ANTIMICROBIAL RESISTANCE OF FOODBORNE PATHOGENS AS A FOOD SAFETY ISSUE

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Summary

Antimicrobial resistance is a daunting public health threat impacting both human and animal health and it is a cause for concern wherever antimicrobial agents are in use.

The usage of antimicrobial drugs in food producing animals could results in significant food safety issue - antimicrobial resistance among zoonotic bacteria in these animals. Resistance monitoring program still does not exist in Serbia, so we made a pilot program to screen the situation in our abattoirs. We found similar situation like the one in EU. The resistance to one or more antimicrobial drugs was found in 25.00% and 40.57% *Salmonella spp* and 40.00% and 75.32% *Campylobacter spp* strains isolated from poultry and pig carcasses, respectively.

The resistant bacteria may then be transmitted to humans through food supply and increase the risk of treatment failures. Resistant zoonotic pathogens in food have to be controlled through a complete, continuous farm-to-fork system.

Key words: antimicrobial resistance, food safety

Antimicrobials are grouped into classes on the basis of chemical structure and mode of action. Most antimicrobials used for the treatment of animals belong to classes that are also used in human medicine. Cloxacillin and dicloxacillin are used both in human and veterinary medicine also as gentamicin, ampicillin, amoxicillin etc. The usage of antimicrobial drugs in food producing animals could results in antimicrobial resistance among pathogenic and commensal bacteria in these animals, and the resistant bacteria may then be transmitted to humans through the food chain and increase risk of treatment failures. The consequence of resistance to certain antimicrobials especially fluoroquinolones as well as to 3rd and 4th generation cephalosporins and macrolides, are of particular concern, since these are critically important for therapy of human systemic bacterial infections.

Antimicrobial resistance in *Salmonella spp*, *Campylobacter spp* and other bacteria is an increasing public health problem. Resistant *Salmonella* and *Campylobacter* involved in human disease are mostly spread through foods. Contaminated poultry meat, eggs, pork and beef are prominent in this regard. There is a temporal association between the introduction of fluoroquinolones for use in poultry and a substantial rise in the prevalence of quinolone-resistant *C. jejuni* isolated in live poultry, poultry meat and infected humans. Moreover, prior to use in poultry treatment, no resistant strains were reported in humans with no previous exposure to quinolones

(6). A correlation between introducing of fluoroquinolones in treatment of animals and cases of resistant *Campylobacter* was noticed in Holland and USA (1, 2). Avoparcin was a frequently used growth promoter in poultry until its ban in Denmark in 1995 because of its association with the development and spread of vancomycin-resistant enterococci (in 80% of poultry). Resistance was also found in humans. After banning avoparcine, cases of vancomicine resistant enteroccoci decreased to 3% (7).

The use of antimicrobials at sub therapeutic levels in food producing animals has long been viewed as undesirable e.g. the Swann report 1969. Since January 2006 the use of all antimicrobial feed additives has been banned within the EU in order to reduce the number of resistant bacteria in farm animals Regulation (EC) No 1831/2003. The effect of this ban on the extent of bacterial antimicrobial resistance both within farm animals, and with regard to human health, however, is as yet unknown (12).

The WHO, FAO, including Codex, and OIE have each (individually or jointly) reviewed the area and provided guidelines, recommendations and lists of clinically important antimicrobials. The latest activity in this area is a Codex Task Force, which aims to assess the risks to human health associated with the presence in food and feed of antimicrobial-resistant organisms, antimicrobial resistance genes and residues of antimicrobials, and to develop risk management advice based on that assessment to reduce such a risk.

Food borne pathogen resistance

Zoonoses are diseases or infections, which are transmissible from animals to humans. The zoonosis which occur most frequently in the developed world today are food-borne infections caused by *Salmonella, Campylobacter, VTEC, Yersinia, Listeria* etc.

Although various foods can serve as a source of food borne illness, meat and meat products are important sources of *Salmonella* and *Campylobacter*. These two pathogens are most frequently reported causes of zoonotic diseases in EU in 2005, incidence of 38.2-51.6 cases per 100 000 population (11). These bacteria's are reported in all meat producing animals, they are widespread in poultry production in Europe. *C. jejuni* is frequent commensal in poultry and cattle, and *C. coli* in swine and poultry. In red meat animals, *Salmonella* was most frequently found in pigs, followed by cattle. Poultry meat and eggs represent an important source of human infection with *Salmonella spp. S. enteritidis* and *S. typhimurium* are the most commonly reported strains isolated from poultry meat products and human cases of salmonelosis. The most common way that humans become infected with zoonotic enteric pathogens is through the ingestion of food contaminated with animal feces (contamination usually occurs during processing). Oral medication of large groups of animals is particularly likely to favor emergence of and selection for resistant microorganisms. Also, in animal production conditions exist that facilitate the spread of bacteria, such as high density and/or poor infection control.

Food borne diseases caused by *Salmonella spp*. and thermopile *Campylobacter spp*. usually do not demand antimicrobial therapy. When antimicrobials are indicated (immuno-deficient patients etc.) for the treatment of campylobacter gastroenteritis, erythromycin or a fluoroquinolone such as ciprofloxacin is the drug of choice (8).

Occurrence of food borne patogen resistance

Antimicrobial drug resistance in food borne pathogens is a common problem in many European countries. Application of antimicrobial drugs in veterinary medicine has been monitored in EU, as well as the resistance of human and zoonotic pathogens (Table 1).

Monitoring program has not been applied in Serbia. Therefore, Veterinary Directorate of the Ministry of Agriculture financed three projects of our Institute to screen the situation in Serbia. Performed examinations have found similar situation like in EU. The occurrence of *Salmonella* and *Campylobacter* is very frequent in pig and poultry carcasses (Table 2 and 3). The occurrence of these bacteria varies depending on the range of factors including the organism, geographical factors, farming and meat production practice. Studies have reported that poultry can become contaminated with *Salmonella* and *Campylobacter* at the farm level (9, 10). These organisms can be transported to the production facility and can contaminate the processing environment and the final product. The influence of farm and production management and is great, since in some abattoirs *Campylobacter spp.* was found on 11.43% carcasses, and in other even on 90.00% carcasses. Similar situation was found in pig abattoirs.

Table 1

Prevalence of resistant *Campylobacter spp.* and *Salmonella spp.* strains isolated from food (3, 13)

Antimicrobial drug	Prevalence of resistant strains (%)			
	Austria		Holland	
	Salmonella spp	*Campylobacter spp*	*Salmonella spp*	*Campylobacter spp*
Fluoroquinolones	42.0	40.7	32.7	26.9
Tetracyclines	33.0	/	21.5	57.1
Streptomycin	27.0	9.7	0.0	51.3
Ampicillin	17.0	8.6	43.9	16.8
Erythromycin	/	4.0	0.1	34.5
Gentamicin	/	1.5	0.0	16.8
Chloramphenicol	/	0.7	0.0	0.0
Trimethoprim	/	/	58.9	/
Trim/Sulfametho	/	/	48.6	37.0
xazole	/	/	0.9	0.0
Florfenicol	/	/	/	37.8
Metronidazole				

Resistant *Salmonella spp.* was isolated from 40.57% poultry samples and 25.0% pig samples. Resistance was most frequently found to amoxicilline and sulphametoxazole. Most of the *S. enteritidis* strains were sensitive (66.67% - poultry samples and 100% - pig samples). Only one strain was resistant to three antimicrobial drugs: doxycycline, chloramphenicol and sulphometoxasole. Resistance to chloramphenicol is very important fact, because this drug is banned for treatment of the food producing animals. Since *S. typhimurium* causes more serious consequences on human health than *S. etneritidis*, alarming information is that the frequency of resistance of this microorganism was very high; 76.92% *S. typhimurim* strains isolated from poultry samples and 33.33% strains isolated from pig samples were resistant. In both pigs and poultry samples resistance was most frequently determined to amoxicilline and sulphometaxazole. Similar data were found by other authors. According to MARAN report in 2002, in Holland *Salmonella* was most frequently resistant to amoxicilline, doxycycline, trimethoprime and sulfamethoxazole (13).

Campylobacter spp. was more frequently isolated from pig carcasses than *Salmonella*. The occurrence of these bacteria in carcasses varies depending on abattoir. On pig carcasses only *C. coli* were isolated while in poultry *C. jejuni* dominated (89.87% of isolated *Campylobacter* strains). Resistance is more frequently found in *Campylobacter* than in *Salmonella*; 75.32% *Campylobacter* strains isolated from poultry samples and 40.00% strains isolated from pig samples were resistant.

Table 2

Occurrence of *Salmonella spp*. and *Campylobacter spp*. on pig carcasses (5)

Occurrence of pathogens on carcasses (%)	Abattoir mark				
	A	B	C	D	E
Salmonella spp	1.85	6.06	3.70	1.85	0.00
Campylobacter spp.	3.70	22.22	14.81	5.55	1.85

Multidrug resistance showed 75% of *Campylobacter* strains isolated from pig samples and 82.79% strains isolated from poultry samples. *Campylobacter* strains isolated from pig samples were resistant to amoxicilline, streptomycine and sulfamethoxazole, while *Campylobacter* strains from poultry carcasses were resistant mostly to trimethoprime 48.734%, ciprofloxacine 46.20%, and amoxicilline 40.51%. Resistance to erythromycin (7.59% strains) has special importance because this antimicrobial is the drug of first choice for the treatment of human enteric infection caused by *C. jejuni* and *C. coli*.

Strains isolated from poultry showed in the MARAN (13) report, as well as study, that *Salmonella* present the highest rate of resistance to amoxicilline and *Campylobacter* to trimethoprime and ciprofloxacine. The resistance patterns of *Campylobacter spp* and *Salmonella spp* strains found in this study are correlated to

the antimicrobial drugs most widely used in veterinary medicine in Serbia (beta-lactam antibiotics and fluoroquinolones).

Table 3

Occurrence of *Salmonella spp*. and *Campylobacter spp*. in poultry samples (4)

Occurrence of pathogens (%)		Abattoir mark						
		A	B	C	D	E	F	G
Salmonella spp.	liver	22.86	32.50	11.43	6.00	60.00	20.00	3.70
	carcasses	2.86	5.00	5.71	2.00	8.57	2.86	0.00
Campylobacter spp.	liver	40.00	5.00	8.56	6.00	34.28	2.86	5.71

Although the multidrug resistance patterns were different, the high frequency of multidrug resistant *Campylobacter spp.* strains found in the present study suggests that strict measures have to be taken to ensure proper use of antimicrobials in veterinary medicine in Serbia. Animals and food of animal origin play an important role in the transmission of campylobacteriosis and salmonelosis to humans. So the findings of multidrug resistant *Campylobacter spp.* and *Salmonella spp.* strains from domestic animals stressed the importance of epidemiological surveillance and preventive actions that may avoid the dissemination of these strains over animal and human populations.

Based on the performed research it may be concluded that the resistance of food borne pathogens is present in Serbia. It may be considered as an important hazard for human health since a considerable number of isolated pathogens were resistant.

Table 4

Prevalence of resistant *Campylobacter spp.* and *Salmonella spp.* strains isolated from poultry and pig abattoirs (4, 5)

Antimicrobial drug	Resistant strains (% of total isolates)			
	Salmonella spp.		*Campylobacter spp*	
	Poultry carcasses	Pig carcasses	Poultry carcasses	Pig carcasses
Amoxicillin 25	23.19	25.00	40.51	10.00
Cefuroxim 30	0.00	0.00	/	/
Imipenem 10	0.00	0.00	1.90	0.00
Gentamicin 10	7.25	0.00	5.69	0.00
Doxycyline 30	4.35	0.00	8.23	0.00
Trimethoprim 5	0.00	0.00	48.73	0.00
Ciprofloxacin 5	0.00	0.00	46.20	0.00
Chloramphenicol 30	1.45	0.00	0.00	0.00
Streptomycin 10	5.80	0.00	16.46	40.00
Sulfamethoxazole 100	13.04	25.00	8.23	30.00
Erythromycin 15	/	/	7.59	0.00

Control strategies

In order to continue decreasing the number of resistant pathogens, partners from the farming, veterinary, medical and public health communities will need to work together to prevent the misuse and overuse of antimicrobials. Besides preventive and hygiene-sanitary measures, the problem of pathogen resistance demands introducing system of surveillance and applying control system in the primary production. This can provide control of distribution, application of antimicrobial drugs and carrying out adequate resistance monitoring program. EU regulates the conditions and paths of monitoring through Directive 2003/99EC, also in 2001 WHO published Global Strategy for Limiting Antimicrobial Resistance where the recommendations and instructions for patients, doctors, public and drug sellers are given.

Conclusion

Zoonotic pathogens in food have to be controlled through a complete, continuous farm-to-fork system. Application of prophylactic and zoosanitary measures in farm breeding, slaughtering, processing of meat and safe disposal of animal wastes may considerably reduce risk from the incidence of zoonotic pathogens.

References

1. **Endtz, H., Ruijs, G., Van Klingeren, B., Jansen, W., Van der Reyden, T., Mouton, R.**, Quinolone resistance in Campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine, J Antimicrob Chemother, 1991, 27, 2, 199-208.
2. **Engberg, J., Aarestrup, F., Taylor, D., Gerner-Smidt, P., Nachamkin, I.**, Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates, Emergening Infectious Deseases, 2001, 7, 1, 24-34.
3. **Mayrhofer, S., Paulsen, P., Smulders, F.**, Hilbert, F., Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. Int J of Food Microbiol., 2004, 87, 1, 23-29.
4. **Petrović, J., Baltić, M., Kljajić, R., Kapetanov, M.**, Rezistencija zoonotskih patogena koji poticu iz mesa živine prema antimikrobnim lekovima, Tehnologija mesa, 2007a, 301, 147-152.
5. **Petrović, J., Karabasil, N., Baltić, M., Kljajić, R., Dimitrijević, M., Krasić, J.**, Ispitivanje osetljivosti na razlicite antimikrobne lekove sojeva salmonella izolovanih u lancu proizvodnje mesa živine i svinja. 1. Međunarodni kongres Tehnologija, kvalitet i bezbednost hrane. I Simpozijum biotehnologije i mikrobiologije hrane, 13-15.10., Novi Sad, 2007b, 105-111.
6. **Piddock, L.**, Quinolone-resistant campylobacetr in the United Kingdom. Use of quinolones in food animals and potential impact on human health. Report and

Proceedings of a WHO Meeting.Geneva, Switzerland, 2-5 June, 1998, 189-198.

7. **Prescott, J., Baggot, J., Walker, R.**, Antimicrobial therapy in veterinary medicine. Third Edition. Iowa State University Press/ Ames, 2000.
8. **Smith, K., Besser, J., Hedberg, C., Leano, F., Bender, J., Wicklund, J., Johnson, B.**, Quinolone-resistant Campylobacter jejuni infections in Minnesota, 1992-1998. Investigation Team. N Engl J Med, 1999, 340, 20, 1525-1532.
9. **Stojanov, I., Orlić, D.**, Infections in parental flock and eggs on Campylobacter and Salmonella strains, Zbornik Matice srpske za prirodne nauke, 2000, 101, 101-107.
10. **Stojanov, I., Orlić, D.**, Types of Campylobaster strains in laying hens with or without diarrhoea, Zbornik Matice srpske za prirodne nauke, 2001, 98, 61-66, 3.
11. EFSA. 2005. EFSA Report of the EFSA Panel on Biological Hazards on the request from the Commission related to Campylobacter in animals and foodstuffs. Annex to The EFSA Journal 173, 1-105,
12. EFSA. 2008. Food borne antimicrobial resistance as a biological hazard. Draft Scientific Opinion of the Panel on Biological Hazards (Question No EFSA-Q-2007-089).Draft endorsed on 6 March 2008;
13. MARAN. 2002. Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2002.