Agronomy Research **18**(4), 2365–2373, 2020
https://doi.org/10.15159/AR.20.170

Special features of *Pseudomonas aeruginosa* strains in animal and poultry farms in the regions with various levels of man-made pollution

I.M. Donnik[1], A.S. Krivonogova[2], A.G. Isaeva[1,2,*], I.A. Shkuratova[2], K.V. Moiseeva[1,2] and N.B. Musikhina[1]

[1]'Ural State Agrarian University' (FSBEI HE Ural SAU), Federal State Budgetary Educational Institution of Higher Education, 42, K. Liebknechta Str., RU620000 Ekaterinburg, Russia
[2]'Ural Federal Agrarian Scientific Research Centre, Ural Branch of the Russian Academy of Sciences' (FSBSI UrFASRC, UrB of RAS), Federal State Budgetary Scientific Institution, 112 A, Belinskogo Str., RU620000 Ekaterinburg, Russia
[*]Correspondence: isaeva.05@bk.ru

Abstract. The research on the *P. aeruginosa* strains in animal and poultry farms located in the areas with various levels of technogenic pollution were done. The content of *P. aeruginosa* in composition of opportunistic pathogenic microflora in dairy, pig-breeding and poultry farms was stated. Susceptibility of *P. aeruginosa* to fluoroquinolone antibiotics and carbapenems was defined. The enterprises were located in the areas with various levels of contamination of agrobiocenosis with Zn, Fe, Cd, Cu, As, Pb, ^{90}Sr, ^{137}Cs of technogenic origin. It was stated that content of *P. aeruginosa* in opportunistic pathogenic microbiota was the most in poultry farms. In man-made polluted areas *P. aeruginosa* was most often found in samples from oral cavity and cloaca of laying hens and broiler chickens, and in 'clean' areas' - mostly in wash-offs from cages and drinking pans. In dairy farms content of *P. aeruginosa* was higher in environmentally friendly areas, as compared to the areas with technogenic pollution. Analysis of antibiotic susceptibility has shown that in dairy farms average level of resistance of *P. aeruginosa* strains to carbapenems and fluoroquinolone was 12% and 6%, in pig-breeding farms - 9% and 13%, and in poultry farms - 6% and 18% correspondingly. At the same time, in environmentally neglected areas significant content of the strains with low susceptibility to the above-mentioned antibiotics was stated. The research is executed at the expense of a grant of the Russian scientific fund (project No. 18-16-00040).

Key words: animal farms, antibiotic susceptibility, opportunistic microbiota, poultry farms, *Pseudomonas aeruginosa*, technogenic pollution.

INTRODUCTION

Pseudomonas aeruginosa is widely spread in opportunistic microbiocenosis of animals and humans. Intensive adaptive potential of this microorganism is explained by a number of its unique special features, such as histolytic activity, genetic flexibility, quorum sensing, and ability to form biofilms, as well as high speed of development of resistance to antibiotics. A significant number of severe nosocomial diseases and complications is caused by *P. aeruginosa*, which is resistant to modern antibiotics. *P. aeruginosa* strains with 100% resistance are more and more often detected in medical enterprises all over the world (Ventola, 2015; von Wintersdorff et al., 2016).

In veterinary enterprises and animal farms *P. Aeruginosa*, as well as *S. aureus*, cause a significant number of purulent-septic infections and complications among fragile, ill and new-born animals, accompanied by injuries, stress, or during pre- or post-operation periods. Highly productive animals are also included into a risk-group because of their chronical physiological deterioration (Donnik et al., 2019). Circulation of *P. aeruginosa* in animal and poultry farms inevitably results in formation of strains resistant to antibiotics. In Russia public supervision over microbial resistance in veterinary and animal farming has been done only for a few last years. Earlier only veterinary specialists monitored microbial antibiotic susceptibility at the local level. Numerous data on opportunistic microbiocenosis in animal farms for the period of 2015–2018 prove a high level of resistant strains in enterprises with various profiles. One of the factors facilitating spread of dangerous strains of opportunistic pathogenic microflora is chronical secondary immunodeficiency of highly productive animals (Koba, 2018). Incompetence of the immune system leads to growing incidence of disease, and need in antibiotic treatment, and results in formation of resistant strains. In a number of regions of the Russian Federation agricultural enterprises are subject to man-made environmental pollution, including pollution of agrobiocenosis. They are mostly regions with developed industries or those damaged after serious man-made accidents (Luo et al., 2012; Alimova et al., 2015; Belykh et al., 2015). High concentrations of metals often are toxic to soil microflora, plants and animals. The toxicity of heavy metals depends on the type of metal element and its bioavailability on the soil (R. Imeri et al., 2019). Content of such man-made pollutants as Cd, Fe, Zn, Cu, Hg, As, Pb, and radionuclides in soil, water, plants, vegetable feedstuff, and in organisms of wild and productive animals is much higher than in the regions with favourable ecological situation (Ghiyasi et al., 2010; Abdulkhaliq et al., 2012). According to the data of various authors, agricultural animals in the regions with man-made pollution have secondary immunodeficiency because of frequent intoxication, chronical metabolitic stress, metabolic disorder and disbalance of processes of humoral regulation (Abdulkhaliq et al., 2012). Such situations cause higher risk of opportunistic infections, and growth of microbial resistance reduces the effect of medical-preventive activities. In order to improve quality of veterinary interventions, protect animals and prevent spread of dangerous resistant strains, a complex of activities, also including monitoring resistance in animal farms, is necessary. The aim of the work was to do the research on *P. aeruginosa* strains in animal and poultry farms located in the regions with various levels of environmental pollution. In order to achieve the aim, the following tasks were set: to determine content of *P. aeruginosa* in the composition of opportunistic pathogenic microflora in dairy, pig and poultry farms of meat- and egg-type; give the description of sensitivity of detected *P. aeruginosa* strains to antibiotics from groups of fluroquinolones and carbapenems.

MATERIALS AND METHODS

In order to achieve the tasks set, we have done the research on genus and species composition of microbiota in poultry farms of meat- and egg-type, dairy farms and pig farms. For the research enterprises with similar technological and production cycles, spices and breeds of animals, homotypic diet and veterinary maintenance were selected. All the enterprises were located in the Ural area, in the regions with various level of man-made contamination of agrobiocenosis with Zn, Fe, Cd, Cu, As, Pb, ^{90}Sr, ^{137}Cs. We did the preliminary analysis of contamination of feedstaff, soil and water with metals, using the methods of atomic absorption and atomic emission spectrometry (AA 6800 FG, Shimadzu); activity of samples of ^{90}Sr, ^{137}Cs were defined by radio-chemical methods. Totally 24 animal farms were researched, including 8 dairy farms (4 farms located in the area of Eastern Ural radioactive trail and border industrial districts, whereas other 4 farms - in the districts located not less than 100 km from the sources of technogenic emission). All the commercial dairy farms used yard housing, had similar technological profile and used their own feedstuff produced at their own agricultural lands. Total livestock numbers was 1,500–1,700 livestock units of Holstein black-and-white cows. The results of preliminary contamination of agrobiocenosis and feedstuff at the farms under research are given in Table 1.

Table 1. Average content of technogenic pollutants in ready-made feed mix at the commercial dairy farms in the regions with various level of man-made pollution

Pollutant	Regions with intensive man-made pollution	Regions with insignificant man-made pollution
^{90}Sr (Bqkg^{-1})	3.94	1.26
^{137}Cs (Bqkg^{-1})	3.15	0.19
Zn (mgkg^{-1})	52.51	33.38
Pb (mgkg^{-1})	6.14	2.23
Cd (mgkg^{-1})	0.42	0.11
Fe (mgkg^{-1})	123.14	49.65

In dairy farms wash-offs from mucosa, coat and udder of cows and calves, wash-offs from equipment, feed boxes, drinking pans, fence, surfaces, wash-offs from tools for cattle' maintenance, samples of litter, manure, water, feedstuff and air were taken. In every dairy farm the following departments were researched: maternity barn, calf-shed, and department for milking herd. 8 pig farms were researched, including the one located in the area of Eastern Ural radioactive trail; three farms located within a 50–80 km radius from large iron and non-ferrous industries, and four pig farms located in relatively favourable area, far from the sources of technogenic pollution. Pig farms were selected according to the following parameters: similar technological profile, bacon-pig production, Large White breed, total livestock numbers of 5,500–6,100 units, and their own feedstuff produced at their own agricultural lands and production facilities. According to the data of preliminary analysis of feedstuff of self-production, content of Cd in feedstuff at the pig farms in environmentally neglected regions was on average 0.48 mg kg^{-1}, Pb - 7.37 mg kg^{-1}, Fe - 69.5 mg kg^{-1}, the same data at the farms in the regions with low level of technogenic pollution were 2.5–6 times lower. In pig farms samples of air, feedstuff and premixes, litter, water, wash-offs from mucosa and dugs of pregnant and farrow sows, wash-offs from mucosa and skin of piggery from weaning cohort, nursery and fattening groups; wash-offs from equipment, fence, surfaces and tools in various places of premises were taken. The research was done on 4 poultry farms of meat-type (production locations of isolated raising with similar technological profile, cage housing, total cage flock of 0.9–1.2 million units of Cobb-500 and Ross-308 and 4 poultry farms of egg-type (with similar technological profile, cage housing, and total cage flock of 1.8–2.0 million of units of Lohman pedigree). Percent of home-grown feeder grain in all the farms under research was not less than 40%. 4 poultry farms were located in environmentally neglected regions with high content of metal pollutants in soil and water. Feeder grain grown in the lands of the poultry farms had high content of Zn (50.5–63.8 mg kg^{-1}), Pb (2.48–5.52 mg kg^{-1}), and Cd (0.26–0.38 mg kg^{-1}) that was 2–14 times higher than the same parameters of grain in the poultry farms located in the regions with low level of technogenic pollution. In poultry farms samples of feedstuff, water, air, litter, poultry manure, and wash-off from cages, feed-throughs, drinking pans and dung tape; wash-off from mucosa, feather and skin cover, and from cloaca of laying chicken from different age groups (in the poultry farms aimed at egg farming) and broiler chicken of antemortem age (in the poultry farms aimed at meat farming) were made. In total, 1,443 samples for microbiological research were taken. Selected samples were analyzed according to standard microbiological methods: inoculations of medium, cultivation, recovery of pure line, identification of microorganisms, and evaluation of their pathogenicity and antibiotic susceptibility by disk-diffusion method (Minimum Inhibitory Concentration) and serial dilution method (Clinical recommendations. Determination of the susceptibility of microorganisms to antimicrobials, 2015). The results obtained in the course of research were analyzed by methods of mathematical statistics in 'STATISTICA 10' including averaging, standard deviation, normality testing with Shapiro-Wilk criteria, estimation of verified differences between the groups according to various parameters by ANOVA methods and Mann-Whitney criteria.

RESULTS AND DISCUSSION

Analysis of structure of opportunistic pathogenic microflora in animal farms has shown that the most typical agents in all the objects were *Enterococcus faecium, Enterococcus faecalis, Enterococcus durans*, *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Proteus vulgaris, Proteus mirabilis, Echerichia coli, Bacillius subtilis, Enterobacter spp., Citrobacter farmeri, Klebsiella spp., Candida albicans, Aspergillus spp., Mucor spp., Penicillium spp*, and *Fusaium spp*. Proportion of a number of detected strains varied depending on profile of an enterprise and its location. In general, content of *P. aeruginosa* was the biggest one in poultry farms of both meat- and egg-type and made up averagely 19% of all the detected microorganisms. In pig farms in environmentally neglected zones *P. aeruginosa* was detected more often (16%) than in dairy farms (12%), but not as often as in poultry farms.

Research on opportunistic pathogenic microflora of poultry farms have shown that the dominating microorganism detected in 63–100% of samples, was Enterococcus faecium. The second one, according to its frequency, was *Pseudomonas aeruginosa* detected in 29–100% of samples, depending on a definite enterprise. At the same time in the regions with man-made pollution *P. Aeruginosa* was mostly often detected in samples from mouth cavity and cloaca of laying chicken and chicken broilers, whereas in 'clean' regions - mostly in wash-offs from cages and drinking pans (Table 2). The fact may imply that in environmentally neglected zones birds have chronical toxic load on their digestive tract, caused by contamination of feedstuff with metal pollutant Fe, Cd, Cu, Pb and others.

In poultry farms of meat-type content of *P. aeruginosa* strains was averagely 18.6% of all the detected opportunistic pathogenic microorganisms. In poultry farms of egg-type - 19.7%. Therefore, in the poultry farms of both meat- and egg-type under research percent of *P. aeruginosa* in general opportunistic microflora was comparatively the same.

Results of the research on dairy farms have shown that structure of opportunistic pathogenic microbiota (without fungal microflora) in various regions significantly differs. Thus, in enterprises located in ecologically favourable regions *S. aureus* (23.2%) and *P. aeruginosa* (22.9%) were most often detected. In farms with high level of contamination of agrobiocenosis with metals and radionuclides of man-made nature proportion was the following: *Ent. faecium* (16.6%), *P. aeruginosa* (12.9%), and *S. aureus* (15.8%) (Table 3). In dairy farms proportion of samples, positive for *P. aeruginosa* was twice more in ecologically favourable regions than in regions with man-made pollution.

It is especially important that more than a half of *Pseudomonas aeruginosa* positive tests were the tests taken from maternity barns of the farms. In calf-sheds and departments for milking herd *P. aeruginosa* was detected less often. *P. aeruginosa* was found in litter, on tools, fences and in manure. At the same time, in wash-offs from calves' coat *P. aeruginosa* with parameters of antibiotic susceptibility, similar to the ones of the strains from maternity barns was detected. This fact speaks for possible risk of insemination of a new-born calf with strains of opportunistic pathogenic

Table 2. Quantity of positive *P. aeruginosa* tests in samples from various objects from poultry farms located in different regions

Object	Region with agrobiocenosis with man-made pollution (n = 221)	Ecologically favourable region (n = 219)
Mouth cavity	26	10
Cloaca	24	6
Outer coverings	14	13
Litter, manure	25	11
Cage	8	24
Feed-through	9	21
Drinking pan	17	26
Total amount of positive tests	123	111

Table 3. Structure of opportunistic pathogenic microflora of dairy farms in ecologically favourable regions and in regions with man-made pollution (2018)*

Microorganism	Man-made pollution on the level of background pollution	Intensive man-made pollution
P.aeruginosa	22.9%	12.9%
Ent. faecalis	11.1%	13.7%
Ent. faecium	8.6%	16.6%
Ent. durans	10.2%	2.4%
P. mirabilis	6.2%	3.2%
P. vulgaris	5.0%	12.4%
E.coli	4.9%	5.3%
S. aureus	23.2%	15.8%
S. epidermidis	3.5%	2.5%
S. saprophyticus	< 0.5%	2.9%
C. farmer	2.3%	< 0.5%
S. marcescens	1.1%	< 0.5%
B. subtillis	< 0.1%	2.8%
Enterobacter	< 0.3%	8.4%
Other microorganisms	< 0.1%	< 0.1%
	100%	100%

**Distribution based on statistical analysis of structure of strains in selected samples, n = 492.*

microflora, which are low sensitive or resistant to antibiotics, that takes place during contact of a calf with litter or surfaces in a maternity barn. Circulation of resistant strains in maternity barn causes high risk of contamination of calves with them.

In samples taken from pig farms, there was the following distribution of strains according to average frequency: Ent. faecium (19%), *P. aeruginosa* (15%), *Aspergillus spp.* (14%), *S. aureus* (11%), *C. albicans* (8%), *Enterobacter* (8%), *Proteus spp.* (7%), *E.coli* (4%), *S. saprophyticus* (4%). Content of *S. epidermidis* and *Ent. faecalis* strains was not more than 0.2%. It was stated that in pig farms located in the regions with man-made pollution total number of detected *P. aeruginosa* strains did not have any statistically significant differences from ecologically favourable regions. All the enterprises under research had high level of contamination of pregnant sows with *P. aeruginosa*: more than a half of wash-offs from mucosa of mouth, nose and vagina were positive for this microorganism. Tools in all the enterprises under research was contaminated with *Enterococcus* and *P. aeruginosa*.

Analysis of antibiotic susceptibility has shown that in dairy farms average level of resistance of P. aeruginosa strains to carbapenems and fluroquinolones was 12% and 6%, in pig farms - 9% и 13%, in poultry farms - 6% and 18% respectively. Resistance of *P. aeruginosa* to carbapenems is explained by activation of a few mechanisms, such as formation of betalactamases, reduction of permeability of a membrane, and efflux-dependent elimination of antibiotic from a bacterial cell. These mechanisms can be activated separately or in complex. Resistance to fluroquinolones is explained by modification of targets, mostly type II and IV topoisomerases, fermentative inactivation of antibiotic, as well as by efflux systems (Jacoby, 2005; Wolter & Lister, 2013; Hong et al., 2015; Chebotar et al., 2017; Rostami, 2018).

In poultry farms there were mostly *P. aeruginosa* strains with low susceptibility to fluroquinolones and carbapenems. At the same time in the regions with intensive man-made pollution no strains with good susceptibility to these antibiotics were detected (Table 4). Resistant strains in environmentally neglected zones was detected twicemore often than in the regions with low man-made load.

Table 4. Proportion of *P. Aeruginosa* strains with various levels of susceptibility to to fluroquinolones and carbapenems in poultry farms from ecologically favourable and environmentally neglected regions

Status of man-made pollution of grobiocenosis	Proportion from all the strains researched in the object		
	Fluroquinolones		
	Susceptibility preserved	Low susceptibility	Resistance
Low	10%	80%	10%
Intensive	0%	70%	30%
	Carbapenems		
Low	20%	80%	0%
Intensive	0%	82.5%	16.5%

In pig farms in all the researched regions there were mostly *P. aeruginosa* with low susceptibility to antibiotics of fluroquinolones type and carbapenems. Reaction to carbapenems (meropenem, imipenem) was relatively similar in all the regions under research: strains with good susceptibility made up 34–36%, with low susceptibility - 56–58%, and resistant - 9%. In general, in pig farms sensitivity of *P. aeruginosa* to meropenem and Tienam was higher than to antibiotics of fluroquinolones type (enrofloxacin, ciprofloxacin, ofloxacin). At the same time, in the regions with man-made pollution proportion of strains resistant to fluroquinolones was less than in ecologically favourable regions. Thus, in polluted regions 10% of detected strains were non-sensitive to enrofloxacin and in conventionally ‘clean’ regions - 17% (Fig. 1).

On the other hand, pig farms located in ecologically favourable regions have more strains with good susceptibility to enrofloxacin (Fig. 2).

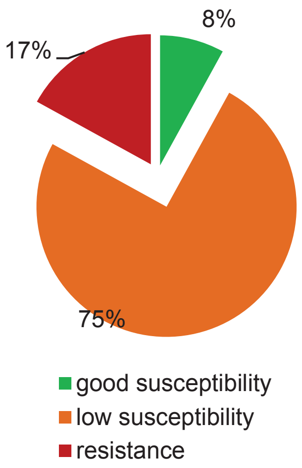

Figure 1. Susceptibility of *P. aeruginosa* strains to enrofloxacin in pig farms located in ecologically favourable regions (2018).

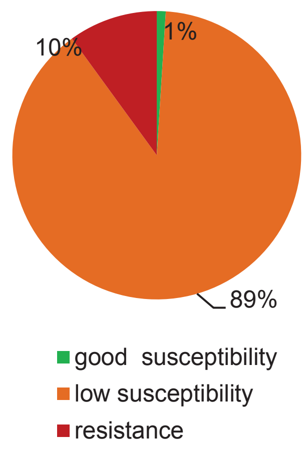

Figure 2. Susceptibility of *P. aeruginosa* strains to enrofloxacin in pig farms in the regions with man-made pollution (2018)

Research on antibiotic susceptibility of *P. aeruginosa* in dairy farms have shown that in the regions with man-made pollution quantity of strains resistant to fluroquinolones was 1.8 times less than in ecologically favourable regions. Both groups of regions had most strains with low susceptibility (their proportion made up 52–58% depending on a farm). Only in two from eight enterprises under research located in the regions with various levels of ecological load, *P. Aeruginosa* strains with good susceptibility to enrofloxacin and ofloxacin formed the most part (53% and 63% from all the detected strains). In these farms no strains resistant to fluroquinolones were detected. Probably, the reason for preserved antibiotic susceptibility of *P. aeruginosa* in these enterprises were special preventive measures taken against microbial resistance.

CONCLUSION

The research done have shown that content of *P. aeruginosa* in composition of opportunistic pathogenic microbiota was highest in poultry farms and lowest - in dairy farms. At the same time ecological well-being of agrobiocenosis correlated to a number of characteristics. Thus, in poultry farms in the regions with man-made pollution *P. aeruginosa* was more often found in mouth cavity and cloaca of chicken, whereas in ecologically favourable regions - in wash-offs from equipment. This fact implicits that in environmentally neglected areas microbiocenosis of poultry is affected by chronical alimentary intoxication related to contamination of feedstuff. In dairy farms difference in percentage of *P. aeruginosa* strains was stated - in ecologically favourable regions content of *P. aeruginosa* in structure of microbiocenosis was almost twice higher than in the regions with man-made pollution. *P. aeruginosa* was most often found in maternity barns - in samples from litter, and wash-offs from tools and equipment. High percentage of positive *P. aeruginosa* tests in maternity barns, as well as detected similarity of antibiotic-resistance profiles of *P. aeruginosa* strains from maternity barns and calf-sheds might speak for the possibility of insemination of newly-born calves with resistant strains of opportunistic microorganisms. In this case, risk of replacement of native microflora of a newly-born calf with more pathogenic and resistant microorganisms is significantly higher, that may cause a negative effect on calves' health and efficiency of antibiotic treatment in farms. Pig farms had high level of contamination of pregnant sows with *P. aeruginosa*. Total number of detected *P. aeruginosa* strains did not have any stastically significant differences depending on ecological status of territory. Average level of resistance of *P. aeruginosa* strains to carbapenems and fluroquinolones was in the commercial dairy farms 12% and 6%, in pig farms - 9% and 13%, in poultry farms - 6% and 18% respectively. In regions with man-made pollution *P. aeruginosa* strains with low susceptibility to carbapenems and fluroquinolones were dominating and there were practically no strains with good susceptibility to these antibiotics. In poultry farms quantity of resistant strains in environmentally neglected zones were detected 2.5 times more often than in the regions with low man-made load. In pig farms and dairy farms located in the regions with man-made pollution content of strains resistant to fluroquinolones and carbapenems was generally lower than in ecologically favourable regions.

ACKNOWLEDGEMENTS. The research was supported by a grant from the Russian Science Foundation (Project No. 18-16-00040).

REFERENCES

Abdulkhaliq, A., Swaileh, K.M. & Hussein, R.M. 2012. Levels of metals (Cd, Pb, Cu and Fe) in cow's milk, dairy products and hen's eggs from the West Bank, Palestine. *International Food Research Journal* **19**(3), 1089–1094.

Alimova, G.S., Korzhavin, A.V., Zemtsova, E.S., Dudareva, I.A., Tokareva, E.I., Popova, E.I. & Sulkarnaeva, R.M. 2015. Heavy metals accumulation in the riverside soil of samsonovskaya, lev and vandras minor rivers. *In the World of Scientific Discoveries*, Series B. **3**(2), pp. 22–33.

Belykh, E.S., Maystrenko, T.A., Grusdev, B.I., Vakhrusheva, O.M., Kaneva, A.V., Zainullin, V.G. & Trapeznikov, A.V. 2015. Species diversity of plant communities from territories anthropogenically contaminated with natural radionuclides. *Russian Journal of Ecology* **5**, 425–430.

Chebotar, I.V. Bocharova, Yu.A. & Maianski, N.A. 2017. Mechanisms of resistance of Pseudomonas aeruginosa to antibiotics and their regulation. *Clinical microbiology and antimicrobial chemotherapy* **19**(4), 308–319.

Clinical recommendations. Determination of the susceptibility of microorganisms to antimicrobials, 2015. Available at: *www.antibiotic.ru/minzdrav/files/docs/clrec-dsma2015*. (in Russian).

Donnik, I.M., Bykova, O., Krivonogova, A.S., Isaeva, A.G., Loretts, O.G., Baranova, A., Musikhina, H. & Romanova, A. 2019. Biological safety of cows' milk under the conditions of technogenic agricultural ecosphere when using biologically active substances *International Transaction Journal of Engineering, Management and Applied Sciences and Technologies* **10**(2), 203–209.

Ghiyasi, S., Karbassi, A., Moattar, F., Modabberi, S. & Sadough, M.B. 2010. Origin – and concentrations of heavy metals in agricultural land around aluminum industrial complex. *Food Agric. Environ*. **8**, 1237–40.

Hong, D.J., Bae, I.K., Jang, I.H., Jeong, S.H., Kang, H.K., Lee, K. 2015. Epidemiology and characteristics of metallo-β-lactamase-producing Pseudomonas aeruginosa. *Infect Chemother*. **47**(2), 81–97.

Imeri, R., Kullaj, E., Duhani, E. & Millaku, L. 2019. Impact of rootstock on heavy metal bioaccumulation in apple plant grown near an industrial source in Obiliq, Kosovo. *Agronomy Research* **17**(1), 100–110. https://doi.org/10.15159/AR.19.012

Jacoby, G.A. 2005. Mechanisms of resistance to quinolones. *Clin Infect Dis*. **41**(2), 120–126.

Koba, I.S., Lysenko, A.A., Koshchaev, A.G., Shantyz, A.K., Donnik, I.M., Dorozhkin, V.I. & Shabunin, S.V. 2018. Prevention of mastitis in dairy cows on industrial farms. *Journal of Pharmaceutical Sciences and Research* **10**(10), 2582–2585.

Luo, X.S., Yu, S, Zhu, Y.G. & Li, X.D. 2012. Trace metal contamination in urban soils of China. *Sci. Total Environ* **17**(30), 421–422.

Rostami, S., Sheikh, A.F., Shoja, S., Farahani, A., Tabatabaiefar, M.A., Jolodar, A., Sheikhi, R. 2018. Investigating of four main carbapenem–resistance mechanisms in high-level carbapenem resistant Pseudomonas aeruginosa isolated from burn patients. *J. Chin. Med. Assoc.* **81**(2), 127–132.

Ventola, L.C. 2015. The antibiotic resistance crisis. Part I: causes and threats. *Pharmacy and Therapeutics* **40**, 1–3.

von Wintersdorff, C.J.H., Penders, J., van Niekerk, J.M., Mills, N.D., Majumder, S., van Alphen, L.B., Savelkoul, P.H.M. & Wolffs, P.F.G. 2016. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in Microbiology* **7**, 173. https://doi.org/10.3389/fmicb.2016.00173

Wolter, D.J. & Lister, P.D. 2013.Mechanisms of β-lactam resistance among Pseudomonas aeruginosa. *Curr. Pharm. Des*. **19**(2), 209–222.