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Survey of antimicrobial susceptibility among some pathogenic and commensal bacteria isolated from pigs in Lithuania

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Summary

Antimicrobial susceptibility of isolated enterotoxigenic E. coli, Salmonella enterica serovar Choleraesuis, Pasteurella multocida and Streptococcus suis from pigs was tested in the Veterinary Institute of the Lithuanian Veterinary Academy. Commensal E. coli and Enterococcus faecalis were also included in the testing as commensal bacteria. Clinical and pathological material was investigated from various regions of the country. Isolation and identification of bacteria was done using common methods. The agar diffusion method according to NCCLS guidelines was applied for antimicrobial susceptibility testing. Enterotoxigenic E. coli showed the highest resistance to tetracycline (67%), ampicillin (52%) and sulfamethoxazole-trimethoprim (43%). Not less than 90% of these bacteria were susceptible to colistin, florfenicol and ceftiofur. Salmonella Choleraesuis demonstrated the highest resistance to tetracycline (53%). Florfenicol, ceftiofur and enrofloxacin were effective against most strains of salmonella. Pasteurella multocida in most cases were susceptible to all the tested antimicrobials, however 20% of the isolates were resistant to sulfamethoxazole-trimethoprim. Streptococcus suis demonstrated the highest resistance to tetracycline (43%), lincomycin (40%), sulfamethoxazole-trimethoprim (40%), and erythromycin (30%). Ceftiofur was the most effective against S. suis. Commensal E. coli showed less resistance than enterotoxigenic E. coli, however not less than 25% of isolates were resistant to tetracycline, sulfamethoxazole-trimethoprim and ampicillin. All the tested Enterococcus faecalis were susceptible to vancomycin and ceftiofur and 80% of enterococci were resistant to tetracycline.

Keywords: antimicrobials, antimicrobial susceptibility, resistance

The resistance of bacteria to antimicrobials has become one of the most important topics both in human and animal medicine. According to EU Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, Member States must implement a monitoring programme that provides comparable data on the occurrence of antimicrobial resistance in zoonotic agents and, in so far as they present a threat to public health, other agents. A number of countries have national surveillance programmes to assess bacterial susceptibility to antimicrobials among zoonotic and commensal bacteria isolated from healthy and sick animals. However there were no sufficient data on the susceptibility of zoonotic and commensal bacteria isolated from animals in Lithuania, as there were no national surveillance programmes on the antimicrobial susceptibility. With the aim to fill this gap some investigations were implemented in Lithuanian Institute of Lithuanian Veterinary Academy.

Pork production is a traditional branch of the husbandry in Lithuania. About one million pigs are breeding in the country every year. A big concentration of animals and intensive production is one of the reasons on spreading highly transmissible diseases. Diagnostics of some infectious diseases of pigs in more cases are complicated. There are the reasons that usage of broad-spectrum antimicrobials to treat animals and also to use some of them, as growing promoters in pork production are more intensive than in other branches. Antimicrobials usually are used by preventive tasks even from the first days of the piglets birth. By these reasons surveillance on antimicrobial susceptibility on a regular basis in pig farms must be implemented.

The aim of the investigations was to determine antimicrobal susceptibility of some pathogenic and commensal bacteria isolated from pigs in Lithuania.

Material and methods

Investigations were carried out in Veterinary Institute of Lithuanian Veterinary Academy, Department of Microbiology and Food Safety. *Salmonella enterica*, enterotoxigenic *Escherichia coli*, *Pasteurella multocida*, and *Streptococcus suis* were tested as pathogenic bacteria. Pathological material was delivered directly to laboratory from all counties. For initial isolation of bacteria, Tryptone Soy Agar, (BBL, England) and analogous media were used. For isolation of *Salmonella enterica* pre-enrichment (Buffered Peptone Water, Oxoid, England) and enrichment media (Rappaport Vassiliadis Media, Oxoid England) were used. Some needful supplements

(such as blood or sera) were used for isolation of fastidious microorganisms.

Commensal bacteria (non pathogenic *E. coli* and *Enterococcus faecalis*) were investigated with the aim to rate common situation of antimicrobial susceptibility due to the Draft monitoring programme on the occurrence of antimicrobial resistance according to EU Directive 2003/99/EC. Samples were collected directly in farms from anus of healthy animals using sterile swabs. Mac Conkey Agar (BBL, USA) was used for isolation of *E. coli.* For isolation of enterococci Slanetz Bartley Agar (Liofilchem, Italy) and Bile Aesculine Agar (Liofilchem, Italy) were used. Investigations were carried out in pursuance of principles and limitations of monitoring antimicrobial resistance among food animal (2).

Identification of isolated bacteria was done using biochemical tests (Crystal, BBL, USA). *Escherichia coli* and *Salmonella enterica* were serotyped by drop on glass or latex agglutination test with commercial sera. For *Salmonella* typing SIFIN (Germany), for *E. coli* – Sanofi Diagnostics Pasteur (France), Oxoid (England) and Bundesinstitut Veterinärmed (Germany) sera were used.

Agar diffusion method according to NCCLS guidelines was applied for antimicrobial susceptibility testing. Mueller Hinton Agar (Oxoid, England) was used as testing media. Turbidometer MSI-5 (Latvia) was used for determination of optical turbidity. 60 isolates of *Salmonella enterica*, 60 isolates of enterotoxigenic *E. coli* (ETEC), 30 isolates of *Pasteurella multocida* and 30 isolates of *Streptococcus suis* were tested for antimicrobial susceptibility. 60 isolates of non-pathogenic *E. coli* and 60 isolates of *Enterococcus faecalis* were tested for antimicrobial susceptibility as commensal bacteria. Antimicrobials were selected according to the guidelines of the Draft Monitoring programme on the occurrence of antimicrobial resistance (2004). The following disks (BBL, USA) were used: ampicillin (10 µg), tetracycline (30 µg), gentamicin (10 µg), neomycin (10 µg), nalidixic acid (30 µg), enrofloxacin (10 µg), ceftiofur (30 µg), sulfamethoxazole/trimethoprim (23.75 µg + 1.25 µg), florfenicol (10 µg) and colistin (10 µg). Results were interpreted by special tables of manufacturer. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis* ATCC 29212 were used as control microorganisms.

Results and discussion

Bacteriological investigations revealed that *Escherichia coli*, *Salmonella* spp., *Streptococcus* spp. and *Pasteurella multocida* are one of the most spread pathogenic bacteria in Lithuanian pig farms. In total 300 isolates were tested. At the period of investigations (2000-2004) 76 strains of enterotoxigenic *E. coli* that contained antigens K88, K99 and 987P were isolated. Susceptibility to antimicrobials of these bacteria is shown in tab. 1. *E. coli* was the most spread species of *Enterobacteriaceae* family in pig herds. This species appears to be more resistant and adapts in various unfavourable conditions. Sixty seven percent of all tested enterotoxigenic *E. coli* were resistant to tetracyclines. Fifty two percent were resistant to broad-spectrum penicillins (ampicillin) and 43% – to sulfonamide and trimethoprim combination. Enterotoxigenic *E. coli* were less resistant to colistin, ceftiofur and florfenicol. High resistance of enterotoxigenic *E. coli* is confirmed and by other authors. For example in England and Wales up to third isolated *E. coli* from pigs are multiresistant (16). In Canada almost all (93%) of tested isolates were resistant to tetracycline, and a similar number (91%) were resistant to sulphonamides. The rates of resistance to ampicillin, neomycin, kanamycin, chloramphenicol, and trimethoprim ranged from 21 to 38%, whereas only 14% of the isolates were resistant to gentamicin (17). Studies of ETEC resistance in Canada by other authors showed the similar results (12).

Tab. 1. Susceptibility of enterotoxigenic *E. coli* to antimicrobials (n = 60)

Antimicrobials	Susceptibility					
	Susceptible	%	Intermediate	%	Resistant	%
Ampicillin	26	43	3	5	31	52
Tetracycline	17	28	3	5	40	67
Neomycin	47	78	5	8	8	13
Florfenicol	57	95	0	0	3	5
Ceftiofur	56	93	2	3	2	3
Enrofloxacin	52	87	2	3	6	10
Colistin	59	98	0	0	1	2
Trimetho/Sulfa	30	50	4	7	26	43

Tab. 2. Susceptibility of *Salmonella* Choleraesuis to antimicrobials (n = 60)

Antimicrobials	Susceptibility					
	Susceptible	%	Intermediate	%	Resistant	%
Ampicillin	35	58	5	8	20	33
Tetracycline	22	37	6	10	32	53
Nalidixic acid	38	63	2	3	20	33
Florfenicol	58	97	0	0	2	3
Ceftiofur	55	92	3	5	2	3
Enrofloxacin	52	87	3	5	5	8
Colistin	48	80	2	3	10	17
Trimetho/Sulfa	45	75	6	10	9	15

Salmonella spp. was common among other pathogenic bacteria, however prevalence of these bacteria had tendency to decrease (23). The most common serovar among *Salmonella enterica* was *Salmonella* Choleraesuis. In Lithuanian pig farms other serovars are uncommon but other epidemiologically important serovars such as *S.* Enteritidis and *S.* Typhimurium are found in other species of animals. Susceptibility of *Salmonella* Choleraesuis is shown in tab. 2. *Salmonella enterica* is one of the most important zoonotic bacteria that often can be characterized by high resistance to antimicrobials (4, 13, 22, 24). Our investigations showed that 53% of *S. enterica* serovar Choleraesuis isolates were resistant to tetracyclines and one-third part of them – to ampicillin and nalidixic acid. Quinolones became one of the most popular antimicrobials used for treating animals and poultry; reduced susceptibility to these compounds are observed worldwide and became serious problem both in human and veterinary medicine (14, 18). Reduced susceptibility of salmonella was observed against enrofloxacin that shows spreading of appropriate genetic structures encoding resistance to fluoroquinolones between bacteria (14). The situation according to resistance of *Salmonella* in different countries differs from the

source of isolation and from serotypes. For example in Lithuania highest resistant demonstrates *Salmonella* that are isolated from pigs (23). In some countries highest resistance demonstrates *Salmonella* isolated from cattle or poultry (4, 13). Multiresistant isolates of *Salmonella* were isolated in Lithuania as and in other countries (data not showed) (23).

Pasteurella multocida is one of the most important agents that causes respiratory diseases, especially in association with other infectious agents, such as *Mycoplasma* spp. Capsulotypes A and D of these bacteria are found in the farms of Lithuania. In this study, toxigenic *Pasteurella multocida* of both capsulotypes were tested for antimicrobial susceptibility. Results are shown in tab. 3. *Pasteurella multocida* as and the other species of family *Pasteurellaceae* is conformist bacteria enable to survive for a long time only in live organisms. By this reason they are well adapted to undergo unfavourable factors and to survive. Our results showed that *Pasteurella multocida* were comparatively susceptible to all classes of antimicrobials. Exception was sulfamethoxazole-trimethoprim – even 20% strains of *P. multocida* were resistant to this combination of antimicrobials. Results obtained in other countries shows that most strains of *P. multocida* is still susceptible to various antimicrobials. For example in Canada findings for *Pasteurella multocida* isolated from the respiratory tract of pigs and cattle reveal resistance in less than 7% of the isolates to many antimicrobials tested, such as ampicillin (0%), ceftiofur (< 1%), and the trimethoprim/sulfamethoxazole combination (1-6%) (9, 20). On the other hand, resistance to tetracycline was greater than 15% (20). In the UK 20% of *Pasteurella multocida* isolated from pigs were resistant to neomycin, 8% to tetracycline, 9% to sulfamethoxazole-trimethoprim and only 3% – to ampicillin. No resistant strains were isolated to enrofloxacin (6, 7). However there are some countries where resistance of *P. multocida* to some antimicrobials are higher. For example in Spain 90% of isolated *Pasteurella multocida* (capsulotype A) were resistant to streptomycin and 60% – to lincomycin (25). *Pasteurella multocida* is comparatively susceptible to various unfavourable conditions, however their mechanism of adaptation differ from other bacteria – this species like and other related species can survive in organisms by their localization (outermost from blood vessels that antimicrobial can not reach them) or surviving in certain blood cells (macrophages).

Streptococcus suis are one of the most important bacteria among streptococcal infections in pigs (15). There are no serological data about *S. suis* serotypes prevalence in Lithuania, however both biotypes are isolated. The results of susceptibility to antimicrobials of *S. suis* are shown in tab. 4. *Streptococcus suis* showed the highest resistance to tetracyclines (43%), lincomycin (40%), sulfonamide-trimethoprim combination (40%) and erythromycin (30%). Thirteen percent of isolates were resistant to enrofloxacin. It should be noted that *S. suis* showed resistance to all tested antimicrobials. These results are in accordance with many other studies that have described *S. suis* as mainly susceptible to these antimicrobial agents. Resistance to macrolides/lincosamides and tetracyclines described in this study has also been described in the literature to varying degrees in different countries. In France, Morvan reported that 19% of 400 *S. suis* strains were susceptible to spiramycin, 38% to lincomycin and 18% to tetracycline (19). In the United Kingdom 94% of isolated *S. suis* were resistant to tetracycline (7). In Italy *S. suis* were less susceptible to oxytetracycline, sulfamethazine and tilmicosin (5). In Spain, Reams *et al.* reported that 33%, 32% and 19% of *S. suis* strains were susceptible to erythromycin, clindamycin and tetracycline, respectively (21). In Denmark, 20% of *S. suis* strains, isolated from 1995 to 1997, were resistant to erythromycin, spiramycin, tylosin and lincomycin and 44% to tetracycline (3). In Brasil *S. suis* were less susceptible to tetracycline and trimeto/sulfa drugs (10). These high rates of resistance to macrolides/lincosamides and to tetracyclines might be explained by intensive use of tylosin (growth promoter) and tetracycline (therapeutic) in pig production (3).

Susceptibility of commensal *E. coli* and *Enterococcus faecalis* is shown it tab. 5 and tab. 6 respectively. Resistance of commensal *E. coli* was similar as to enterotoxigenic *E. coli*. However less number of isolates was resistant to all antimicrobials, especially to ampicillin (25%). There were no resistant isolates to ceftiofur and colistin among non-pathogenic *E. coli*. Only one strain was resistant to enrofloxacin and two strains were resistant to florfenicol and neomycin. Investigations in other European countries show that resistance of commensal *E. coli* may very differ. For example in Sweden only 3.4% of isolated *E. coli* was resistant to ampicillin, however in Spain more than a half of isolates were resistant to this antibiotic. Resistance to fluoroquinolones (ciprofloxacin) in Spain was more than 50%, however in Germany, Denmark and Sweden only

Tab. 3. Susceptibility of *Pasteurella multocida* to antimicrobials (n = 30)

Antimicrobials	Susceptibility					
	Susceptible	%	Intermediate	%	Resistant	%
Ampicillin	27	90	0	0	3	10
Tetracycline	29	97	0	0	1	3
Lincomycin-spectinomycin	27	90	0	0	3	10
Ceftiofur	29	97	0	0	1	3
Enrofloxacin	27	90	3	10	0	0
Neomycin	26	87	3	10	1	3
Trimetho/Sulfa	20	67	4	13	6	20

Tab. 4. Susceptibility of *Streptococcus suis* to antimicrobials (n = 30)

Antimicrobials	Susceptibility					
	Susceptible	%	Intermediate	%	Resistant	%
Ampicillin	26	87	3	10	1	3
Tetracycline	15	50	2	7	13	43
Lincomycin	18	60	0	0	12	40
Ceftiofur	29	97	0	0	1	3
Enrofloxacin	26	87	0	0	4	13
Gentamicin	26	87	2	7	2	7
Trimetho/Sulfa	16	53	2	7	12	40
Erythromycin	21	70	0	0	9	30

Tab. 5. Susceptibility of *Enterococcus faecalis* to antimicrobials (n = 60)

Antimicrobials	Susceptibility					
	Susceptible	%	Intermediate	%	Resistant	%
Penicillin	57	95	0	0	3	5
Tetracycline	10	17	2	3	48	80
Vancomycin	60	100	0	0	0	0
Ceftiofur	60	100	0	0	0	0
Enrofloxacin	54	90	2	3	4	7
Neomycin	56	93	1	2	3	5
Gentamicin	58	97	0	0	2	3

Tab. 6. Susceptibility of commensal *E. coli* to antimicrobials (n = 60)

Antimicrobials	Susceptibility					
	Susceptible	%	Intermediate	%	Resistant	%
Ampicillin	43	72	2	3	15	25
Tetracycline	32	53	3	5	25	42
Neomycin	57	95	1	2	2	3
Florfenicol	58	97	0	0	2	3
Ceftiofur	60	100	0	0	0	0
Enrofloxacin	59	98	0	0	1	2
Colistin	60	100	0	0	0	0
Trimetho/Sulfa	38	63	5	8	17	28

14%, 3.5% and 0.5% of isolates were resistant respectively (8, 11).

Enterococci are one the most spread bacteria in pig farms. Sometimes they can be isolated from infected organs and likely participate in aetiology of various infections. Our results showed that 80% of *Enterococcus faecalis* isolates were resistant to tetracycline. High resistance to tetracycline of *E. faecalis* are found and in other countries. For example in Sweden 63% of isolated *E. faecalis* from pigs were resistant to this antibiotic (1). Susceptibility to other tested antimicrobials was higher, but 100% susceptibility was only to vancomycin and ceftiofur. VRE (vancomycin resistant enterococci) is a serious problem in human medicine, however ban of some related antibiotics used as feed additives conserved highest susceptibility of some bacteria important in human and veterinary medicine.

There are few reasons of reduced susceptibility of non-pathogenic bacteria to some antimicrobials. Results showed that non-pathogenic isolates of *E. coli* and *E. faecalis* had resistance patterns only to separate classes of antimicrobials. Highest resistance was observed against these antimicrobials that were used for a long period (tetracyclines, sulphonamides, trimethoprim). Less resistance was observed against other antibiotics that were used intensive, such as β-lactams and aminoglycosides. It could be explained that different antimicrobials have different action and different mechanisms of bacterial resistance exist. There are some antimicrobials that still are very effective (colistin, ceftiofur).

According to the obtained data may be outlined that the situation of antimicrobial susceptibility in Lithuanian pig farms is not conspicuous in comparison with the data obtained in other countries.

References

1. *Anon*.: Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM). National Veterinary Institute, Uppsala, Sweden 2003.
2. *Aarestrup F. M. L.*: Monitoring of antimicrobial resistance among food animals: principles and limitations. J. Vet. Med. 2004, 51, 3800-3808.
3. *Aarestrup F. M., Rasmussen S. R., Artursson K., Jensen N. E.*: Trends in the resistance to antimicrobial agents of Streptococcus suis isolates from Denmark and Sweden. Vet. Microbiol. 1998, 63, 71-80.
4. *Baggesen D. L., Sandvang D., Aarestrup F. M.*: Characterization of Salmonella enterica serovar Typhimurium DT104 isolated from Denmark and comparison with isolates from Europe and the United States. J. Clin. Microbiol. 2000, 38, 1581-1586.
5. *Barrigazi G., Candotti P., Foni L., Martinelli L., Raffo A.*: Proc. 14th Internat. Pig Vet. Soc. Congress, Bologna, Italy 1997, p. 308.
6. *Burch D. G. S.*: Pharmacokinetics – antimicrobial sensitivity and resistance. Pig Journal 2003, 52, 150-165.
7. *Burch D.*: Problems of antibiotic resistance in pigs in the United Kingdom. In Practice 2005, 27, 37-43.
8. *Bywater R., Deluyker H., Deroover E., deJong A., Marion H., McConville M., Rowan T., Shryock T., Shuster D., Thomas V., Vale M., Walters J.*: A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. J. Antimicrob. Chemother. 2004, 54, 744--754.
9. *Daignault D., Higgins R., Messier S., CoutureY.*: Sensibilité des isolats de Pasteurella multocida et Pasteurella haemolytica envers différents agents antibactériens. Méd. Vét. Québec 1997, 27, 154-155.
10. *Del'Arco A. E., Guimarães W. V.*: Resistance and sensitivity of Streptococcus suis to drugs. Proc. 17th Internat. Pig Vet. Soc. Congress, USA 2002, p. 335.
11. *Guerra B., Junker E., Schroeter A., Malorny B., Lehmann S., Helmuth R.*: Phenotypic and genotypic characterization of antimicrobial resistance in German Escherichia coli isolates from cattle, swine and poultry. J. Antimicrob. Chemother. 2003, 52, 489-492.
12. *Hariharan H., Coles M., Poole D., Page R.*: Antibiotic resistance among enterotoxigenic Escherichia coli from piglets and calves with diarrhea. Can. Vet. J. 2004, 45, 605-606.
13. *Hoszowski A., Wasyl D.*: Salmonella prevalence and resistance to antibiotics in Poland. Medycyna Wet. 2005, 61, 660-663.
14. *Hoszowski A., Wasyl D.*: Salmonella serovars found in animals and feeding stuffs in 2001 and their antimicrobial resistance. Bull. Vet. Inst. Pulawy 2002, 46, 165-178.
15. *King S. J., Leigh J. A., Heath P. J., Luque I., Tarradas C., Dowson C. G., Whatmore A. M.*: Development of a multilocus sequence typing scheme for the pig pathogen Streptococcus suis: identification of virulent clones and potential capsular serotype exchange. J. Clin. Microbiol. 2002, 40, 3671-3680.
16. *Mathew A. G., Saxton A. M., Upchurch W. G., Chattin S. E.*: Multiple antibiotic resistance patterns of Escherichia coli isolates from swine farms. Appl. Environ. Microbiol. 1999, 65, 2770-2772.
17. *Maynard C., Fairbrother J. M., Bekal S., Sanschagrin F., Levesque R. C., Brousseau R., Masson L., Larivičre S., Harel J.*: Antimicrobial resistance genes in enterotoxigenic Escherichia coli O149:K91 isolates obtained over a 23 year period from pigs. Antimicrob. Agents Chemother. 2003, 47, 3214-3221.
18. *Mølbak K., Gerner-Smidt P., Wegener H. C.*: Increasing quinolone resistance in Salmonella enterica serotype Enteritidis. Emerg. Infect. Dis. 2002, 8, 514-519.
19. *Morvan H.*: Sensibilité aux antibiotiques en élevage industriel. Institut Supérieur des Productions animales et des Industries Agro-alimentaires, Ploufragan, France 1994, p. 209-222.
20. *Nadeau M., Coté G., Higgins R.*: Surveillance de l'antibiorésistance chez des bactéries d'origine aviaire et porcine de 1993 ŕ 1999 au Québec. Méd. Vét. Québec 2001, 30, 1995-1999.
21. *Reams R. Y., Glickman L. T., Harrington D. D., Bowersock T. L., Thacker H. L.*: Streptococcus suis infection in swine: a retrospective study of 256 cases. Part I. Epidemiologic factors and antibiotic susceptibility patterns. J. Vet. Diag. Invest. 1993, 5, 363-367.
22. *Ruzauskas M., Amrozevičienė Č. B.*: Most common pathogenic bacteria and their antimicrobial resistance in pigs. Žemės ūkio mokslai 2004, 2, 44-51.
23. *Ruzauskas M., Virgailis M., Spakauskas V.*: Serological diversity and antimicrobial resistance of Salmonella isolated from different sources in Lithuania. Vet. Arhiv 2005, 75, 211-221.
24. *Wasyl D., Hoszowski A.*: Antibiotic susceptibility in Salmonella swine isolates. Salinpork, 4th International Symposium on the epidemiology and control of Salmonella and other food borne pathogens in pork. Leipzig, Germany 2001.
25. *Williams J. J., Salazar F. M. R., Ramírez P. R., Mosqueda A. Z.*: Sensibilidad in vitro de cepas de Actinobacillus pleuropneumoniae y Pasteurella multocida tipo „A" ante diferentes antimicrobianos. Rev Biomed. 2001, 12, 172-179.

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