

PRESENCE OF *Campylobacter coli* IN SLAUGHTERED PIGS AND ITS RESISTANCE TO ANTIBIOTICS**

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Abstract: *Campylobacter coli* is zoonotic bacterium. As saprophyte it persists in pigs and they are its common reservoir. It transmits to human by meat and meat products. *Campylobacter coli* is sensitive to antibiotics but in one moment could become resistant. According to literature data, the aim of our investigations was to affirm the sensitivity of *Campylobacter coli*, isolated from the samples collected from clinically healthy pigs, on slaughter line, to following antibiotics: Erythromycin, Ampycillin and Tetracycline. From all of identified isolates it was selected 18 strains and they are examined to antibiotic sensitivity by both of dilution and diffusion methods. Obtained results were interpreted according to proposition of European Working Group for investigation of antibiotic resistance of bacteria (1996). Using both methods it was found out: all strains were sensitive to erythromycin; all strains were resistant to tetracycline, 16 strains were resistant to ampicillin, two strains were intermediate to ampicillin (strain 4 and 13). Usage of antibiotics in prophylactic purposes is common practice in intensive animal husbandry. This usage change microbial flora in treated animals and its inadequate application often cause bacterial resistance to them.

Key words: *Campylobacter coli*, pork, antibiotics, resistance

Introduction

Domestic animals, including pigs, act as natural reservoirs of these bacteria, and infection of human may occur through the ingestion of contaminated foodstuff, especially the consumption of pork and poultry meat

(Beckers, 1986; Fransen *et al.*, 1996; Moore and Madden, 2001). The most common disease pattern is enteritis accompanied by fever, malaise, stomach pain and headache (Stern and Kazmi, 1989; Malakauskas *i sar.*, 2006). The complications caused by *Campylobacter* spp. are appendicitis and cholecystitis, pancreatitis, hepatitis, mega colon toxicosis, erythema nodosum, arthritis, myocarditis, meningitis and septicemia. Symptoms of gastroenteritis do not develop in everyone that is exposed to infection; minimal infective dose that leads to infection is 500 bacteria in 1g of food (Heisick, 1984).

Gastroenteritis is mainly caused in 90-95% of cases by *Campylobacter jejuni* while *Campylobacter coli* cause the majority of the remainder (Madden *et al.*, 2000). Enteritis caused by *Campylobacter* spp. has become a worldwide problem.

Poisonings caused by *Campylobacter* spp. are also common, and even more frequent than those caused by *Salmonella* spp., according to data from the World Health Organization (WHO). It is known that *Campylobacter* spp. produce thermolabile endotoxins, enterotoxins and cytotoxins (Buzler, 1984.; Mc Cardell *et al.*, 1984). The antibiotic resistance of *Campylobacter* spp. is topic of many investigations. These investigations have importance from both veterinary and humane medicine aspect, in order to suppress infections by target therapy. Observing the structure of *Campylobacter* cell, Elwel (1985) found out that plasmids determine virulence of many bacteria. According to his data, plasmids influence on possibility of binding of bacteria with epithelial cells, to invasive toxin production, exclusion of iron, resistance to bactericide serum effects and resistance to bactericide complexes.

Many researchers have isolated plasmids from *Campylobacter jejuni* and affirmed plasmid as chain length 1-86 mega Daltons. Antibiotic resistance and production of enterotoxins in some specific cases, they have scrambled by plasmids.

Brandbury and Monroe (1995) have found out serological difference between *Campylobacter coli* in healthy pigs and in pigs with diarrhea. Examining 200 samples, they affirmed in 116 (53%) DNA plasmid. Percentage of plasmids in *Campylobacter coli* isolated from clinically healthy pigs was 73, 80% and in pigs with diarrhea was less (30%).

DNA plasmids contain *Campylobacter jejuni* isolated from 13,60% clinically healthy cattle and 31,20% in cattle with diarrhea. Isolates was sensitive to kanamycin, erythromycin, gentamycin, tetracycline and mixture of sulphonamides. *Campylobacter* strains that contain plasmids were more

resistant to ampicillin, tetracycline and gentamicin than those they do not.

Austin et al. (1990) have isolated *Campylobacter jejuni* from different regions. They have found that 19% of colonies originated from different locations contain plasmids. Only one isolate contains more than one plasmid. Molecular weight of plasmids was 5-70 million Dalton. Some of isolates were resistant to antibiotics. Authors have not found direct connection between resistance and plasmids size. They have identified transmissible plasmids resistant to tetracycline. Also, they have found that if mother cell contains plasmid responsible for antibiotic resistance, by dividing it can be transmit to daughter cells, but not to other species of microorganisms (*Taylor et al.*, 1991).

Wang et al. (1984) have compared the resistance of *Campylobacter jejuni* and *coli* to antibiotics. They have defined MIC for 12 antibiotics for isolates from 104 of human samples and from all of 74 samples collected from pigs. From 74 samples collected from pigs, five (7%) were hypurat positive and 93 (89%) of 104 of human isolates. All of isolates they have treated with 12 antibiotics: ampicillin, amoxicillin, clindomycin, chloramphenicol, erythromycin, furazolidon, norfloxacin, nalidixic acid, rosoxacin, rosaramycin, tetracycline and Sch 32063. Human isolates were significantly more sensitive from isolates of samples collected from pigs as following: clindomycin, erythromycin, rosaramycin and Sch 32063. From 11 hypurat negative human samples, 3 (27%) were resistant to clindomycin, erythromycin, rasaramycin and Sch 32063. Almost all isolates from human samples and from pigs were sensitive to furazolidon and nalidixic acid. *Campylobacter* isolates from human and pigs had different antibiogram and antibiotic resistance.

According to literature data the aim of our examination was to affirm antibiotic resistance of *Campylobacter coli* strains isolated from samples collected from clinically healthy pigs, to antibiotic that commonly use in therapy in pigs, but also in humans.

Material and methods

As a material the pig carcasses on the slaughter line were used. Pigs were slaughtered in the same slaughterhouse, originated from the same farm and fed in the same way. They were not treated with antibiotics and were clinically health.

Swab samples were taken from peritoneum, bill bladder and caecal mucosa. Methods for isolation of *Campylobacter coli* is described earlier (S.

Ivanović, 2005).

From all of identified isolates were picked 18 strains. For examination of sensitivity of *Campylobacter coli* on antibiotics (erythromycin, ampicillin and tetracycline) both dilution and diffusion methods on the solid medium Columbia agar were used.

For diffusion method were used antibiogram tablets with: tetracycline 30 mcg, ampicillin 10 mcg and erythromycin 15 mcg, added in Columbia agar.

In dilution method (method of double dissolving of antibiotics) examined minimal inhibitory concentration (MIC) in Columbia agar with 5% horse blood and examined concentration of antibiotic.

Tetracycline was dissolved in sterile distilled water. Start solution for preparing of other solutions was 500 mcg/mL. Used concentrations were: 25 mcg/mL, 6.25 mcg/mL, 3.12 mcg/mL and 0.78 mcg/mL.

Ampicillin was dissolved in 0.1 M phosphate puffer at pH 8. Start solution was 500 mcg/mL. Used concentrations were 25 mcg/mL, 12.5 mcg/mL, 6.25 mcg/mL, 3.12 mcg/mL and 1.56 mcg/mL.

Erythromycin was dissolved in 96% ethanol. Start solution was 31.2 mcg/mL. Used concentration was 1.56 mcg/mL, 0.78 mcg/mL, 0.39 mcg/mL and 0.19 mcg/mL.

Solid medium, Columbia agar, was inoculated by bacteriological loop with 10^6 bacteria/mL and incubated under microaerophilic conditions. Interpretation of obtained results was in according to proposition of European Work Group (1996):

Diffusion method inhibition zone in mm:

Ampicillin: sensitive 21 and more; intermediary 19-20; resistant 18 and bellow

Erythromycin: sensitive 26 and more; intermediary 23-25 ; resistant 22 and bellow

Tetracyclin: sensitive 26 and more; intermediary 23-25 ; resistant 22 and bellow

Dilution method MIC in mcg/ml:

Ampicillin: sensitive 6,25 and less; intermediary 12,5; resistant 25

Erythromycin: osetljiv 1,56 and less; intermediary 3,13; resistant 6,25 and more

Tetracyclin: osetljiv 1 3,13 and less; intermediary 6,25 ; resistant 12,5 and more

Results and discussion

Based on obtained results of examination the reliability and sensitivity both dilution and diffusion methods were compared.

Using diffusion method, according to proposition of European Work Group:

- All strains were sensitive to erythromycin,
- All strains were resistant to tetracycline,
- 16 strains were sensitive to ampicillin, and
- 2 strains were intermediate to ampicillin (strain 4 and 12)

Using dilution method, according to proposition of European Work Group:

- All strains were sensitive to erythromycin,
- All strains were resistant to tetracycline,
- 16 strains were sensitive to ampicillin, and
- 2 strains were intermediate to ampicillin (strain 4 and 13)

Obtained results can be compared with literature data, particularly with data of *Hariharan et al.*, (1990). They have examined the sensitivity of *Campylobacter jejuni* and *coli* to some of antibiotics. As samples they have used caecum of slaughtered pigs, taken from the store. From all of 109 samples they have got 62 isolates of *Campylobacter jejuni* and *coli*. Authors were tested sensitivity to erythromycin, tetracycline, kanamycin and ampicillin. Eleven isolates have shown multiple resistances. These isolates were sensitive also to nitrofurantoin, gentamycin and chloramphenicol.

In our country, *S. Ivanović* (2000), has investigated antibiotic sensitivity of *Campylobacter coli* by both dilution and diffusion methods to erythromycin, tetracycline and ampicillin. Tests have shown in both method sensitivity to erythromycin, resistance to tetracycline. Eight strains were sensitive to ampicillin and one was intermediate.

Partly disagreement of results obtained in this paper with literature data can be consequence of environment and mode of breeding of animals.

Results from this paper we compared with data of antibiotic sensitivity of humane isolates of *Campylobacter jejuni/coli* that we got in earlier period from Institute of public health Belgrade. During 1998 it was examined the antibiotic sensitivity of 105 strains of *Campylobacter jejuni/coli* by diffusion method, to antibiotic that were commonly used in therapy (erythromycin, tetracycline and gentamycin) and chloramphenicol

 Antibiotic sensitivity of humane isolates of *Campylobacter jejuni/coli*

<i>Antibiotic</i>	<i>R(%)</i>	<i>S(%)</i>
<i>Erythromycin</i>	3	97
<i>Tetracyclin</i>	0	100
<i>Gentamycin</i>	9	91
<i>Ampicillin</i>	53,9	46,1
<i>Chloramphenicol</i>	0	100

R-resistant, S-sensitive

Use of some antibiotic in preventive purposes is common practice in intensive animal breeding. This use change microbial flora of treated animals. In inadequate use of antibiotics can often develop the resistance of bacteria. Use of antibiotics in therapy of diseases often have weakly activity or do not show their specific activity.

Less sensitivity of humane strains is probably consequence of nonselective use of penicillin in therapy of bacterial infections.

Similarities and differences between examined strains of *Campylobacter* spp. isolated from pigs and humans make the question if human infections results as consumption of contaminated meat.

In some animals, *Campylobacter* spp. persist as facultative pathogens, but in others it has role of infection source and vector of infection is presented as meat of these animals. Passing of these bacteria through organisms both animals and humans can be influenced by positive correlation of agents virulence and macro organism, as well appearance of diseases.

Conclusion

Obtained results show that the tolerance of some antibiotics can be changed and resulted by partly resistance to antibiotics that commonly used in therapy of animals and humans. *Campylobacter coli* is resistant to tetracycline isolated from animal and human isolates. Strains isolated from pigs and humans are sensitive to erythromycin. 11.11% of strains isolated from pigs are resistant to ampicillin and 53.9% from humans.

NALAZ *Campylobacter coli* KOD ZAKLANIH SVINJA I NJEGOVA OSETLJIVOST NA ANTIBIOTIKE

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Rezime

Campylobacter coli je zoonotska bakterija i kao saprofit perzistira kod svinja koje čine prirodni rezervoar. Ova bakterija prenosi se na ljude mesom i proizvodima od mesa. *Campylobacter coli* je osetljiv na antibiotike, ali u jednom momentu može postati i rezistentan. Cilj naših istraživanja bio je da se potvrdi osetljivost *Campylobacter coli*, izolovanog iz uzoraka prikupljenih od klinički zdravih svinja, na liniji klanja, prema sledećim antibioticima: eritromicin, ampicilin i tetraciklin. Od svih identifikovanih izolata odabrano je 18 sojeva i oni su ispitivani na osetljivost na antibiotike metodom dilucije i difuzije. Dobijeni rezultati interpretirani su u skladu sa predlogom Evropske radne grupe za ispitivanje rezistencije bakterije na antibiotike (1996). Korišćenjem obe metode dobijeni su sledeći rezultati: svi sojevi su osetljivi na eritromicin, svi sojevi su rezistentni na tetraciklin, 16 sojeva je rezistentno na ampicilin, dva soja su intermedijarna prema ampicilinu (soj 4 i 13). Upotreba antibiotika u profilaktičke svrhe intenzivna je u uobičajenoj praksi uzgoja životinja. Njihovo korišćenje može da promeni mikrobiološku floru kod tretiranih životinja i njihova neadekvatna aplikacija često može da dovede do rezistentnosti bakterija.

Ključne reči: *Campylobacter coli*, svinjsko meso, antibiotici, rezistentnost

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