

ANTIBIOTIC RESISTANCE IN BACTERIA ISOLATED FROM FISH IN SERBIA

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Abstract

Introduction. Bacteria isolated from skin, gills, and fish intestines from aquaculture ponds, ornamental fish stores/aquariums and live fish markets were investigated.

Materials and Methods. Disk diffusion and E-test were used for susceptibility testing to carbapenems, ureidopenicillins with or without β -lactamase inhibitor, 3rd and 4th generation cephalosporins, aminoglycosides, colistin, fluoroquinolones, and chloramphenicol. PCR was used to detect resistance genes in the bacterial isolates.

Results and Conclusions. Among the total number of bacteria tested, regardless of the genus and species, 56.7% of isolates were found to be sensitive to all antibiotics, 23.1% of isolates were resistant to one or two antibiotics, and 20.2% of isolates were resistant to three and up to 16 antibiotics. In *A. hydrophila* isolated from a guppy (*Poecilia reticulata*) sampled in an ornamental fish store aquarium, 16S rRNA methyltransferase was confirmed by finding the *rmtB* gene. *Pseudomonas* isolates showing phenotypic resistance to carbapenems, ureidopenicillins with or without β -lactamase inhibitor and 3rd and 4th generation cephalosporins were tested and found negative for different resistance genes by PCR (MBL, ESBL, KPC, OXA-23, OXA-24, OXA-40, OXA-58, VIM, IMP, SPM, GIM, NDM, TEM, SHV, CTX-M-1, CTX-M-9, OXA-1, OXA-9 and the AmpC group, as well as single genes, MOXM, CITM, ACCM, EBCM, FOXM, DHAM). Based on E-test results, three *Pseudomonas* isolates from common carp (*Cyprinus carpio*) were found to be resistant to colistin with MIC values of 4 μ g/mL.

Key Words: *A. hydrophila*, antimicrobial agents, *Pseudomonas*, *Poecilia reticulata*

INTRODUCTION

Although modern fisheries rely on vaccinations and improvement of growth technology in order to prevent diseases (Midtlyng *et al.*, 2011), many bacterial infections in fish are

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treated with antibiotics. Therefore, all fish in fish-farm ponds can come into contact with administered antibiotics.

Fish-farm ponds as a specific ecological niche have a huge influence on the accumulation and spread of resistant bacteria and their genes. Therefore, the use of antibiotics in aquaculture is very undesirable. In 2007, the World Health Organization compiled a list of antibiotics labelled as “critically important” that should not be used other than to treat people (WHO, 2007). Seven antibiotics from this list (amoxicillin, ampicillin, tetracycline, erythromycin, flumequin, sulphonamides and enoxacin) are commonly used in aquaculture worldwide (Soonthornchaikul & Garelick, 2009; Cabello, 2006).

Antimicrobial susceptibility testing in bacteria isolated from fish is complex. Fish are colonised by bacteria that are intrinsically resistant to a large number of antibiotics and, therefore, do not have any significance when it comes to assay of most resistance mechanisms (Aksentijević *et al.*, 2016).

In a document (EUCAST, 2016) that serves as a guide to distinguish intrinsic from acquired resistance in various types of bacteria, only a small number of species that can be found in fish are listed (*Pseudomonas*, *Stenotrophomonas*, *Yersinia*, *Enterobacter*).

Data on intrinsic resistance of *Aeromonas* were published jointly by EUCAST and Comité de l'antibiogramme de la Société Française de Microbiologie (the French Association of Microbiologists), (2015). For almost all other bacterial species that live inside the body of fish, including *Vibrio*, there is no information as to which antibiotics they are intrinsically resistant, and which resistances may be considered acquired and, therefore, essential for the spread of resistance genes in natural environments.

Compared with the rest of the world, fish farming in Serbia is an underdeveloped sector of the economy, despite its centuries-long tradition. Most often, carp ponds can be found in Vojvodina region, while other parts of Serbia are suitable for growing trout. Although Serbia has legislation on the use of antibiotics in animals, there is no official monitoring.

The aim of this study was to determine the presence of multi-resistant strains of bacteria from farmed fish in Serbia, with emphasis on the mechanisms of resistance to antibiotics that are of significant importance in human and veterinary clinical practice.

MATERIALS AND METHODS

The strains of bacteria were isolated from the common carp (*Cyprinus carpio*) from six different ponds that get their water from three rivers (Tisa, Sava and Danube) in Vojvodina region. From each of the ponds, samples were taken from 35 fish from different age categories and from different facilities. Material was sampled from healthy, live fish for human consumption. The fish were not killed or injured. Swabs of the skin, gills and rectum were taken. Also, 10 carp were taken from three fish markets in Belgrade, and in addition to swabs of the gills, skin and rectum, samples of

intestines, liver and spleen were taken when fish was being prepared for consumers. A total of 240 carp were sampled. Also, a total of 10 guppy fish (*Poecilia reticulata*) from several different randomly selected aquarium stores were sampled.

The swab and organ samples were streaked directly on plates containing UTI agar (Urogenital tract infections chromogenic agar, HI Media, India). Pure cultures were subcultured and maintained on Columbia agar supplemented with 5% sheep blood (bioMérieux, France). The plates were incubated for 18-24 hours at a temperature of 27°C. Preliminary identification of the isolated strains was performed using API 20 NE strips (bioMérieux, France). The bacterial isolates were identified using polymerase chain reaction (PCR), 16S rRNA gene sequencing, MALDI-TOF (Vitek MS, bioMérieux Industry, France) and MALDI TOF/TOF 4800 Plus (AB SCIEX, USA).

For antimicrobial susceptibility testing, only Gram-negative species were selected in order to test sensitivity to antibiotics important in human and/or veterinary clinical practice. Antimicrobial susceptibility testing in bacteria originating from fish was conducted using the disk diffusion and Epsilon (E)-test for *Pseudomonas*, *Stenotrophomonas*, *Aeromonas* and *Acinetobacter* and for the family of *Enterobacteriaceae*.

Discs of amoxicillin/clavulanic acid (20/10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), piperacillin (100 µg), piperacillin/tazobactam (100/10 µg), imipenem (10 µg), meropenem (10 µg), aztreonam (30 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), polymyxin B (300 U), sulfamethoxazole/trimethoprim (23.75/1.25 µg) and chloramphenicol (30 µg) (Becton Dickinson, USA), were used. The choice of antibiotics was based on the CLSI and EUCAST recommendations from 2017. Incubation of the inoculated Mueller-Hinton (MH) agar disc lasted 16 to 18 hours at 27 °C.

Reading and interpretation of the results were carried out as recommended by CLSI, so each isolate was categorized in qualitative interpretative category as sensitive (S), intermediate (I) or resistant (R) (CLSI, 2017). E-test strips were used according to the manufacturer's instructions.

PCR was used to determine the presence of genes as follows: 16S rRNA methyltransferase according to the protocol described by Doi & Arakawa (2007), extended-spectrum β-lactamase (TEM, SHV, CTX-M-1, CTX-M-9), OXA-1 and OXA-9 β-lactamase, carbapenemases (OXA-23, OXA-24, OXA-40, OXA-58) which include metallo-β-lactamase (VIM, IMP, SPM, GIM) according to the protocol described by Geyer & Hanson (2013), the carbapenemases (KPC) according to the protocol described by Shanmugam et al. (2013), a metallo-β-lactamase (NDM) according to the protocol of Fallah et al. (2014), and AmpC-β-lactamases – the AmpC group – according to the protocol of Jamali et al. (2015), as well as single genes MOXM, CITM, ACCM, EBCM, FOXM, and DHAM according to the protocol of Perez-Perez & Hanson (2002), and finally, *mcr-1* gene which encodes resistance to colistin according to the protocol described by Cavaco & Hendriksen (2015). Positive

controls for all of the aforementioned genes were received from the Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb and Scientific Veterinary Institute Novi Sad. Extraction of DNA from bacterial isolates was performed using a commercial extraction kit GeneJET Genomic DNA Purification Kit (Thermo Scientific). DreamTaq polymerase, dNTPs, DreamTaq buffer were purchased from Thermo Scientific. Visualization of obtained PCR products was performed using horizontal electrophoresis in 1% agarose gel (Serva) in 1xTBE buffer (Thermo Scientific) supplemented with ethidium bromide to a final concentration of 5µg/ml.

RESULTS

Altogether, 134 bacterial isolates from fish were chosen for testing. These isolates belonged to the species that are important in clinical practice, and CLSI and EUCAST documents give limit values for inhibition zones and MIC values, and interpretive categories for these genera/species.

Out of the 134 isolates, 48 (35.8%) belonged to the genus *Pseudomonas*, including *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. veroni*, *P. geniculata*, *P. oleovorans*, *P. stutzeri*, *P. mandeli*, *P. libaneasis* and *P. hibiscicola*. A total of 18 (13.4%) isolates were identified as *Stenotrophomonas maltophilia*. Numerous isolates (22; 16.4%) belonged to the genus *Aeromonas*, including: *A. hydrophila*, *A. sobria*, *A. cavia* and *A. salmonicida* subspecies *salmonicida*. Six (4.5%) isolates belonged to genus *Acinetobacter* were identified as *A. baumannii* and *A. lwoffii* complex. A total of 40 (29.9%) isolates belonged to the *Enterobacteriaceae* family. Of these, 25 (62.5%) isolates belonged to the genus *Enterobacter*, and were *E. amnigenus*, *E. cloacae*, *E. asburiae* and *E. gergoviae*. Seven (17.5%) isolates belonged to the genus *Citrobacter*: *C. youngae*, *C. freundii*, *C. koseri* and *C. braakii*. There were two (5%) isolates of each, *Hafnia alvei* and *Leclercia adecarboxylata*, and one strain (2.5%) belonged to each of the following: *Serratia fonticola*, *Erwinia rhapontici*, *Rahnella aquatilis* and *Raoultella amnigenus*.

Antibiotic susceptibility testing performed with disk diffusion on *Pseudomonas* isolated from fish

Out of the 48 isolates of *Pseudomonas* species, 11 (22.9%) were sensitive to all antibiotics tested. Other *Pseudomonas* were resistant to at least one, and at most, nine antibiotics. Multidrug resistance (resistance to antibiotics from three or more classes) was found in 39.6% of *Pseudomonas* isolates. Three isolates (6.25%) resistant to nine antibiotics were identified as *Pseudomonas aeruginosa*.

The greatest incidence of resistance, 16.7%, was to ceftazidime, and lowest incidence of resistance, 2.1%, was to meropenem. None of the *Pseudomonas* isolates examined were resistant to polymyxin B. Resistance to aminoglycosides and fluoroquinolones was found in 6.25% and 16.7% of *Pseudomonas* strains.

Susceptibility testing to sulfamethoxazole/trimethoprim of *Stenotrophomonas* strains isolated from fish

All of the assayed *Stenotrophomonas maltophilia* strains (100%) were sensitive to sulfamethoxazole/trimethoprim.

Antibiotic susceptibility testing performed with disk diffusion on *Aeromonas* isolated from fish

From 22 strains of *Aeromonas* species isolated from carp, 19 strains (86.4%) were sensitive to all antibiotics. In one tested *Aeromonas* originating from carp, resistance to carbapenems and piperacillin was found. In one strain of *Aeromonas hydrophila* isolated from an aquarium guppy, resistance to all (total of 16) tested antibiotics was determined, including fluoroquinolones, carbapenems, ureidopenicillin, 3rd and 4th generation cephalosporins, sulfamethoxazole/trimethoprim and aminoglycosides. The highest incidence of resistance was detected in the piperacillin and piperacillin/tazobactam, 13.6%, and the lowest, 9.1%, to meropenem.

Antibiotic susceptibility testing performed with disk diffusion method on *Acinetobacter* isolated from fish

A total of six isolates of *Acinetobacter* species isolated from fish were tested. Out of these, five *Acinetobacter* were sensitive to all antibiotics, and one isolate was resistant to piperacillin.

Antibiotic susceptibility testing performed with disk diffusion on *Enterobacteriaceae* isolated from fish

A total of 40 isolates belonging to the family *Enterobacteriaceae* were examined. It was discovered that 38 strains (95%) were sensitive to all antibiotics tested. Two strains of *Enterobacter amnigenus* were resistant to piperacillin and aztreonam.

Looking at the total number of bacterial isolates assayed in this study, regardless of the genus and strain of bacteria, it was found that a total of 56.7% of the isolates were sensitive to all antibiotics, while 20.2% of the bacteria were resistant to 3 to 16 antibiotics, including antibiotics that are used only in human medicine (carbapenems, ureidopenicillin, 3rd and 4th generation cephalosporins). A total of 23.1% of the bacteria were resistant to one to two types of antibiotics, but even among these isolates, some were found to be resistant to antibiotics registered for use in only human medicine (ceftazidime, piperacillin) (Table 1).

Antibiotic susceptibility testing performed with the E-test

For all of the bacteria that were found to be resistant to imipenem, ceftazidime, piperacillin, piperacillin/tazobactam, ciprofloxacin and chloramphenicol in the disc diffusion method, E-test confirmed the resistance of these isolates to the aforementioned antibiotics, with the following obtained MIC values: for imipenem

12 to 32 µg/mL, for ceftazidime 24 to >256 µg/mL, for piperacillin 16 to >256 µg/mL, for piperacillin/tazobactam 48 to >256 µg/mL, for ciprofloxacin 4 to >32 µg/mL. Susceptibility to colistin was not tested using disk diffusion due to the unreliability of the method, and instead, only the E-test was performed, as recommended by EUCAST. Based on the results of the E-test, three *Pseudomonas* isolates from carp were found to be resistant to colistin, with obtained MIC values of 4 µg/mL. In all other bacterial isolates, tested MIC values for colistin were <1 µg/mL (Figure 1).

Table 1. Resistance to antibiotics (%) in isolates belonging to genera *Pseudomonas*, *Stenotrophomonas*, *Aeromonas*, *Acinetobacter* and family *Enterobacteriaceae*

	<i>Pseudomonas</i>	<i>Stenotrophomonas</i>	<i>Aeromonas</i>	<i>Acinetobacter</i>	<i>Enterobacteriaceae</i>
Amoxicillin/clavulanic acid (20/10 µg)	NT	NT	9.1	NT	NT
ceftriaxone (30 µg)	NT	NT	4.5	NT	0
ceftazidime (30 µg)	16.7	NT	4.5	0	0
cefepime (30 µg)	6.3	NT	9.1	0	0
piperacillin (100 µg)	14.6	NT	13.6	16.7	5
Piperacillin/tazobactam (100/10 µg)	14.6	NT	13.6	0	0
imipenem (10 µg)	6.3	NT	4.5	0	0
meropenem (10 µg)	2.1	NT	9.1	0	0
aztreonam (30 µg)	NT	NT	4.5	NT	5
gentamicin (10 µg)	6.3	NT	4.5	0	0
tobramycin (10 µg)	NT	NT	4.5	0	0
amikacin (30 µg)	6.3	NT	4.5	0	0
ciprofloxacin (5 µg)	10.4	22.2	4.5	0	0
polymyxin B (300 U)	0	0	NT	0	0
Sulfamethoxazole/trimethoprim (23.75/1.25 µg)	NT	0	4.5	NT	0
tetracycline (30 µg)	NT	22.2	4.5	NT	0
chloramphenicol (30 µg)	NT	38.9	4.5	0	0

NT – not tested

PCR investigation of 16S rRNA methyltransferase gene

Three *Pseudomonas aeruginosa* isolates from common carp and one of *Aeromonas hydrophila* isolate from aquarium (guppy) fish had gentamicin and amikacin MIC values of >1024 µg/mL, and these bacteria were suspected of having 16S rRNA methyltransferase.

Three *P. aeruginosa* strains that were suspected of having the 16S rRNA methyltransferase mechanism present, were found to not have *armA*, *rmtA*, *rmtB*, *rmtC* *rmtD*, or *rmtE*

genes that encode this mechanism. In *A. hydrophila* isolated from a guppy, 16S rRNA methyltransferase was confirmed by finding *rmB* gene, as we have reported (Blaset al., 2016). Among all of the isolates that were resistant to carbapenems, ureidopenicillin with or without β -lactamase inhibitors, as well as 3rd and 4th generation cephalosporins, none of the following were found: genes for extended spectrum β -lactamases (TEM, SHV, CTX-M-1, CTX- M-9), OXA-1 and OXA-9 β -lactamase, AmpC β -lactamase (the AmpC group), as well as MOXM, CITM, ACCM, EBCM, FOXM, DHAM), carbapenemases (KPC, OXA-23, OXA-24, OXA-40, OXA-58) and metallo- β -lactamase (NDM, VIM, IMP, SPM, GIM). Mcr-1 gene was not found in *Pseudomonas* isolates that were resistant to colistin.

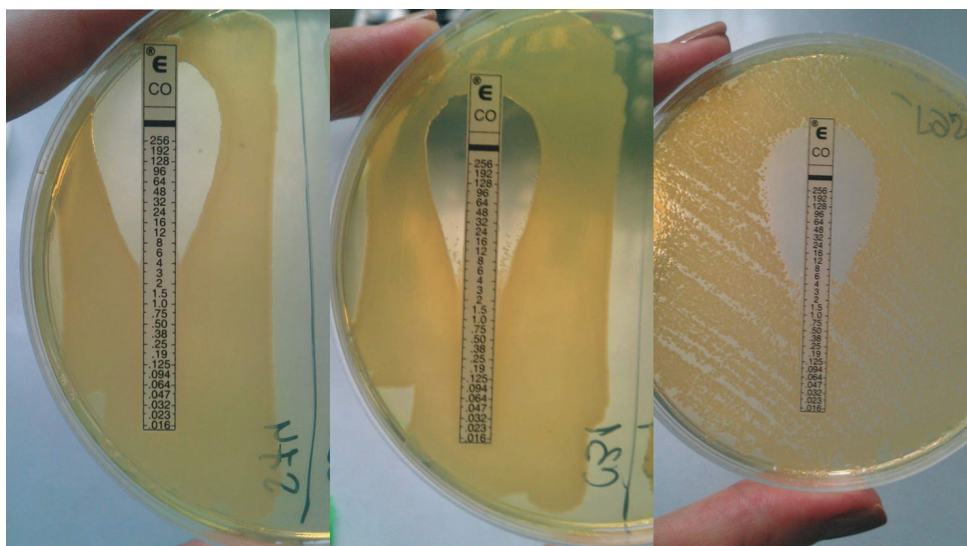


Figure 1. Results of colistin E-test for three isolates of *Pseudomonas* sp.

DISCUSSION

In order to be able to interpret the results of the sensitivity of bacteria to antibiotics, especially that due to intrinsic resistance, it is necessary to accurately identify the bacterial species. In bacteria isolated from fish, this is an extremely difficult task even with the application of sophisticated methods such as MALDI-TOF and sequencing the 16S rRNA gene. The fact is that the MALDI TOF and 16S rRNA sequencing databases are not sufficiently updated with data related to bacteria originating from fish. Therefore, this kind of research is still very limited, as only a small number of bacteria can be precisely identified. Despite all the available means and diagnostic supplies, it remains unclear what the role of bacteria originating from fish is when it comes to the spread of resistance genes.

The phenotypic resistance profile of *Pseudomonas* isolates in our research is very similar to those found in *Pseudomonas* isolates from humans. For example, in one very

extensive study, which ran from 1998 to 2001 in the United States and that included 76,211 strains of *Pseudomonas aeruginosa*, it was found that 90% of the strains were sensitive to amikacin and piperacillin/tazobactam, 80-90% of the strains were sensitive to cefepime, ceftazidime, imipenem and meropenem and 70-80% of the strains were sensitive to ciprofloxacin, gentamicin (Karlowsky *et al.*, 2003). Colistin, which because of its toxicity, has not been used in systematic therapy of people for a long time, has again been introduced as a treatment of severe infections in human population. Finding three strains of the genus *Pseudomonas* resistant to colistin in this study is alarming, but the *mcr-1* gene was not found, and this gene has a particularly great epidemiological importance (since colistin is the last drug for the treatment of infections caused by multi-drug resistant strains of the genus *Pseudomonas*). The resistance to colistin in these strains is obviously based on different mechanisms that were not proven in this study. Pragasam *et al.* (2016) found rare carbapenem-resistant phenotypes, such as imipenem resistant but meropenem sensitive (IRMS), but also meropenem-resistant, but imipenem sensitive (MRIS). These have been further assayed in detail, and it was found that the IRMS phenotype is a consequence of a mutation in different regions of the gene for porin, *OprD*, and that the MRIS phenotype happened due to overexpression of Mexabeta efflux pumps. It was also confirmed that these rare phenotypes are a consequence of intrinsic/chromosome-mediated mechanisms, which occur as a consequence of the selective pressure of the antibiotic. In our research, we found several isolates of the genus *Pseudomonas* that showed IRMS or MRIS phenotype, but it was not possible to determine the precise resistance mechanism.

Finding the *rmtB* gene, which is transmitted through plasmids, in *A. hydrophila* from a guppy indicates the danger of possible spread of this rare and severe case of resistance from fish (and aquatic environments) to humans and other ecological niches. It is unclear whether the fish was a primary carrier or whether this gene was acquired from bacteria originating from humans.

CONCLUSION

Most of the discovered phenotypes of antibiotic resistance in this study could not be confirmed by finding relevant genes. This suggests that further detailed studies on the resistance mechanisms are necessary, to elucidate the intrinsic resistance mechanisms to antibiotics such as carbapenems and ureidopenicillins, which is possible in bacteria isolated from fish.

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Authors' contributions

AK, AJ, NJ, MM, MD, MD carried out the antimicrobial susceptibility testing in bacteria, the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. AK, AJ, NJ, MM, MD, MD participated in the design of the study and performed the statistical analysis. AK, AJ, NJ, MM, MD, MD conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

Hereby we disclose any financial and personal relationships with other people or organisations that could inappropriately influence our work.

REFERENCES

- Aksentijević, K., Ašanin, J., Milivojević, D., Čolović, S., Butorac, A., Cindrić, M., Mišić, D. 2016. Differentiation between *Pseudomonas* and *Stenotrophomonas* species isolated from fish using molecular and MALDI-TOF method. *Acta Veterinaria – Beograd* 66 (3): 304-316. doi: <https://doi.org/10.1515/acve-2016-0027>.
- Blas, J. F. D., Gutierrez, B., Carrilero, L., Santos-Lopez, A., Lopez-Thomas, D., Moyano, A. G., Bernabe, C., Montero, N., Aksentijevic, K., Mistic, D., Gonzalez-Zorn, B. 2016. Identification and characterization of methyltransferases in aminoglycoside resistant human and animal clinical isolates from Serbia, 26th ECCMID, 9 - 12 Apri 2016, Amsterdam, Netherlands, poster PO772.
- Cabello, F. C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing for human and animal health and for the environment. *Environmental Microbiology* 8(7): 1137-44. doi: <http://dx.doi.org/10.1111/j.1462-2920.2006.01054.x>.
- Cavaco, L., Hendriksen, R. 2015. PCR for plasmid-mediated colistin resistance gene mcr-1 (protocol optimized at Statens Serum Institut, Copenhagen, Denmark). Version 1. http://www.crl-ar.eu/data/images/protocols/mcr-1_pcr_protocol_v1_dec2015.pdf
- CLSI. Clinical and Laboratory Standards Institute. 2017. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100 (ISBN 1-56238-804-5 [Print]; ISBN 1-56238-805-3 [Electronic]).
- Doi, Y., Arakawa, Y. 2007. 16S Ribosomal RNA Methylation: Emerging Resistance Mechanism against Aminoglycosides. *Clinical Infectious Diseases* 45 (1): 88-94. doi: <https://doi.org/10.1086/518605>.
- EUCAST – The European Committee on Antimicrobial Susceptibility Testing. Intrinsic resistance and exceptional phenotypes, Expert Rules version 3.1, 2016. <http://www.eucast.org>
- EUCAST – The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.0, 2017. <http://www.eucast.org>
- EUCAST and Comite de l'antibiogramme de la Societe Francaise de Microbiologie. 2015. Recommandations 2015.V1.0 janvier 2015.
- Fallah, F., Noori, M., Hashemi, A., Goudarzi, H., Karimi, A., Erfanimanesh, S., Alimehr, S. 2014. Prevalence of *bla*_{NDM}, *bla*_{PER}, *bla*_{VEB}, *bla*_{IMP} and *bla*_{VIM} genes among *Acinetobacter*

- baumannii* isolated from two hospitals of Tehran, Iran. *Scientifica*, vol. 2014, Article ID 245162, 6 pages. doi: <http://dx.doi.org/10.1155/2014/245162>.
- Jamali, S., Shahid, M., Farrukh, S., Singh, A., Khan, H. M. 2015. Molecular characterization of genes encoding AmpC beta-lactamases in clinical isolates of *Pseudomonas* and *Acinetobacter* species. *Journal of Pharmaceutical Sciences* 5(10): 048-051. doi: <http://dx.doi.org/10.7324/JAPS.2015.501009>.
- Karlowsky, A. J., Draghi, C. D., Jones, E. M., Thornsberry, C., Friedland, R. I., Sahm, F. D. 2003. Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States, 1998-2001. *Antimicrobial Agents and Chemotherapy* 47: 1681-1688. doi: <http://dx.doi.org/10.1128/AAC.47.5.1681-1688.2003>.
- Midtløyg, P. J., Grave, K., Horsberg, T. E. 2011. What has been done to minimize the use of antibacterial and antiparasitic drugs in Norwegian aquaculture. *Aquaculture Research* 42: 28-34. doi: <http://dx.doi.org/10.1111/j.1365-2109.2010.02726.x>.
- Pérez-Pérez, F. J., Hanson, N. D. 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *Journal of Clinical Microbiology* 40: 2153-62. doi: <http://dx.doi.org/10.1128/JCM.40.6.2153-2162.2002>.
- Pragasam, A. G., Raghavivedha, M., Anandan, S., Veeraraghavan, B. 2016. Characterization of *Pseudomonas aeruginosa* with discrepant carbapenem susceptibility profile. *Annals of Clinical Microbiology and Antimicrobials* 15: 12. doi: <http://dx.doi.org/10.1186/s12941-016-0127-3>.
- Shanmugam, P., Meenakshisundaram, J., Jayaraman, P. 2013. bla_{KPC} gene detection in clinical isolates of carbapenem resistant *Enterobacteriaceae* in a tertiary care hospital. *Journal of Clinical and Diagnostic Research* 7(12): 2736-2738. doi: <http://dx.doi.org/10.7860/JCDR/2013/7759.3747>
- Soonthornchaikul, N., Garelick, H. 2009. Antimicrobial resistance of *Campylobacter* species isolated from edible bivalve molluscs purchased from Bangkok markets, Thailand. *Foodborne Pathogens and Disease* 6(8): 947-951. doi: <http://dx.doi.org/10.1089/fpd.2008.0236>.
- WHO. 2007. Critically important antimicrobials for human medicine, 1st revision, ISBN: 978 92 4 159574 2.

OTPORNOST NA ANTIBIOTIKE KOD BAKTERIJA IZOLOVANIH OD RIBA IZ SRBIJE

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Kratak sadržaj

Uvod. U ovom ispitivanju izolovane su bakterija koje su sastavni deo mikrobioma kože, škrge i creva riba iz različitih sredina (ribnjaci, akvarijumi, riblje pijace).

Materijal i metode. Primenom disk difuzionog testa i E testa ispitivano je fenotipsko ispoljavanje rezistencije na karbapeneme, ureidopeniciline sa i bez inhibitora beta-laktamaza, cefalosporine III i IV generacije, aminoglikozide, tetraciklin, kolistin,

flurohinolone i hloramfenikol. Prisustvo gena rezistencije vršeno je primenom metode PCR.

Rezultati i zaključak. Posmatrano na ukupan broj ispitanih sojeva, bez obzira na rod i vrstu bakterija, 56,7% sojeva je osetljivo na sve antibiotike, 23,1% sojeva je rezistentno na 1 do 2 antibiotika a 20,2% sojeva je rezistentno na 3 do 16 antibiotika. Kod soja *A. hydrophila* izolovanom iz akvarijumske ribice gupi nalazom gena *rmtB* potvrđeno je prisustvo 16S rRNK metiltransferaze odgovornim za rezistenciju na aminoglikozide. *Pseudomonas* izolati koji su pokazali fenotipsku rezistenciju na karbapeneme, ureidopeniciline sa i bez inhibitora beta-laktamaza, kao i na cefalosporine III i IV generacije, testirani su i bili negativni na sledeće gene (MBL, ESBL, KPC, OXA-23, OXA-24, OXA-40, OXA-58, VIM, IMP, SPM, GIM, NDM, TEM, SHV, CTX-M-1, CTX-M-9, OXA-1, OXA-9, AmpC grupni kao i pojedinačni geni, MOXM, CITM, ACCM, EBCM, FOXM, DHAM). Na osnovu dobijenih rezultata primenom E testa, kod 3 soja iz roda *Pseudomonas* izolovanih od šarana nađena je rezistencija na kolistin sa dobijenim vrednostima MIK 4 µg/mL.

Ključne reči: *A. hydrophila*, antimikrobna sredstva, *Pseudomonas*, *Poecilia reticulata*