Antimicrobial resistance observed in *Escherichia coli* isolates from pigs

Jadranka Žutić[1], V. Radosavljević[1†], O. Radanović[1], S. Mrenoski[2], D. Bacić[3], M. Žutić[1]

1 Institute of Veterinary Medicine of Serbia, 11000 Belgrade, Serbia; 2 Faculty of Veterinary Medicine Skopje, 1000 Skopje, Macedonia; 3 Faculty of Veterinary Medicine, 11000 Belgrade ,Serbia

SUMMARY

A total of 74 *Escherichia coli* strains isolated from piglets were examined for sensitivity to 10 antimicrobials, using the disc diffusion method. The samples for the investigations were rectal swabs from piglets with clinical signs of diarrhea or parts small intestine with pathoanatomical changes from piglets that died. All the samples originated from suckling piglets aged 1-30 days. *E. coli* isolates showed highest of resistance to tetracycline (91%) and ampicillin (81%). Resistance to streptomycin, neomycin, trimethoprim/sulfamethoxazole and gentamicin was established in 69%, 64%, 61% and 54% strains of *E. coli*. The lowest percentage of resistance to ceftiofur was established in 5% strains. The results of this study indicate a high resistance of isolated strains of *E. coli* to the antimicrobials that are often used in veterinary practice. These results have important implications with regards to the spread and persistence of resistance in bacterial populations and to the prudent use of antimicrobial agents

Keywords: pig, *E.coli*, resistance, antimicrobials

INTRODUCTION

There are numerous factors in contemporary pig production that affect the presence of different infective diseases in piglets maintained in large agglomerations. Diseases of the digestive system are among the major causes of death in piglets (Genovese et al., 2001).

In countries with an intensive pig production, including Serbia, diarrhoea caused by enterotoxic strains of *E. coli* (ETEC) is one of the most frequent and economically most significant diseases (Zutic et al., 2010). Occurrence of diarrhoea during the neonatal period implies the disease in susceptible piglets

† Corresponding author e-mail: vladimiradosavljevic@yahoo.co.uk

infected with ETEC. Different efforts have been made to control diarrhoea in neonatal piglets so far, and, consequently, the obtained results also have varied. Maternal vaccination has been one of the most effective ways to prevent neonatal ETEC diarrhoea in piglets (Eggen, 2009).

Among numerous measures, the use of antibiotics is one of the options undertaken for the treatment (Brito and Tagliari, 2000). But, the occurrence of multiresistant *E.coli* isolates from pigs has been rapidly increased in recent years (Boerlin et al., 2005; Costa et al., 2010).

A relationship has been demonstrated between the use of antimicrobials in pig herds and the increased occurrence of resistant bacterial strains in the digestive tracts of pigs (Bibbal et al., 2009). Antimicrobial-resistant bacteria are now frequently isolated from the commensal gut microbiota of food animals (Aalbaek et al., 1991; Amyes 1987). While resistance in such commensal bacteria is not a problem in itself, the possible transfer of resistance elements to zoonotic pathogens within the gut has serious implications for public health. Consequently, the need to reduce antimicrobial consumption within a veterinary context has received much attention (McEwen et al. 2002; Turnidge 2004).

Large quantities of antimicrobial agents are still being used in modern swine production in many countries around the world. Especially in the intensive and large-scale production, the routine use of antimicrobials has become an integrated part of the production system (Aarestrup et al., 2008). The aim of this study was to determine and compare the prevalence and patterns of antimicrobial resistance among 74 *E. coli* isolates from Landrace pigs in four pig farm in Serbia.

MATERIAL AND METHODS

The material for the study was taken from Landrace piglets showing clinical or pathoanatomical changes indicative for infection caused by *E. coli.* Piglets, 1 to 30 days old originated from sows non-vaccinated against *E. coli* infections.

Samples of the small intestine were taken from dead piglets, and rectal swabs from diseased animals.

Due to a rapid progression of disease, diseased piglets were treated usually at a late stage of disease with different antibiotics (streptomycin, neomycin, gentamycin, enrofloxacin, Trimethoprim/sulfamethoxazole). Rectal swabs were taken before the start of therapy.

The samples were inoculated on blood agar plate (5% sheep's blood) and MacConkey agar (HiMedia) by surface streaking. After overnight incubation at 37^{o}C under aerobic condition, cultures were examined for the presence of

characteristic *E. coli* colonies. After pure cultures were obtained, colonies were inoculated on TSI and identification of the bacteria was accomplished by biochemical tests, including IMVIC test. The identification was confirmed using BBL Crystal, E/N, ID kit (Becton Dickinson).

All isolates of *E. coli* were tested for susceptibility to 10 antimicrobial agents. Antimicrobial susceptibility testing was done by disc diffusion on Mueller-Hinton (MH) agar (bioMerieux) following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2008). The antimicrobial agent discs used in this study were: ampicillin (10µg), ceftiofur (30 µg), colistin (10µg), tetracycline (30µg), streptomycin (10µg), neomycin (30µg), gentamicin (30µg), enrofloxacin (5µg), trimethoprim-sulfamethoxazole (1.25/23.75µg) and florfenicol (30µg). The zone diameters around all the discs were interpreted according to the CLSI (2008). *E. coli* ATCC 25922 was used as a reference strain for quality control of the antimicrobial susceptibility testing.

RESULTS AND DISCUSSION

A total of 74 (57%) isolates of *E. coli* strains were isolated from 129 piglets located in four pig farms located in central region of Serbia. In the present study, 41 (61%) strain of *E. coli* was isolated from the 67 samples of small intestine in pure culture, and 33 (53%) strains were isolated in pure culture from 62 rectal swabs.

In mixed cultures, *E. coli* strains are not taken for further research. Among them, besides *E. coli*, *Isospora suis* and *Clostridium perfringens* were the most frequently found. Virological examination of those samples was not performed in this study.

Results of our study reveal a high resistance of isolated strains to the antimicrobials that are often used in veterinary practice. Prevalence of antimicrobial resistance of the *E. coli* isolates is shown in Figure 1.

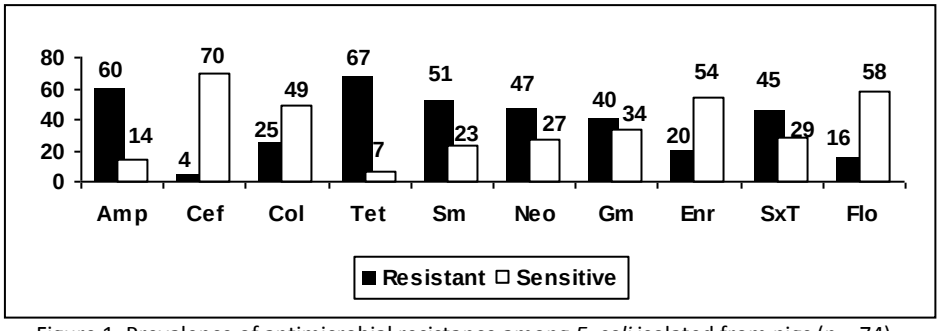

Figure 1. Prevalence of antimicrobial resistance among *E. coli* isolated from pigs (n = 74); Antimicrobial agents: Amp, ampicillin; Cef, ceftiofur; Col, colistin; Tet, tetracycline; Sm, streptomycin; Neo, neomycin; Gm, gentamicin; Enr, enrofloxacin; SxT, trimethoprim/ sulphamethoxazole; Flo, florfenicol

High prevalence of resistance was found to tetracycline (91%). This agrees with Asanin et al. (2009) who had reported resistance of 89% and Burch (2005) 85%.

Approximately 81% of the *E. coli* isolates showed resistance to ampicillin. This result is similar to 85% found by Habrun et al. (2010). On the other hand, the present findings was higher than 42% in Brazil (Costa et al., 2010) and 59% (Choi et al., 2002) in *E. coli* isolates from pigs in Korea. Resistance to streptomycin, neomycin and trimethoprim/sulfamethoxazol was 69%, 64% and 61%, respectively. However, present finding was lower than 83% for streptomycin reported by Wang et al. (2011) and 64% for trimethoprim/sulfamethoxazol (Choi et al., 2002).

Table 1. Percentage of susceptibility to antibiotics of isolated E. coli strains. R, resistant; S, sensitive. Corresponding antibiotic concentrations are listed in Materials and methods

Antibiotic	S	R
Ampicillin	19 %	81 %
Ceftiofur	95 %	5 %
Colistin	66 %	34 %
Tetracycline	9 %	91 %
Streptomycin	31 %	69 %
Neomycin	36 %	64 %
Gentamicin	46 %	54 %
Enrofloxacin	73 %	27 %
Trimethoprim/sulfamethoxazol	39 %	61 %
Florfenicol	78 %	22 %

Significantly lower rates of resistance to streptomycin have recently been reported among *E. coli* isolates from swine faeces in France (Bibbal et al., 2009). Resistance to gentamicin was found in 54% of isolated *E. coli* strains, which is higher than 39% founded in pigs with septicaemic lesions in Romania (Herman et al., 2010).

Lower prevalence of resistance was found to colistin (34%), enrofloxacin (27%) and florfenicol (22%). This is considerably higher than the results from Lyutskanov and Urumova (2010) who had reported 6% resistance for colistin and 19% for enrofloxacin in suckling pigs in Bulgaria and 12% in isolates from clinical samples in Brasil (Costa et al., 2010). However, some studies have previously reported 78% percent of resistant isolates to enrofloxacin in pathogenic *E. coli* isolates from diseased pigs (Wang et al., 2011). In contrast, Burch (2005) reported 11% of resistant *E. coli* strains to enrofloxacin. Only five percent of isolates were resistant to ceftiofur. Ceftiofur was also active against *E. coli* isolates in many cases (Choi et al., 2002; Boerlin et al., 2005).

Certain classes of antimicriobials which are recently introduced in veterinary medicine, such as cephalosporins and fluoroquinolones, are still very effective against most of the isolates, and can be used in most cases. However, these antimicrobial classes are also considered critically important for human health and their use in food animal production should be limited or avoided as far as possible (Aarestrup et al., 2008).

Out of total number strains, 1% of *E. coli* isolates were resistant to one and 27 % to 2-4 antimicrobials. Highest resistance (32%) was established to five antimicrobials. The lower levels of resistance, 27% and 12%, were found to 6, and 7-8 antimicrobials, respectively. This is in accordance with Akwar et al. (2008), who found the most multi-drug resistance patterns to 2 to 6 antimicrobials. The most prevalent resistance patterns are shown in Figure 2.

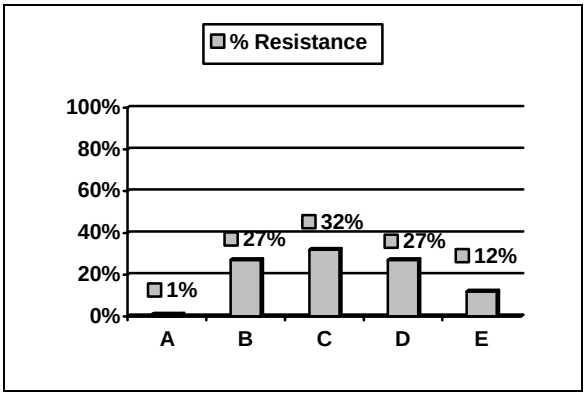

Figure 2. Resistance patterns among *E. coli* isolates from pigs. A-resistance to one antibiotic, B-resistance to 2-4 antibiotics, C-resistance to 5 antibiotics, D-resistance to 6 antibiotics, E-resistance to 7-8 antibiotics

It is an undeniable fact that today, in intensive pig production, neonatal diarrhoea caused by *E. coli* is one of the most present and economically most significant diseases that lead to major losses in the early postnatal period (Zutic et al., 2010).

Cautious use of antimicrobial agents for treatment of infections requires knowledge about the susceptibility of the infectious strain. Antimicrobials are used in swine production for therapeutic, metaphylactic and prophylactic purposes.

Various antimicrobial resistance patterns have been observed in several studies among strains of *E. coli* from different geographical regions. While most *E. coli* isolates are reported to be susceptible toward ceftiofur and florfenicol, resistances toward tetracycline and ampicillin was detected in many cases.

However, it is not possible to predict for *E. coli* whether an isolate is susceptible or resistant, and choice of empiric treatment has to be made on the basis of knowledge of the individual herd and local data on resistance patterns. This is why routine submission of samples to a microbiological laboratory is important to generate records on susceptibility data (Guardabassil et al., 2008).

E. coli infections can be controlled by means of good management practices and high hygiene standards, as well as by vaccination of sows, but also to a great extent to the programmes for medication control.

Bacteria have well-developed genetic adaptation mechanisms, which lead to the development of resistance. This is particularly common on farms, due to the fact that it is not possible to treat only diseased animals, but also those that are in direct contact, and thus exposed to infection. When treating an infection, antibiotics cannot distinguish between pathogenic bacteria and the normal microbiota, and in this case resistance develops in non-pathogenic bacteria, creating a reservoir of resistance genes in nature. There are many evidences that the degree of resistance to some antibiotics depends on the consumption of antibiotics. Consumption of certain antibiotic varies among farms, regions and countries, and it is directly reflected in the intensity of resistance to this antibiotic. Therefore, it is necessary to continuously monitor the resistance of *E. coli* isolates in piglets to the antibiotics commonly used in specific area.

CONCLUSIONS

Our results showed high prevalence of antimicrobial resistance among *E. coli* strains isolated from piglets. These results suggest the possibility of spreading of resistance not only among strains of *E. coli*, but also to other bacteria. The findings also provide further evidence that use of antimicrobial substances in intensive swine production represents selective pressure for antimicrobial resistance of *E. coli* in pigs.

ACKNOWLEDGEMENTS

This research has been financially supported by Ministry of Education, Science and Technological Development of the Republic of Serbia, Project No. TR31079.

REFERENCES

Aalbaek, B., Rasmussen, J., Nielsen, B., and Olsen, J. E., 1991. Prevalence of antibiotic-resistant *Escherichia coli* in Danish pigs and cattle. APMIS 99**:**1103-1110.

Aarestrup F.M., Duran C.O., Burch D.G.S., 2008. Antimicrobial resistance in swine production. Animal Health Research Reviews 9(2); 135–148

Akwar H.T., Poppe C., Wilson J., Reid-Smith RJ., Dyck M., Waddington J., Shang D., McEwen SA., 2008. Prevalence and patterns of antimicrobial resistance of fecal *Escherichia coli* among pigs on 47 farrow-to-finish farms with different in-feed medication policies in Ontario and British Columbia. Can J Vet Res. 72(2): 195–201.

Amyes, S. G. B., 1987. Trimethoprim resistance in commensal bacteria isolated from farm-animals. Epidemiol. Infect. 98**:**87-96.

Asanin R., Zutic M., Asanin J., Misic D., Zutic J., Jakic-Dimic D., Milić N., Nišavić N., 2009. Ispitivanje prisustva novih oblika rezistencije na neke antibiotike kod sojeva *E.coli* izolovanih od prasadi. Veterinarski Glasnik 63 (5-6).311 - 320.

Bibbal, D., Dupouy, V., Prere, M. F., Toutain, P. L., Bousquet-Melou, A., 2009. Relatedness of *Escherichia coli* Strains with Different Susceptibility Phenotypes Isolated from Swine Feces during Ampicillin Treatment. Applied and Environmental Microbiology.p. 2999–3006.

Boerlin P, Travis R, Gyles C.L., Reid-Smith R., Janecko N., Lim H., Nicholson V., McEwen, R., Archambault M., 2005. Antimicrobial Resistance and Virulence Genes of *Escherichia coli* Isolates from Swine in Ontario.Applied and Environmental Microbiology p. 6753-6761.

Brito B.G., Tagliari K.C., 2000. Antimicrobial sensitivity of *Escherichia coli* strains isolated from suckling piglets with diarrhoea. Revista Brasileira de Ciência Veterinária (2): 117-119.

Burch D., 2005. Problems of antibiotic resistance in pigs in the United Kingdom, Practice 27**:**37**-**43.

Costa M.M., Drescher G., Maboni F., Weber S.S., Schrank A., Vainstein M.H., Schrank I.S., Vargas A.C., 2010. Virulence factors, antimicrobial resistance and plasmid content of *Escherichia coli* isolated in swine commercial farms. Arch. Bras. Med. Vet. Zootec. Vol.62 (1):30-36.

Choi C., Ham H.J., Kwon D.,Kim J., Cheon D.S., Min K., Cho W.S., Chung H.K., Jung T., Jung K., Chae C., 2002. Antimicrobial susceptibility of Pathogenic *Escherichia coli* isolated from pigs in Korea. J Vet Med Sci. 64(1) 71-73.

Eggen A.S., 2009. Maternally derived antibodies: Background, function and importance, *Pig Progress*, 25, 2, 1-4.

Genovese K.J., Harvey RB., Anderson RC.,Nisbet DJ. 2001. Protection of Suckling Neonatal Pigs Against Infection with an Enterotoxigenic

Escherichia coli Expressing 987P Fimbriae by the Administration of a Bacterial Competitive Exclusion Culture. Microbial Ecology in Health and Disease, 13: 223–228

Guardabassil L., Jensen L.B., Kruse H. Guide to antimicrobial use in Animals. Blackwell. 2008.

Habrun B., Kompes G., Cvetnić Z., Spicic S., Benic M., Mitak M., 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. Veterinarski Arhiv 80(5): 571-583.

Herman V, Pascu C., Costinar L., Faur B., Vaduva I., Surpat A., Irimie S., Serbescu M., 2010. *E.coli* strains characterization isolated from pig septicemic colibacilosis. Lucrari Stiintifice Medicina Veterinara vol XLIII(1):93-96.

Lyutskanov M., Urumova V., 2010. Etiology, epidemiological monitoring and therapeutic control of bacterial enteritis among suckling pigs in Bulgaria. Bulgarian Journal of Veterinary Medicine. 13, No 4: 227−234.

McEwen, S. A., and P. J. Fedorka-Cray., 2002. Antimicrobial use and resistance in animals. Clin. Infect. Dis. 34**:** S93-S106.

Turnidge, J., 2004. Antibiotic use in animals-prejudices, perceptions and realities. J. Antimicrob. Chemother. 53**:**26-27.

Wang X.M., Liao X.P., Liu S.G., Zhang W.J., Jiang H.X., Zhang M.J., Zhu H.Q., Sun Y., Sun J., Li A.X., Liu Y.H., 2011. Serotypes, virulence genes, and antimicrobial susceptibility of *Escherichia coli* isolates from pigs. Foodborne Pathog Dis. 8(6):687-92

Zutic J, Asanin J., Misic, D., Jakic-Dimic, D., Milic, N., Asanin, R., Stojanović, D., Zutic, M., 2010. Isolation of ETEC strains from piglets with diarrhea in the neonatal period and their typization based on somatic and fimbrial antigens. Acta Veterinaria (Beograd) 60(5-6), 497-506.