VETERINARSKI ARHIV 80 (5), 571-583, 2010

Antimicrobial sensitivity of *Escherichia coli*, *Salmonella* spp., *Pasteurella multocida, Streptococcus suis* and *Actinobacillus pleuropneumoniae* isolated from diagnostic samples from large pig breeding farms in Croatia

Boris Habrun[1]*, Gordan Kompes[1], Željko Cvetnić[1], Silvio Špičić[1], Miroslav Benić[1], and Mario Mitak[2]

[1]*Department of Bacteriology and Parasitology, Croatian Veterinary Institute, Zagreb, Croatia*

[2]*Department for Veterinary Public Health, Croatian Veterinary Institute, Zagreb, Croatia*

HABRUN, B., G. KOMPES, Ž. CVETNIĆ, S. ŠPIČIĆ, M. BENIĆ, M. MITAK: Antimicrobial susceptibility of *Escherichia coli*, *Salmonella* spp., *Pasteurella multocida*, *Streptococcus suis* and *Actinobacillus pleuropneumoniae* isolated from diagnostic samples from large pig breeding farms in Croatia. Vet. arhiv 80, 571-583, 2010.

ABSTRACT

The disk diffusion method was employed to assess the sensitivity of 256 *Escherichia (E.) coli*, 42 *Salmonella* spp. and 62 *Streptococcus (S.) suis* isolates to 13 antimicrobials, and of 108 *Pasteurella (P.) multocida* and 44 *Actinobacillus (A.) pleuropneumoniae* isolates to 14 antimicrobials. All study isolates were obtained from diagnostic material collected at 8 large pig breeding farms in Croatia. *E. coli* isolates showed the highest rate of resistance to oxytetracycline, streptomycin and ampicillin (98%, 91% and 85% of isolates, respectively), whereas 87% of the isolates were resistant to 4 or more antimicrobials. *Salmonella* spp. isolates were most sensitive to enrofloxacin and colistin (all isolates), whereas highest rate of resistance was recorded to oxytetracycline and streptomycin (86% and 67% of isolates, respectively). More than 90% of *P. multocida* isolates were sensitive to ampicillin, amoxicillin with clavulanic acid, cefotaxime, colistin, florfenicol and enrofloxacin, whereas the highest rate of resistance was observed to streptomycin and nalidixic acid (59% and 43%, respectively). *S. suis* isolates were most sensitive to cefotaxime and florfenicol (94% of isolates both), whereas the highest resistance was recorded to streptomycin (100%). All *A. pleuropneumoniae* isolates showed sensitivity to amoxicillin with clavulanic acid, cefotaxime, florfenicol and enrofloxacin, and more than 90% of these isolates were sensitive to penicillin G, ampicillin, colistin, gentamicin and spectinomycin, whereas the highest rate of resistance was recorded to nalidixic acid and streptomycin (59% and 36%, respectively). Sensitivity to all antimicrobials tested was recorded in 27% of *A. pleuropneumoniae* isolates, whereas 41% of these isolates were resistant to one, and 27% to 2-4 of the antimicrobials tested. Of the bacterial species

*Corresponding author:

Dr. Boris Habrun, PhD, Department of Bacteriology and Parasitology, Croatian Veterinary Institute, Savska c. 143, 10000 Zagreb, Croatia; Phone: +385 1 6123 604; Fax +385 1 6190 841; E-mail: habrun@veinst.hr

ISSN 0372-5480
Printed in Croatia

included in the study, the highest rate of antimicrobial resistance was recorded in *E. coli*, and lowest rate in *A. pleuropneumoniae* isolates.

Key words: antimicrobial sensitivity, *E. coli, Salmonella* spp., *P. multocida, S. suis, A. pleuropneumoniae*

Introduction

Antimicrobial resistance has emerged in the past few years as a major problem and many programs have been set up for its surveillance in human and veterinary medicine. These programs are aimed mainly at human pathogens, agents of zoonoses, and indicator bacteria of the normal intestinal flora from animals (LANZ et al., 2003). Little attention has been paid to the resistance in specific animal pathogens (LANZ et al., 2003).

Large swine farms comprise the majority of intensive pig breeding in Croatia. These farms have between 600 and 5000 sows per farm with an adequate number of boars for artificial insemination (HABRUN et al., 1998a), resulting in a large number of animals in a relatively small area. The majority of farms are farrowing - finishing units and all technological phases are based on the all-in-all-out principle.

Such farms have a high prevalence of so-called breeding diseases caused by opportunistic bacteria (BILIĆ and HABRUN, 1996). Although new subunit and polyvalent vaccines have recently been intensively developed, there is no efficient immunoprophylaxis for all bacterial diseases occurring in intensive pig breeding, where the use of antibiotics for prevention and therapy of some dominant bacterial diseases remains necessary.

The aim of this study was to assess the sensitivity of the strains *Escherichia (E.) coli*, *Salmonella* spp., *Pasteurella (P.) multocida*, *Streptococcus (S.) suis* and *Actinobacillus (A.) pleuropneumoniae* isolated in diagnostic material from large pig breeding farms in Croatia.

Materials and methods

Bacterial isolates. Bacterial isolates from different pig organs were collected from 8 large pig breeding farms in Croatia during 2005, 2006 and 2007. All *E. coli* isolates (n=256) were obtained from suckling and weaned piglets that had died with clinical signs of diarrhea. The isolates were identified by use of rapid Bactident *E. coli* test (Merck, Germany). Atypical isolates were identified by use of BBL Crystal Enteric-Nonfermenter biochemical gallery (Becton Dickinson, USA). Hemolytic activity of *E. coli* isolates was tested on 5% sheep blood agar. The hemolytic strains containing F4, F6 and F18 fimbriae were included in this study. Fimbriae were determined by the method of coagglutination using home-made sera.

Salmonella spp. isolates (n=42) were isolated from carcasses of pigs that died with symptoms of septicemia and diarrhea from three pig farms. *Salmonella* (*S.*) Typhimurium was sporadically isolated at one farm, and *S.* Choleraesuis var. Kunzendorf at the other two

farms. Bacterial isolation was performed by conventional methods (BAGER and PETERSEN, 1991; DAVIES et al., 1998; FEDORKA-CRAY et al., 1998). Biochemical characteristics were assessed by TSI agar culture and the BBL Crystal Enteric-Nonfermenter biochemical gallery (Becton Dickinson, USA). Strain typing was done by slide agglutination with respective antisera (Institute of Immunology, Croatia).

P. multocida isolates were isolated from the lungs of pigs that died with symptoms of respiratory disease at all eight pig farms. The isolates were identified by use of BBL Crystal Enteric-Nonfermenter biochemical gallery (Becton Dickinson, USA).

A. pleuropneumoniae isolates were obtained from the lungs of pigs that died with clinical and pathoanatomical symptoms of pleuropneumonia. Primary isolation was performed in 5% sheep blood agar inoculated with the strain Staphylococcus (*S.*) aureus, where CAMP phenomenon, satellite growth and hemolysis of the isolate were determined. Serotyping was performed by the gel diffusion test as described elsewhere (NIELSEN and O'CONNOR, 1984). Reference strains were provided by courtesy of Professor J. Nicolet.

Streptococcus suis isolates were collected from pigs that died with symptoms of pneumonia, septicemia and central nervous system disorder (meningitis). The isolates were isolated on 5% sheep blood agar and identified by use of amylase and Voges-Proskauer (acetoin) tests (DEVRIESE et al., 1991) and API Strep system gallery (BioMerieux, France).

All isolates were stored in Luria-Bartrani broth with glycerin at -70 °C until sensitivity testing.

Sensitivity testing. Susceptibility testing was performed by the disk diffusion method according to CSLI standards (Clinical and Laboratory Standards Institute, M31-A3, ANONYM., 2008) for the following antimicrobial agents: penicillin G (10 IU), ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), cefotaxime (30 μg), colistin (10 μg), chloramphenicol (30 μg), oxytetracycline (30 μg), streptomycin (10 μg), neomycin (30 μg), gentamicin (10 μg), nalidixic acid (30 μg), enrofloxacin (5 μg), spectinomycin (100 μg) and florfenicol (30 μg).

Penicillin G and florfenicol were tested only in *P. multocida, S. suis* and *A. pleuropneumoniae,* and chloramphenicol in *E. coli* and *Salmonella* spp. Colistin was not tested in *S. suis* isolates.

According to CSLI, Mueller-Hinton agar was used as a culture medium (Merck 1.05435), with the addition of 5% defibrinated sheep blood on *S. suis* isolate testing. *S. suis* isolate sensitivity to penicillin G was also tested on Mueller-Hinton agar with the addition of 5% horse serum instead of sheep blood. Chocolate Mueller-Hinton agar was used on *A. pleuropneumoniae* isolate testing, according to CSLI recommendation (M37-

A3). *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *Enterococcus faecalis* ATCC 29212 were used as control strains.

The zone of growth inhibition was interpreted as sensitive, moderately sensitive and resistant, as recommended by CSLI M31 A3.

Results

Serotyping. Forty-two *Salmonella* spp. isolates included 29 *S.* Choleraesuis var. Kunzendorf and 13 *S.* Typhimurium isolates. Forty-four *A. pleuropneumoniae* isolates included 21 serovar 2, 15 serovar 9, and 8 serovar 7 isolates.

Antimicrobial susceptibility testing. The results are presented in Tables 1-5. Table 1 shows the sensitivity of the *E. coli* strains tested, which exhibited the highest resistance, especially to ampicillin, streptomycin and oxytetracycline (85%, 91% and 98% of resistant strains, respectively). The highest sensitivity rate was recorded to colistin and cefotaxime (94% and 86% of sensitive strains, respectively). Only 2 (less than 1%) isolates were sensitive to all antimicrobials tested, while 12% of isolates showed sensitivity to 1-3, 69% to 4-9 and 18% to 10-12 antimicrobials tested.

Table 1. Antimicrobial susceptibility of enterotoxigenic *Escherichia coli* (n = 256) by agar disc diffusion method*

Antimicrobial agent	Susceptible	Intermediate susceptible	Resistant
Ampicillin	15	0	85
Amoxicillin/clavulanic acid	55	16	29
Cefotaxime	86	0	14
Colistin	94	0	6
Cloramphenicol	54	0	46
Oxitetracycline	2	0	98
Streptomycin	8	1	91
Neomycin	19	11	70
Gentamycin	41	6	53
Nalidixic acid	20	0	80
Enrofloxacin	50	11	39
Spectinomycin	41	1	58
Sulfametoxazome/trimethoprim	12	1	87

*Results are expressed as a percentage of 256 *E. coli* isolates susceptible, intermediate/moderately susceptible and resistant, respectively, for each antimicrobial

Table 2. Antimicrobial susceptibility of *Salmonella* spp. (n = 44) by agar disc diffusion method*

Antimicrobial agent	Susceptible	Intermediate susceptible	Resistant
Ampicillin	67	0	33
Amoxicillin/clavulanic acid	76	10	14
Cefotaxime	95	0	5
Colistin	100	0	0
Cloramphenicol	67	0	33
Oxitetracycline	14	0	86
Streptomycin	14	19	67
Neomycin	71	9	20
Gentamycin	95	0	5
Nalidixic acid	81	0	19
Enrofloxacin	100	0	0
Spectinomycin	81	0	19
Sulfametoxazome/trimethoprim	48	0	52

*Results are expressed as a percentage of 44 *Salmonella* spp. isolates susceptible, intermediate/moderately susceptible and resistant, respectively, for each antimicrobial

Table 3. Antimicrobial susceptibility of *Pasteurella multocida* (n = 108) by agar disc diffusion method*

Antimicrobial agent	Susceptible	Intermediate susceptible	Resistant
Penicillin G	72	0	28
Ampicillin	91	0	9
Amoxicillin/clavulanic acid	96	0	4
Cefotaxime	93	0	7
Colistin	100	0	0
Florfenicol	100	0	0
Oxitetracycline	76	0	24
Streptomycin	39	2	59
Neomycin	54	24	22
Gentamycin	76	6	18
Nalidixic acid	57		43
Enrofloxacin	96		4
Spectinomycin	88		12
Sulfametoxazome/trimethoprim	63	2	35

*Results are expressed as a percentage of 108 *Pasteurella multocida* isolates susceptible, intermediate/moderately susceptible and resistant, respectively, for each antimicrobial

Table 4. Antimicrobial susceptibility of *Streptococcus suis* (n = 62) by agar disc diffusion method*

Antimicrobial agent	Susceptible	Intermediate susceptible	Resistant
Penicillin G	81[a] (94[b])	0	19
Ampicillin	94	0	13
Amoxicillin/clavulanic acid	94	0	13
Cefotaxime	94	0	6
Colistin	0	0	100
Florfenicol	94	0	6
Oxitetracycline	13	0	87
Streptomycin	0	0	100
Neomycin	16	26	58
Gentamycin	26	13	61
Nalidixic acid	19	0	81
Enrofloxacin	71	10	19
Spectinomycin	58	0	42
Sulfametoxazome/trimethoprim	45	0	55

*Results are expressed as a percentage of 62 *Streptococcus suis* isolates susceptible, intermediate/moderately susceptible and resistant, respectively, for each antimicrobial; [a]Mueller-Hinton agar with the addition of 5% defibrinated sheep blood; [b] Mueller-Hinton agar with the addition of 5% horse serum

Table 5. Antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* (n = 44) by agar disc diffusion method*

Antimicrobial agent	Susceptible	Intermediate susceptible	Resistant
Penicillin G	91	0	9
Ampicillin	95	0	5
Amoxicillin/clavulanic acid	100	0	0
Cefotaxime	100	0	0
Colistin	95	0	5
Florfenicol	100	0	0
Oxitetracycline	82	0	18
Streptomycin	59	5	36
Neomycin	82	9	9
Gentamycin	96	4	0
Nalidixic acid	41	0	59
Enrofloxacin	100	0	0
Spectinomycin	95	0	5
Sulfametoxazome/trimethoprim	68	5	27

*Results are expressed as a percentage of 44 *Actinobacillus pleuropneumoniae* isolates susceptible, intermediate/moderately susceptible and resistant, respectively, for each antimicrobial

The sensitivity of *Salmonella* spp. isolates is presented in Table 2. The highest sensitivity rate was recorded to colistin and enrofloxacin (100% both), and the highest resistance rate to oxytetracycline (86%) and streptomycin (67%). None of the *Salmonella* spp. isolates exhibited sensitivity to all antimicrobials tested, whereas 76% of isolates showed resistance to 2-4 and 24% to 5-7 antimicrobials. None of the *Salmonella* spp. isolates showed resistance to more than 7 antimicrobials.

The sensitivity of *P. multocida* isolates is presented in Table 3. More than 90% of these isolates showed sensitivity to ampicillin (91%), amoxicillin with clavulanic acid (96%), cefotaxime (93%), colistin (100%), florfenicol (100%) and enrofloxacin (96%). The highest rate of resistance was recorded to streptomycin (59%) and nalidixic acid (43%). Eleven percent of these isolates were sensitive to all antimicrobials tested, whereas 24% of isolates were resistant to 1, 48% to 2-4 and 17% to 5-7 antimicrobials. None of the *P. multocida* isolates showed resistance to more than 7 antimicrobials tested.

The *Streptococcus suis* isolates showed highest rate of sensitivity to cefotaxime and florfenicol (94% both), and highest rate of resistance to colistin and streptomycin (100% both) (Table 4). None of the *Streptococcus suis* isolates exhibited sensitivity to all antimicrobials tested, whereas 42% of these isolates were sensitive to 2-4, 26% to 5-7 and 26% to 8-12 antimicrobials.

The sensitivity of *A. pleuropneumoniae* isolates is presented in Table 5. All *A. pleuropneumoniae* isolates showed sensitivity to amoxicillin with clavulanic acid, cefotaxime, florfenicol and enrofloxacin, whereas more than 90% of these isolates were sensitive to penicillin G (91%), ampicillin (95%), colistin (95%), gentamicin (96%) and spectinomycin (95%). The highest rate of resistance was recorded to nalidixic acid and streptomycin (59% and 36%, respectively). Twenty-seven percent of *A. pleuropneumoniae* isolates were sensitive to all antimicrobials, 41% to 1 antimicrobial and 27% to 2-4 antimicrobials tested. Only 2 strains showed sensitivity to 7 antimicrobials.

Discussion

Beta-hemolytic *E. coli* is the most common bacterial etiologic agent of diarrhoea in neonatal and postweaning pigs. Treatment of enteric *E. coli* infections in swine commonly includes the use of broad-spectrum antibiotics (BISCHOFF et al., 2002). In the present study, the sensitivity of 256 *E. coli* isolates to 13 antimicrobials was tested (Table 1). The highest rate of resistance was recorded to tetracycline (98%), streptomycin (91%), sulfamethoxazole/trimethoprim (87%) and ampicillin (85%). Multidrug resistance was frequently observed, with 87% of *E. coli* isolates resistant to 4 or more antibiotics. The swine *E. coli* isolates showed a pattern similar to other clinical veterinary *E. coli* strains in terms of their increased resistance to tetracycline, streptomycin, sulfamethoxazole/trimethoprim and gentamicin (COATES and HOOPES, 1980; LIBAL and GATES, 1982;

HARNET and GYLES, 1984; NIJSTEN et al., 1996; KEYES *et al*., 2000; BISCHOFF et al., 2002).

A steady decrease has been observed in sensitivity to fluoroquinolones (enrofloxacin), as only 58% of the strains were sensitive to this drug. The studies conducted in Croatia in 1990 revealed no *E. coli* strains resistant to fluoroquinolones, whereas those carried out in 1997-1998 found 29.7% of *E. coli* isolates collected from swine to be resistant to enrofloxacin (BILIĆ et al., 1999). Comparison of the present and previous study results (BILIĆ et al., 1999) also indicates a steady increase in the bacterial resistance to neomycin (22% and 56% of resistant strains in 1990 and 1997-1998, respectively, *vs*. 70% in the present study) and gentamycin (27.5% and 49.7% of resistant strains in 1990 and 1997-1998, respectively, *vs*. 53% in the present study) (Table 1).

The high rate of sensitivity to colistin (94% of study strains) is attributed to the fact that colistin has not been used in veterinary medicine in Croatia to date, and more common use of colistin in intensive pig breeding may result in an increased resistance to this antimicrobial in the future. Although there has been a ban on the systemic use of cloramphenicol in Croatia for more than 10 years now, 46% of study isolates were resistant to this antibiotic.

The high multidrug resistance of *E. coli* strains isolated from large swine agglomerations was most probably due to obsolete farming technology, where economically profitable productivity could hardly be maintained without the systemic use of antibiotics administered in the critical steps of production.

Over the last few years, bacteria of the genus *Salmonella* isolated from porcine organs were collected from only three large pig breeding farms in Croatia. At one of these farms, *S.* Typhimurium was only sporadically isolated from dead animal organs, whereas *S.* Choleraesuis var. Kunzendorf was sporadically isolated at the other two farms (HABRUN et al., 2002).

The study results showed all the bacterial strains tested to be sensitive to enrofloxacin and colistin, whereas 95% of the strains were sensitive to gentamicin and cefotaxime (Table 2). The highest rate of bacterial resistance was recorded to oxytetracycline (86%) and streptomycin (67% of resistant strains and 19% of moderately resistant strains) (Table 2). These results are consistent with literature reports on the sensitivity studies of *Salmonella* spp. isolated from pigs (WONDWOSSEN et al., 2000; OLIVEIRA et al., 2002). In comparison with other studies, our isolates showed quite good sensitivity to ampicillin (67%) and amoxicillin with clavulanic acid (76%).

The present study revealed the resistance of *Salmonella* spp. in Croatian intensive pig breeding to be by far lower than the resistance of *E. coli*, which is quite conceivable considering the markedly lower presence of the former at Croatian pig breeding farms. Only 4 strains exhibited pentadrug resistance to ampicillin, chloramphenicol, streptomycin,

tetracycline and sulfamethoxazole, characteristic of the multidrug resistance DT104 phagotype (THRELFALL et al., 1996), however, additional studies are needed to confirm these findings.

P. multocida was the second most common secondary bacterial infective agent isolated from porcine respiratory disease complex. All isolates showed sensitivity to colistin and florfenicol, whereas more than 90% of isolates were sensitive to ampicillin, amoxicillin with clavulanic acid, cefotaxime and enrofloxacin, which is comparable with the results reported by HORMANSDORFER and BAUER (1998). The highest rate of isolate resistance was recorded to streptomycin and nalidixic acid (59% and 43%, respectively) (Table 4).

In Croatia, an increasing rate of *S. suis* isolation from dead swine organs has been recorded since the occurrence of porcine reproductive and respiratory syndrome in 1996. As testing on Mueller-Hinton agar with sheep blood revealed 81% of isolates to be sensitive to penicillin G and 94% to ampicillin and amoxicillin, the testing of bacterial sensitivity to penicillin G was repeated on Mueller-Hinton agar with horse serum, which allows for a greater zone of growth inhibition to obtain thus a higher proportion of sensitive strains to detect (MARIE et al., 2002). Bacterial testing on Mueller-Hinton agar with horse serum showed 94% of isolates to be sensitive to penicillin G (Table 4). The same percentage of *S. suis* isolates showed sensitivity to florfenicol and cefotaxime. These findings are consistent with literature reports on the highest rate of *S. suis* sensitivity to these particular antibiotics (SHYROCK et al., 1992; STUART et al., 1992; TARRADAS et al., 1994; AARESTRUP et al., 1998a; KATAOKA et al., 2000; MARIE et al., 2002).

Bacterial resistance to oxytetracycline (87% of resistant isolates) was also in agreement with literature data (AARESTRUP et al., 1998a,b), as was resistance to streptomycin (all isolates) (STUART et al., 1992; TURGEON et al., 1994; AARESTRUP et al., 1998b; KATAOKA et al., 2000; MARIE et al., 2002).

A. pleuropneumoniae serovars 2, 7, 8 and 9 had previously been isolated from porcine pleuropneumonia in Croatia (HABRUN et al., 1998b). All the strains tested here showed sensitivity to amoxicillin with clavulanic acid, cefotaxime, florfenicol and enrofloxacin, whereas 95% of these isolates exhibited sensitivity to ampicillin and spectinomycin, and 91% to penicillin G (Table 5). These findings indicate a considerably higher isolate sensitivity in Croatia than in Taiwan (CHANG et al., 2002), where 73% and only 17% of isolates were sensitive to enrofloxacin and ampicillin, respectively.

In the present study, as many as 82% of *A. pleuropneumoniae* isolates were found to be sensitive to oxytetracycline, in spite of the high rate of *A. pleuropneumoniae* resistance to this antibiotic reported (NADEAU et al., 1988) and the relatively wide use of oxytetracycline in Croatian pig breeding. The highest rate of resistance was recorded to streptomycin (36% of resistant isolates and 5% of moderately sensitive isolates), whereas the rate of bacterial sensitivity to other aminoglycosides under study was considerably

higher (neomycin 82% and gentamicin 96%). Only 2 study strains showed sensitivity to 7 antimicrobials tested, whereas 27% of isolates were sensitive to all antibacterials tested.

Conclusions

Of the bacterial strains tested, *E. coli* isolates showed the highest rate of resistance. Due to the rather high rate of resistance, technological conditions and feeding procedures should be systematically improved and sow immunoprophylaxis routinely performed to prevent neonatal colibacillosis, while limiting the use of antibiotics that should be so utilized as to reduce the development of resistance. Although the rate of *Salmonella* spp. resistance was much lower than of *E. coli*, the occurrence of a number of multidrug resistant strains indicates that the sensitivity of *Salmonella* spp. should be more closely monitored and efforts invested to identify the genes responsible for this resistance. The species *S. suis, P. multocida* and *A. pleuropneumoniae* showed considerably better sensitivity than *E. coli* and *Salmonella* spp.

References

AARESTRUP, F. M., S. E. JORSAL, N. E. JENSEN (1998a): Serological characterisation and antimicrobial susceptibility of *Streptococcus suis* isolates from diagnostic samples in Denmark during 1995 and 1996. Vet. Microbiol. 60, 59-66.

AARESTRUP, F. M., S. R. RASMUNSEN, K. ARTURRSON, E. N. JENSEN (1998b): Trends in the resistance to antimicrobial agents of *Streptococcus suis* isolates from Danmark and Sweden. Vet. Microbiol. 63, 71-80.

ANONYMOUS, (2008): Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. Third Ed. M31-A3. Vol 22, No. 6., Clinical and Laboratory Standards Institute Wayne, PA.

BAGER, F., J. PETERSEN (1991): Sensitivity and specificity of different methods for the isolation of *Salmonella* from pigs. Acta Vet. Scand. 32, 473-481.

BILIĆ, V., B. HABRUN (1996): Control of diseases during growth of pigs in large herds. Praxis vet. 44, 53-57.

BILIĆ, V., B. HABRUN, A. HUMSKI (1999): Sensitivity of bacteria isolated from pigs. Praxis vet. 47, 39-46.

BISCHOFF, K. M., D. G. WHITE, P. F. MCDERMONT, S. ZHAO, S. GAINES, J. J. MAURER, D. J. NISBET (2002): Characterisation of chloramphenicol resistance in beta-hemolytic *Escherichia col*i associated with diarrhea in neonatal swine. J. Clin. Microbiol. 40, 389-394.

CHANG, C. F., T. M. YEH, C. C. CHOU, Y. F. CHANG, T. S. CHIANG (2002): Antimicrobial susceptibility and plasmid analysis of *Actinobacillus pleuropneumoniae* isolated in Taiwan. Vet. Microbiol. 84, 169-177.

COATES, S. R., HOOPES K. H. (1980): Sensitivities of *Escherichia coli* isolated from bovine and porcine enteric infections to antimicrobial antibiotics. Am. J. Vet. Res. 41, 1882-1883.

DAVIES, P. R., F. G. E. M. BOVEE, J. A. FUNK, F. T. JONES, E. M. MORROW, J. DEEN (1998): Isolation of *Salmonella* serotypes from feces of pigs raised in a multiple-site production system. J. Am. Vet. Med. Assoc. 212, 1925-1929.

DEVRIESE, L. A., K. CEYSSENS, J. HOMMEZ, R. KILPPER-BALZ, K. H. SCHLEIFER (1991): Characteristics of different *Streptococcus suis* serovars and description of a simplifed identification method. Vet. Microbiol. 26, 141-150.

FEDORKA-CRAY, P. J., D. A. DARGATZ, L. A. THOMAS, J. T. GAY (1998): Survey of *Salmonella* serotypes in feedlot cattle. J. Food Prot. 61, 525-530.

HABRUN, B., V. BILIĆ, A. HUMSKI (1998a): Comparison of ELISA and 2-META assays used in serological diagnosis of infection with *Actinobacillus pleuropneumoniae* serotypes 2 and 4-7 in breeding pigs in Croatia. Prev. Vet. Med. 36, 179-186.

HABRUN, B., J. FREY, V. BILIĆ, J. NICOLET, A. HUMSKI (1998b): Prevalence of serotypes and toxin types of *Actinobacillus pleuropneumoniae* in pigs in Croatia. Vet. Rec. 143, 255-256.

HABRUN, B., A. HUMSKI, V. BILIĆ, A. PREMZL (2002): Bacterial diseases of swine in Croatia in 2001. Croatian Veterinary Congress. Proceedings, pp. 23-24.

HARNET, N. M., C. L. GYLES (1984): Resistance to drugs and heavy metals, colicin production and biochemical characteristics of selected bovine and porcine *Escherichia coli* strains. Appl. Environ. Microbiol. 48, 930-935.

HORMANSDORFER, S., J. BAUER (1998): Resistance of bovine and porcine *Pasteurella* to florfenicol and other antibiotics. Berl. Münch. Tierärtzl. Wochenschr. 111, 422-426.

KATAOKA, Y., T. YOSHIDA, T. SAWADA (2000): A 10-year survey of antimicrobial susceptibility of *Streptococcus suis* isolates from swine in Japan. J. Vet. Med. Sci. 62, 1053-1057.

KEYES, K., C. HUDSON, J. J. MAUER, S. THAYER, D. G. WHITE, M. D. LEE (2000): Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. Antimicrob. Agents Chemother. 44, 421-424.

LANZ, R., P. KUHNERT, P. BOERLIN (2003): Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet. Microbiol. 91, 73-84.

LIBAL, M. C., C. E. GATES (1982): Antimicrobial resistance in *Escherichia coli* strains isolated from pigs with diarrhea. J. Am. Vet. Med. Assoc. 180, 908-909.

MARIE, J., H. MORVAN, F. BERTHELOT-HERAUT, P. SANDERS, I. KEMPF, A. V. GAUTIER-BOUCHARDON, E. JOUJ, M. KOBISCH (2002): Antimicrobial susceptibility of *Streptococcus suis* isolated from swine in France and from humans in different countries between 1996 and 2000. J. Antimicrob. Chemother. 50, 201-209.

NADEAU, M., S. LARIVIERE, R. HIGGINS, G. P. MARTINEAU (1988): Minimal inhibitory concentrations of antimicrobial agents against *Actinobacillus pleuropneumoniae*. Can. J. Vet. Res. 52, 315-318.

NIELSEN, R., P. J. O'CONNOR (1984): Serological characterisation of 8 *Haemophilus pleuropeumoniae* strains and proposal of a new serotype: serotype 8. Acta Vet. Scand. 25, 96-106.

NIJSTEN, R., N. LONDON, A. VAN DEN BOGAARD, E. STOBBERINGH (1996): Antibiotic resistance among *Escherichia coli* isolated from faecal samples of pig farmers and pigs. J. Antimicrob. Chemother. 37, 1131-1140.

OLIVEIRA, C. J., L. F. CARVALHO, S. A. FERNANDES, A. T. TAVECHIO, C. C. MENEZES, F. J. DOMINGUES JR. (2002): Antimicrobial resistance of *Salmonella* serotypes isolated from slaughter age pigs and environmental samples. Microb. Drug Resist. 8, 407-411.

SHYROCK, T. R., J. E. MORTENSEN, L. R. RHOADS (1992): Antibiotic susceptibility in *Streptococcus suis*. Curr. Ther. Res. 52, 419-424.

STUART, J. G., E. J. ZIMMERER, L. R. MADDUX (1992): Conjugation of antibiotic resistance in *Streptococcus suis.* Vet. Microbiol. 30, 213-222.

TARRADAS, M. C., A. ARENAS, A. MALDONADO, S. VICENTE, A. MIRANDA, A. PEREA (1994): Susceptibility of *Streptococcus suis* to various antimicrobial agents. Zentralbl. Veterinärmed. B 41, 685-688.

THRELFALL, E. J., J. A FROST, L. R. WARD, B. ROWE (1996): Increasing spectrum of resistance in multiresistant *Salmonella typhimurium*. Lancet 347, 1053-1054.

TURGEON, P. L., R. HIGGINS, M. GOTTSCHALK, M. BEAUDOIN (1994): Antimicrobial susceptibility of *Streptococcus suis* isolates. Br. Vet. J. 150, 263-269.

WONDWOSSEN, A. G., P. R. DAVIES,. W. E. M. MORROW,. J. A. FUNK, C. ALTIER (2000): Antimicrobial resistance of *Salmonella* isolates from swine. J. Clin. Microbiol. 38, 4633-4636.

Received: 13 April 2009
Accepted: 29 January 2010

HABRUN, B., G. KOMPES, Ž. CVETNIĆ, S. ŠPIČIĆ, M. BENIĆ, M. MITAK: Antimikrobna osjetljivost bakterija *Escherichia coli*, *Salmonella* spp., *Pasteurella multocida, Streptococcus suis* i *Actinobacillus pleuropneumoniae* izdvojenih iz dijagnostičkih uzoraka iz velikih svinjogojskih farmi u Hrvatskoj. Vet. arhiv 80, 571-583, 2010.

SAŽETAK

Disk difuzijskom metodom istražena je osjetljivost 256 izolata *Escherichia* (*E.*) *coli*, 42 izolata *Salmonella* spp. i 62 izolata *Streptococcus* (*S.*) *suis* na 13 antimikrobnih lijekova, te 108 izolata *Pasteurella* (*P.*) *multocida*, i 44 izolata *Actinobacillus* (*A.*) *pleuropneumoniae* na 14 antimikrobnih lijekova. Svi su izolati bili izdvojeni iz dijagnostičkoga materijala s 8 velikih svinjogojskih farmi u Hrvatskoj. U *E. coli* najveći stupanj rezistencije utvrđen je na oksitetraciklin (98% izolata), streptomicin (91% izolata) i ampicilin (85% izolata), te je 87% izolata bilo rezistentno na 4 i više antimikrobnih lijekova. Izolati *Salmonella* spp. bili su najosjetljiviji na enrofloksacin i kolistin (svi izolati), a najveći postotak otpornosti utvrđen je na oksiteraciklin (86%) i streptomicin (67%). Više od 90% izolata *P. multocida* bilo je osjetljivo na ampicilin, amoksicilin s klavulanskom kiselinom, cefotaksim, kolistin, florfenikol i enrofloksacin. Najveća rezistencija bakterije *P. multocida* utvrđena je na streptomicin (59%) i nalidiksičnu kiselinu (43%). Izolati vrste *Streptococcus suis* bili su najosjeljiviji na cefotaksim i florfenikol (94% osjetljivih izolata), a najveća rezistencija utvrđena je na streptomicin (100%). Svi

testirani izolati vrste *A. pleuropneumoniae* bili su osjetljivi amoksicilin s klavulanskom kiselinom, cefotaksim, florfenikol i enrofloksacin, a više od 90% izolata bilo je osjetljivo na penicilin G, ampicilin, kolistin, gentamicin i spektinomicin. Najveća rezistencija utvrđena je na nalidiksičnu kiselinu (59%) i streptomicin (36%). Na sve testirane antimikrobne lijekove bilo je osjetljivo 27% izolata *A. pleuropneumoniae*, a 41% izolata bilo je rezistentno na jedan testirani antimikrobni lijek, dok je 27% izolata bilo otporno na 2 do 4 antimikrobna lijeka. Od testiranih bakterijskih vrsta najveći stupanj rezistencije imali su izolati *E. coli*, dok su izolati *A. pleuropneumoniae* imali najmanji stupanj rezistencije.

Ključne riječi: antimikrobna osjetljivost, *E. coli, Salmonella* spp., *P. multocida, S. suis, A. pleuropneumoniae*