aureus* isolates were confirmed using BBL Gram positive kit (BD USA).

Results

On 53 samples ESBL test on rectal swabs showed one positive isolate (1.9%) and it was from farm A. *Staphylococcus spp.* were presumably found in 18 swabs (35,85%) , where 3 of them (15.78%) are presumably identified as *Staphylococcus aureus* and 7 (36,85%) as *Staphylococcus epidermidis*. Other 8 (42,1%) *Staphylococci* are identified as other Staphlococci or *Staphylococcus spp.* Results of disc diffusion method (in mm) are shown in table 1.

Table 1: results of diffusion method of 7 randomly selected MRS (in mm), grey fields show resistance, white fields show susceptible and intermediate decisions by rules of CLSI protocol

Isolate/ Antibiotic	1	2	3	4	5	6	7
Penicillin	15	15	12	0	16	0	28
Cefoxitin	10	12	8	12	13	15	12
Oxacillin	15	0	0	0	0	0	0
SXT	20	18	8	0	13	9	10
Neomycin	0	0	0	0	0	0	0
AMC	23	0	17	13	20	24	25
Gentamicin	0	0	0	0	0	0	0
Eriyromycin	0	20	0	0	0	0	0
Fusidic acid	30	29	30	39	30	30	23
Vancomycin	20	21	21	19	24	22	15
Ciprofloxacin	12	12	12	11	10	15	8
Azithromycin	0	17	0	0	0	0	0

Discussion

This is small scale, pilot study of presence antimicrobial resistance in sows. On one farm both MRS and ESBL models have been proven, and on other only MRS. Both resistance models have been found in low levels.

Incidence of ESBL is lower than reported in Switzerland pig farms, where it goes to (15.3%) in faecal samples (N. Geser, R. Stephan & H. Hächler, 2012). On Dutch pig farms 55% is ESBL positive (Dohmen, et al., 2012),. These results are much higher than we could suggest in our research.

Our results show lower incidence of MRSA than in Canadian pigs (25%) without significance in maturity age (T. Khanna, R. Friendship, C. Deweya & J. Weese, 2007) also our results are lower than MRSA testing in Holland (36%) (Neeling et al 2007). Low prevalence of MRSA shown by Velebit (Velebit, Mirilović, Teodorović, Fletsch, & Jovanović, 2010) fin Serbia of 7% is according to our results. Very high prevalence shown by Broens of up to 81% (Broens n.d) is not suspected in Serbia. Our results on 2 pig farms may be considered near results on wildlife studies as in wild boars 8 isolates of MRSA are retrieved from 117 samples in Germany, but none was carrying CC398 type which is associated to pigs in industrial farming (Meemken, et al., 2012 in print). MRSA in humans are lower in Serbia than European average (Jovanović & Cirković 2011) or occurence in India (INSAR 2013)

On 7 random selected staphylococci, disk diffusion zones of inhibition showed susceptibility to vankomycin, fusidic acid and azithromycin. Resistance to penicillin was expected regarding the MRS nature of isolates. Two of seven isolates showed resistance to amoxicillin with clavulonic acid and four of them to sulfametoxasole with trimetoprim. Total resistance to neomycin and gentamicin was expected as these antibiotics are in wide use in Serbia. Similar to these susceptibility of one MRS isolate to eritromycin could be considered exception. Those founding are somewhat similar to previously reported (Neeling at al. 2007)

Conclusion

This study show low ESBL and low MRS level in sows in Serbia. However this sampling population is narrow and more on precise study requires for larger sample material as well as more tests like microdilution method, PCR, MLST, and spa analysis as well as widening test population to all categories and not exclusively to sows. These results should be supplemented in future by analysis of SCCmec regions in MRS as well as typing on ESBL positive isolate to determine relation between pig origin of bacterial isolate and origins of antimicrobial resistance genes (i.e. Human or animal).

These resistance mechanisms show necessity for testing carriage of resistance mechanisms in live animals as possible spread centre for food and farm workers.

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