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MONITORING OF ANTIMICROBIAL RESISTANCE UNDER DIRECTIVE 2020/1729 IN EU COUNTRIES

Dalibor Todorović[1*], Marko Pajić[1], Đorđe Marjanović[2], Dragana Ljubojević Pelić[1], Suzana Vidaković Knežević[1], Biljana Đurđević[1], Maja Velhner[1]

[1]*Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia*

[2]*University of Belgrade, Faculty of Veterinary Medicine, Department of Pharmacology, Belgrade, Serbia*

[*]*e-mail: dalibor@niv.ns.ac.rs*

Abstract

Monitoring of antimicrobial resistance is mandatory in all EU member states and is carried out according to the Decision of the European Commission No. 2020/1729. Member states are required to test annually the resistance of 170 isolates of *Escehrichia coli*, *Salmonella* spp., *Campylobacter jejuni*/*coli* and *Enterococcus faecalis*/*fecium*, by determining the minimum inhibitory concentration of antibiotic that inhibits bacterial growth (MIC). Also, they are obliged to test 300 isolates of *Escherichia coli* and *Salmonella* spp. for the production of ESBL, AmpC, or CP enzymes. Monitoring shall be carried out in accordance with the procedures described by the European Committee for Antimicrobial Susceptibility Testing (EUCAST), according to the international reference method ISO 20776-1: 2021 (E) entitled "Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices - Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases". Directive EU2020/1729 stipulates that isolates should come from healthy animals, so for these reasons, sampling at the slaughter line in slaughterhouses, retail outlets, as well as on disease-free farms is most appropriate.

Key words: antimicrobial resistance, monitoring, food, microdilution, EU directives

Introduction

According to the European food safety authority (EFSA), the number of people with infections caused by multidrug-resistant bacteria is increasing every year. Public health is most concerned about increasing the resistance of bacteria to antibiotics used in human medicine, such as fluoroquinolones, third and fourth generation cephalosporins, carbapenems and colistin. For these reasons, it is very

important to monitor antibiotic resistance in indicator bacteria in the food production chain, which also includes testing the mechanisms of resistance, which can see the risks of spreading "super" bacteria in humans and animals (EFSA Journal, 2021).

Monitoring and reporting on the occurrence of resistance in bacteria that pose a threat to public health began in 2003 in the countries of the European Union (EU) according to Directive 2003/99/EC. This directive stipulated that EU member states must assess and analyze the movement and sources of antimicrobial resistance in their territory and submit an annual report to the EU Commission, which includes all data collected in accordance with that directive (Directive 2003/99/EC). The EU Commission Decision of 2013 introduced Directive 2013/652/EU, which lays down detailed rules for harmonized monitoring of antimicrobial resistance and reporting in zoonotic and commensal bacteria originating from domestic animals, and from food originating from animals used for human consumption (Directive 2013/652/EU).

In 2019, the European Food Safety Authority (EFSA) published a report "Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from foodproducing animals and food" recommending certain changes to the current monitoring of antimicrobial resistance under the Directive 2013/652/EU. These changes concerned the animal populations for food production and the categories of food to be sampled, the sampling plan to be followed, the types of zoonotic and commensal bacteria whose antimicrobial resistance should be tested, and the analytical methods to be used by laboratories designated to test bacterial resistance to antibiotics (EFSA Journal, 2019).

On November 17, 2020, the European Commission decided to use the new Directive 2020/1729 for monitoring and reporting antibiotic resistance of zoonotic and commensal bacteria for the period 2021-2027 years. This Directive is in line with the recommendations of the 2019 EFSA report, as well as the procedures described by the European Committee for Antimicrobial Susceptibility Testing – EUCAST according to the international reference method ISO 20776-1: 2021 entitled "Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices - Part 1: Broth microdilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases" (Directive 2020/1729). Also, this directive authorizes the use of whole genome sequencing (WGS) techniques as an alternative to phenotypic analysis, but on a voluntary basis because a limited number of EU member states are able to use WGS for routine monitoring and analysis of antimicrobial resistance. WGS can be used in the future, but only according to the technical conditions and instructions prescribed by the European Reference laboratory for

antimicrobial resistance (EURL-AMR) in Denmark, so that the results are comparable, ie monitoring is harmonized (Directive 2020/1729).

EU member states are required to test the susceptibility of bacteria to antibiotics annually in the commensal bacteria *Escherichia coli* and *Enterococcus fecalis/fecium* and zoonotic bacteria *Salmonella* spp., and *Campylobacter jejuni/coli*, by determining the minimum inhibitory concentration of antibiotics (MIC). In addition to the listed commensal and zoonotic bacteria, EU member states are obliged to test 300 isolates of *Escherichia coli* and *Salmonella* spp. to determine whether they have the ability to produce the enzyme extended spectrum ß-lactamase (ESBL), the enzyme AmpC ß-lactamase (AmpC) and the enzyme carbapenemase (CP) (Directive 2020/1729).

Subject and scope of amr monitoring

The number of test isolates depends on the annual production of poultry, pork and beef of the monitoring country. If the annual production of broiler and pork is higher than 100,000 t, beef higher than 50,000 t and turkey higher than 10,000 t, 170 isolates of commensal and zoonotic bacteria are tested. For testing ESBL/AmpC/CP producing *Escherichia coli* and *Salmonella* spp. it is necessary to test 300 isolates annually (Table 1). EU member states that have lower annual production of poultry, pork and beef than prescribed, test 85 isolates of commensal and zoonotic bacteria, while for testing ESBL/AmpC/CP producing *Escherichia coli* and *Salmonella* spp. test 150 isolates. EU member states that failed to collect 170 isolates of commensal and zoonotic bacteria or 300 isolates of potential ESBL/AmpC/CP *Escherichia coli* and *Salmonella* spp. include in the monitoring of antimicrobial resistance the number of isolates collected that year (Directive 2020/1729).

Samples for monitoring antimicrobial resistance must be origin of healthy animal, so EU Directive 2020/1729 prescribes sampling at the slaughter line in slaughterhouses, retail outlets, border crossings, as well as on disease-free poultry farms (Directive 2020/1729). For testing the antimicrobial resistance of *Salmonella* spp. in the case of broilers, laying hens and fattening turkeys, EU member states may use isolates already obtained from the national control programs provided for in Regulation (EC) No 2160/2003 (Directive 2020/1729). Categories of animals by species, origin and number of samples are shown in Table 1. Accredited laboratories designated by the EU member state are responsible for sampling materials at slaughterhouses, retail outlets, border crossings and farms, isolation and strain identification, and for adequate transport of isolates to the National reference laboratory in designated to conduct of antimicrobial resistance testing. The National reference laboratory for antimicrobial resistance is responsible for testing the

susceptibility of the obtained bacterial isolates to antibiotics, followed by monitoring and analysis of *Escherichia coli* and *Salmonella* spp. which produce ESBL/AmpC/CP enzymes, as well as alternative methods (WGS) in ESBL/AmpC/CP producing strains of *Escherichia coli* and *Salmonella* spp. (Directive 2020/1729).

Table 1. Type and categories of animals, origin and number of isolates covered by antimicrobial resistance monitoring according to Directive 2020/1729

Bacterial species	Number of isolates	Animal species	Sample
E. coli	170	Broilers	Caecal content, fresh meat
		Fattening turkeys	Caecal content, fresh meat
		Fattening pigs	Caecal content, fresh meat
		Cattle up to 1 year of age	Caecal content, fresh meat
Salmonella spp.	170	Broilers	Caecal content, neck skin, fresh meat
		Fattening turkeys	Caecal content, neck skin, fresh meat
		Broilers, fattening turkeys, laying hens	Overshoes
		Fattening pigs	Caecal content
		Cattle up to 1 year of age	Caecal content
Campylobacter jejuni/coli	170	Broilers	Caecal content
		Fattening turkeys	Caecal content
		Fattening pigs	Caecal content
		Cattle up to 1 year of age	Caecal content
Enterococcus faecalis/fecium	170	Broilers	Caecal content
		Fattening turkeys	Caecal content
		Fattening pigs	Caecal content
		Cattle up to 1 year of age	Caecal content
ESBL/AmpC/CP produce *E. coli* and *Salomenlla spp.*	300	Broilers	Caecal content, fresh meat
		Fattening turkeys	Caecal content, fresh meat
		Fattening pigs	Caecal content, fresh meat
		Cattle up to 1 year of age	Caecal content, fresh meat

Microdilution method in broth

Broth microdilution is performed according to the reference method ISO 20776-1:2021, which determines MIC (ISO 20776-1: 2021). This method allows the tested bacterial strains to be classified as susceptible or resistant by determining the epidemiological limit value of the MIC according to EU Directive 2020/1729 (EU Directive 2020/1929). In order to harmonize the monitoring of antimicrobial resistance, EU member states are required to perform the method in sensititre Muller-Hinton broth (Thermo Scientific, United Kingdom) and sensititre plates with flat bottom (Thermo Scientific, United Kingdom) in which double-diluted antibiotics are impregnated (Table 2) (EU Directive 2020/1929). The microdilution method is intended to test pure cultures of bacteria that grow easily after overnight incubation on Columbia agar with 5% defibrinated sheep blood (ISO 20776-1: 2021). For susceptibility testing of *Escherichia coli* and *Salmonella spp*. two antibiotic panels are used, the first panel (EUVSEC3) to determine the multiresistance of the test strain (MDR) and the second panel (EUVSEC2) to determine whether the test strain has the ability to produce ESBL/AMPC/CP enzymes (Table 2). Only one panel of antibiotics is used for susceptibility testing in *Campylobacter jejuni/coli* (EUCAMP3) and *Enterococcus faecalis/fecium* (EUVENC) (Table 2) (EU Directive 2020/1729).

Table 2. Antibiotics used in monitoring of antimicrobial resistance according to EU Directive 2020/1729.

No	*Escherichia coli*	*Salmonella spp.*	*Campylobacter jejuni/coli*	*Enterococcus faecalis/fecium*
	First panel	**First panel**	**First panel**	**First panel**
	EUVSEC3	**EUVSEC3**	**EUCAMP3**	**EUVENC**
1.	Amikacin	Amikacin	Chloramphenicole	Ampicilin
2.	Ampicilin	Ampicilin	Ciprofloxacin	Chloramphenicole
3.	Azitromycin	Azitromycin	Ertapenem	Ciprofloxacin
4.	Cefotaksime	Cefotaksime	Eritromycin	Daptomicin
5.	Ceftazidime	Ceftazidime	Gentamicine	Eritromycin
6.	Choramphenicol	Choramphenicole	Tetracycline	Gentamicin
7.	Ciprofloxacin	Ciprofloxacin	/	Linezolid
8.	Colistin	Colistin	/	Quinupristin/Dalfopristin
9.	Gentamicin	Gentamicine	/	Teikopalanin
10.	Meropenem	Meropenem	/	Tetracycline
11.	Nalidixic ac.	Nalidixic ac.	/	Tigecycline
12.	Sulfametoksazole	Sulfametoksazole	/	Vankomycin
13.	Tetracycline	Tetracycline	/	/
14.	Tigecycline	Tigecycline	/	/
15.	Trimetoprim	Trimetoprim	/	/

	Second panel	Second panel	Second panel	Second panel
	EUVSEC2	**EUVSEC2**	**/**	**/**
16.	Cefepime	Cefepime	/	/
17.	Cefotaxime	Cefotaxime	/	/
18.	Cefotaxime/clav.ac.	Cefotaxime/clav.ac.	/	/
19.	Cefoxitin	Cefoxitin	/	/
20.	Ceftazidime	Ceftazidime	/	/
21.	Ceftazidime/clav.ac.	Ceftazidime/clav.ac.	/	/
22.	Ertapenem	Ertapenem	/	/
23.	Imipenem	Imipenem	/	/
24.	Meropenem	Meropenem	/	/
25.	Temocilin	Temocilin	/	/

Quality control, storage of isolates and testing for certificate

EU member states are obliged to carry out quality control in accredited laboratories for sampling, isolation and identification of bacteria, as well as in National reference laboratories designated for monitoring antimicrobial resistance according to Directive 2020/1729. Quality control includes verification of professional competence and quality control of performing methods for sampling, isolation and identification of bacteria, as well as testing for sensitivity to antibiotics. The National reference laboratory is required to store for five years at -80 ºC all isolates determined to be resistant during monitoring. In the event that EFSA or the EURL-AMR deems it necessary and relevant to confirm or further test an isolate, the National reference laboratory of an EU member state is obliged to adequately label the isolate and send it to the EURL-AMR (Directive 2020/1729).

Preparing reports and reporting

EU member states are obliged to analyze the results of one-year monitoring of antimicrobial resistance and prepare a report to the EFSA (EFSA Journal, 2021). The report should contain all relevant data on the movement and sources of zoonosis, as well as data on samples and isolates (for each isolate separately) that have been tested for antimicrobial resistance according to the instructions prescribed in Directive 2020/1729. EU member states must also describe in their reports the sampling plans, stratification and randomization procedures by animal population and food category (Directive 2020/1729). The sampling frequency was made according to the rotation model, the results are analyzed and a report is prepared for isolates originating from broilers, laying hens, broilers and fresh meat of broilers and turkeys (example 2020, 2022, 2024, 2026), and for isolates originating from fattening pigs and cattle up to one year old and fresh meat from fattening pigs and cattle up to one year old (example 2021, 2023, 2025, 2027) (Directive 2020/1729). The obtained monitoring results are reported to EFSA from 1 April to 30 May of the current year for the previous year. Upon receipt of the report from the national reference laboratories, EFSA produces a final report and mapping containing national quantitative data on antibiotic resistance

based on the results and analyzes reported by all EU member states included in the antimicrobial resistance monitoring network (EFSA Journal, 2021).

Conclusion

Continuous monitoring of bacterial resistance contributes to a better understanding of the development of resistance, its transmission between bacteria and its maintenance in nature. The food production chain is one of the main sources of pathogenic or non-pathogenic resistant bacteria for humans. Taking into account one health, EU member states have implemented monitoring of antimicrobial resistance in veterinary medicine, in order to reduce the high percentage of resistance with the rational use of antibiotics. In order to implement resistance monitoring in Republic of Serbia, it is important to establish adequate sampling strategy and collection of data on samples, as well as adequate methods of storing and sending isolates to the National reference laboratory.

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Authors contributions

Writing – original draft preparation DT, MP, ĐM, DLjP, SVK and BĐ; Writing – review and editing DT and MV; Supervision – MV. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they have no competing interests

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