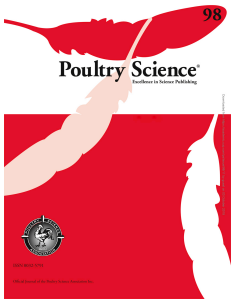

Study of the antibiotic residues in poultry meat in some of the EU countries and selection of the best compositions of lactic acid bacteria and essential oils against *Salmonella enterica*

Elena Bartkiene, Modestas Ruzauskas, Vadims Bartkevics, Iveta Pugajeva, Paulina Zavistanaviciute, Vytaute Starkute, Egle Zokaityte, Vita Lele, Agila Dauksiene, Michael Grashorn, Ludwig E. Hoelzle, Anara Mendybayeva, Raushan Ryshyanova, Romas Gruzauskas

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Study of the antibiotic residues in poultry meat in some of the EU countries and selection of the best compositions of lactic acid bacteria and essential oils against *Salmonella enterica*

Running title: Different origin antimicrobial compositions for the poultry

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ABSTRACT

In this study, the presence of antibiotics (ANB) residues was evaluated in poultry meat purchased from German and Lithuanian markets. In addition, the antimicrobial activity of thirteen lactic acid bacteria (LAB) strains, two essential oils (EOs) (*Thymus vulgaris* and *Origanum vulgare* L.), and their compositions were tested for the purpose of inhibiting antibiotic-resistant *Salmonella* spp. ANB residues were found in 3 out of the 20 analysed poultry meat samples: sample No. 8 contained enrofloxacin (0.46 µg/kg), sample No. 14 contained both enrofloxacin and doxycycline (0.05 and 16.8 µg/kg, respectively), and sample No. 18 contained enrofloxacin (2.06 µg/kg). The maximum residue limits (MRLs) for the sum of enrofloxacin and ciprofloxacin and for doxycycline in poultry muscle are 100 µg/kg. Finally, none of the tested poultry meat samples exceeded the suggested MRLs, however, the issue of ANB residues still requires monitoring of the poultry industry in Germany, Poland, and Lithuania, despite the currently established low ANB concentrations. These findings can be explained by the increased use of alternatives to ANB in the poultry industry. Our results showed that an effective alternative to ANB, which can help to reduce the occurrence of antibiotic-resistant salmonella, is a composition containing 1.0% of thyme EO and the following LAB strains: *Lactobacillus plantrum* LUHS122, *Enteroccocus pseudoavium* LUHS242, *Lactobacillus casei* LUHS210, *Lactobacillus paracasei* LUHS244, *Lactobacillus plantarum* LUHS135, *Lactobacillus coryniformins* LUHS71, and *Lactobacillus uvarum* LUHS245, which can be recommended for poultry industry as components of feed or for the treatment of surfaces, in order to control the contamination with *Salmonella* strains. However, it should be mentioned that most of the tested LAB strains were inhibited by thyme EO at the concentrations of 0.5 and 1.0%, except for LUHS122, LUHS210, and LUHS245. Finally, it can be noted that the agents responsible for the inhibitory effect on *Salmonella* are not the viable LAB strains but rather their metabolites, and further studies are needed to identify which metabolites are the most important.

Key words: poultry, meat, antibiotic residues, antimicrobial activity, *Salmonella*

INTRODUCTION

The European Union (EU) imposed a complete ban of all antibiotics (ANB) as growth promoters (GP) in animal feed since January 2006, and according to the regulations by Food and Drug Administration (FDA), ANB cannot be used for growth promoting purposes across the United States of America (USA) from 2017. The restriction of ANB use in animal feed is a controversial global issue, because the presence of ANB in feed formulations is known to promote the growth of broilers (Gadde et al., 2018; Wealleans et al., 2018) which is explained with the timely control of infections in poultry farms (Singer and Hofacre, 2006). However, the exposure to ANB can lead to the spread of drug resistant infections in humans and animals, which are projected to cause 10 million human deaths the loss of 100 trillion USD by 2050 if the current trends in ANB consumption will continue (O'Neill, 2014; Mellor et al., 2019). The widespread clinical and agricultural use of antimicrobials has facilitated the emergence of antimicrobial resistance in bacteria (Laxminarayan and Heymann, 2012). Some opportunistic and pathogenic bacteria are more virulent than others. Thus, over 100,000 cases of enterocolitis in the EU, causing annual losses of €3 billion, are attributed to non-typhoidal *Salmonella* infections, of which *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* is the second most common serovar (EFSA, 2017). It has been reported that poultry and its products are a potential source of resistant *Salmonella* strains (de Oliveira et al., 2005; Singh et al., 2010; Velasquez et al., 2018). The control of *Salmonella* in poultry production is very complicated, because birds can be exposed to *Salmonella* not only from wild birds, but also from flies (Wales et al., 2010; Andrés et al., 2013). Also, it should be mentioned that the presence of pathogenic bacteria in the microbiota of broilers is an important biosafety factor in the poultry industry (Clavijo et al., 2019).

Salmonella is a common pathogen that can survive and pass through the technological steps of poultry production (Vinueza-Burgos et al., 2019). Human gastrointestinal infections caused by *Salmonella* usually are associated with the consumption of poultry products, therefore the control of

this type of pathogens is of great importance (Wegener et al., 2003). Three possible routes of *Salmonella* contamination in chicken meat have been identified, including initial presence, cross-contamination from broilers carrying *Salmonella* that have been slaughtered on the same day, and contamination from resident flora in the slaughterhouse, with the last route being the most common (Shang et al., 2019).

However, the treatment of poultry with ANB is not an acceptable solution, as the use of ANB promotes the resistance of pathogenic strains, as well as ANB residues can directly affect the human immune system, growth, and metabolism processes (Muhammad et al., 2019). In order to reduce the health risks due to ANB use, a search for alternatives continues. It has been suggested that xylanase and amylase produced by *Aspergillus niger* during solid state fermentation of apple pomace can be used as alternatives to ANB growth promoters (GP) in poultry feed (Suresh et al., 2019). Also, the use of probiotics (PRO) has been suggested to reduce the presence of ANB in poultry farming (Patterson and Burkholder, 2003; Gaggia et al., 2010). Most PRO are bacteria that already exist in the digestive tract of animals, and have the properties of bacterial community stabilizers or antimicrobials against undesirable bacterial species (de Vrese and Schrezenmeir, 2008; Kabir, 2009). Our previous studies have shown that lactic acid bacteria (LAB) can inhibit methicillin-resistant *Staphylococcus aureus* (Bartkiene et al., 2019). In addition, LAB has various properties, which are desirable in poultry farms. For example, phosphatase excreted by LAB can lead to improving of phosphate digestion (Neveling et al., 2020). The LAB, which possessing PRO properties, showed ability to attach to intestinal epithelial cells and to reduce pathogens colonization, as well as to increase growth performance and improve the immune system of the poultry (Salehizadeh et al., 2020; Soomro et al., 2019; Mohammadreza et al., 2020). In addition to above mentioned probiotic properties, LAB can reduce mycotoxins in feed (Haquea et al., 2020).

Also, our previous studies showed strong antimicrobial properties of some essential oils (EOs), which do not inhibit LAB, while inhibiting pathogenic bacteria (Bartkiene et al., 2019; Bartkiene et al., 2018a). EOs typically contain a combination of volatiles that produce cumulative antimicrobial effects. EOs have a great potential as alternatives to ANB in poultry industry and are generally favoured as natural antimicrobials that are less toxic and free from residues (Zhai et al., 2018).

Finally, even though LAB and EOs are well known for their antimicrobial properties in the poultry industry, studies regarding the antimicrobial activity of these very different agents are scarce. For this reason, we set out to test our hypothesis that these antimicrobials with different mechanisms of action can produce a synergic antimicrobial effect. In this study, the presence of ANB residues was evaluated in poultry meat purchased from the German and Lithuanian markets. In addition, the antimicrobial activity of thirteen different LAB strains, two Eos, and their compositions against ANB-resistant *Salmonella* spp. was tested.

MATERIALS AND METHODS

Poultry Meat Samples, Salmonella and Lactic Acid Bacteria Strains, Essential Oils

A total of 20 poultry meat samples were purchased from different hypermarkets and central markets in Germany and Lithuania (Table 1). The obtained meat samples originated from different countries: Germany (purchased in Germany), Lithuania, Latvia, Poland, and France (purchased in Lithuania).

The *Salmonella* strains were isolated from raw poultry products (chicken) in the Northern region of Kazakhstan in years 2018-2019 (the project was supported by the Ministry of Education and Science of the Republic of Kazakhstan, Project number AP05131447). All isolates belonged to the Enteritidis serotype of *Salmonella enterica*. Susceptibility testing was performed using disk-diffusion method at

the Kostanay State University (Kazakhstan) according to clinical breakpoints set by EUCAST (whenever possible) and the applicable national standard. The *Salmonella* resistance profiles are given in Table 2.

The LAB strains (*Leuconostoc mesenteroides* LUHS225, *Lactobacillus plantarum* LUHS122, *Enteroccocus pseudoavium* LUHS242, *Lactobacillus casei* LUHS210, *Lactobacillus curvatus* LUHS51, *Lactobacillus farraginis* LUHS206, *Pediococcus pentosaceus* LUHS183, *Pediococcus acidilactici* LUHS29, *Lactobacillus paracasei* LUHS244, *Lactobacillus plantarum* LUHS135, *Lactobacillus coryniformis* LUHS71, *Lactobacillus brevis* LUHS173, and *Lactobacillus uvarum* LUHS245) were acquired from the Lithuanian University of Health Sciences collection (Kaunas, Lithuania). The LAB strains were selected according to their inhibiting properties against pathogenic and opportunistic bacterial strains (Bartkiene et al., 2019; Bartkiene et al., 2018b; Lele et al., 2018). The tested LAB strains were grown in the MRS medium (Biolife, Italy) at 30°C. Two percent of the MRS solution (v/v) in which the strains were multiplied were inoculated into fresh medium and propagated for 18 h. The multiplied LAB samples were used for the determination of their antimicrobial activities against the aforementioned *Salmonella* strains.

The EOs of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare* L.) were purchased from Sigma-Aldrich (Saint-Louis, MO, USA).

Evaluation of Antibiotic Residues in Poultry Meat Samples by UHPLC-MS/MS Method

The following antibiotics were analysed in this study: cephalosporins (cefacetrile, cefalexin, cefoperazone, cefalonium, cefaprim, cefazolin, cefquinome, ceftiofur), penicillins (amoxicillin, ampicillin, benzylpenicillin, cloxacillin, dicloxacillin, nafcillin, oxacillin, phenoxymethylpenicillin, penicillin V), quinolones (ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, flumequine, marbofloxacin, nalidixic acid, norfloxacin, orbifloxacin, oxolinic acid, sarafloxacin), sulfonamides

(sulfachloropyridazine, sulfadimethoxine, sulfadimidine, sulfadoxine, sulfamerazine, sulfamethizole, sulfathiazole, sulfamonomethoxine, sulfanilamide), tetracyclines (chlortetracycline, doxycycline, oxytetracycline, tetracycline), macrolides and lincosamides (erythromycin A, josamycin, kitasamycin, lincomycin, neospiramycin, pirlimycin, spiramycin, tildipirosin, tilmicosin, tylosin A, tulathromycin A), and other antibiotics (thiamphenicol, bacitracin, novobiocin, rifaxamin, tiamulin, tylvalosin, valnemulin and trimethoprim).

The analyses were performed according to a previously published method by Reinholds et al., (2016). According to this method, a 2 g sample was weighed into a 15 mL centrifuge tube. Quality control samples were fortified with the appropriate volume of standard solution in order to obtain levels corresponding to 10% of EU MRLs for muscles. Then 3 mL of acetonitrile was added to each sample. The samples were vigorously shaken for 20 min and centrifuged for 15 min at 4500 rpm. The supernatant was collected and loaded onto a Phree™ phospholipid removal tube (1 mL) that was pre-conditioned with 0.5 mL of acetonitrile. The obtained extracts (2 mL) were collected into clean sample tubes, while the Phree™ tubes were washed with additional 0.3 mL of acetonitrile. The combined acetonitrile extracts were evaporated to dryness under nitrogen stream at 55°C. The residues were dissolved in 1 mL of 0.1% formic acid solution in water/methanol (90:10, v/v). The samples were then filtered through 0.22 µm centrifuge filters at 3000 rpm and transferred to autosampler vials for further analysis. A 10 µL aliquot of each sample was injected into the UHPLC-MS/MS system.

The obtained low level concentrations of enrofloxacin and ciprofloxacin were confirmed using the method described by Pugajeva et al., 2018. According to that method, a sample of muscle tissue (10 g) was spiked with 50 µL of 0.01 µg L^{-1} internal standard solution (concentration in samples was 0.05 µg kg^{-1}). The analytes were extracted by adding 20 mL of acetonitrile, than shaken for 20 min and sonicated for 10 min in ultrasonic bath. After centrifugation at 4000 rpm for 10 min, 15 mL of the supernatant was transferred into another centrifuge tube and evaporated under nitrogen stream at 50°C.

The sample was reconstituted in 5 mL of water and centrifuged for 10 min at 4000 rpm at 4°C. The supernatant was loaded into a Strata X cartridge (500 mg / 6 mL) previously conditioned with methanol (5 mL) and deionised water (5 mL). The column was washed with aqueous 50% methanol solution. The elution of analytes was achieved with 5 mL of 1% ammonia solution in methanol. The eluate was evaporated to dryness under nitrogen stream at 50°C. The residue was dissolved in aqueous 50% methanol solution (200 µL), then transferred into a vial for UHPLC-MS/MS analysis.

Chromatographic separation of target compounds was achieved using an UltiMate 3000 UHPLC system (Thermo Scientific, Waltham, MA, USA). The separation was performed on a 100 mm × 2.1 mm i.d., 1.9 µm Hypersil Gold analytical column (Thermo Scientific). The mobile phase component A was water and the component B was methanol, both containing 0.1% of formic acid. The flow rate was 300 µL min^{-1}. The effective gradient began at the initial mobile phase composition of 90% A and 10% B. The percentage of mobile phase component B was linearly raised from 10% to 30% until 4.0 min, then maintained for 1.0 min. From 5.0 min to 10 min the percentage of component B was linearly raised up to 95% and was held constant until 10.5 min. Then the percentage of component B was sharply decreased to 10% over 0.5 min and was kept at this level until 15 min. The column and sample temperatures were 30°C and 10°C, respectively.

The UHPLC system was coupled to a Thermo Scientific TSQ Quantiva mass spectrometer equipped with a heated electrospray ionisation probe (HESI) used in the positive ionisation mode. Sample analysis was performed in the selected reaction monitoring (SRM) mode, by selecting one precursor and two product ions for each compound with a dwell time of 100 ms per channel, using resolution of 0.7 FWHM for Q1 and Q3 and setting the collision gas (argon) pressure at 1.5 mTorr. The following general ionisation source parameters were applied: spray voltage 4.0 kV, vapouriser temperature 320°C, ion transfer tube temperature 280°C, sheath gas (N_2) 40 arbitrary units (arb), auxiliary gas (N_2)

15 (arb), and sweep gas (N_2) 5 (arb). The data processing was carried out with TraceFinderEFS software (Thermo Fisher Scientific).

Evaluation of Lactic Acid Bacteria and Essential Oils Antimicrobial Properties against Salmonella Strains

An agar well diffusion assay was used for testing the antimicrobial activity of LAB. For this purpose, 0.5 McFarland turbidity suspension of each *Salmonella* strain was inoculated onto the surface of cooled Mueller Hinton Agar (Oxoid, UK) using sterile cotton swabs. Wells with 6 mm diameter were punched in the agar and filled with 50 µL of the tested LAB suspension. The antimicrobial activity against the tested bacteria was determined by measuring the DIZ (mm). The experiments were repeated three times and the average value of DIZ was calculated.

In addition, the Minimal Inhibitory Concentrations (MIC) of the LAB and EOs against the aforementioned *Salmonella* strains were determined according to the Clinical and Laboratory Standards Institute (CLSI) microdilution method (CLSI, 2015). MIC was defined as the concentration of LAB or EOs that inhibited visible microbial growth. Two concentrations of LAB and four concentration of EOs were tested against the *Salmonella* strains (suspension of 0.5 McFarland turbidity) were tested: (i) 0.5 mL LAB + 0.1 mL of *Salmonella* suspension, (ii) 0.5 mL LAB + 0.01 mL of *Salmonella* suspension, and i) 0.01 mL EOs + 0.01 mL of *Salmonella* suspension, (ii) 0.02 mL EOs + 0.1 mL of *Salmonella* suspension, (iii) 0.05 mL EOs + 0.01 mL of *Salmonella* suspension, (iiii) 0.1 mL EOs + 0.1 mL of *Salmonella* suspension. The experiments were performed in triplicate.

Evaluation of Essential Oil Antimicrobial Properties against Lactic Acid Bacteria

The LAB strains selected for the highest antimicrobial activity were multiplied in MRS broth (Biolife, Italy) at 30°C. Then, 500 µL of the selected LAB strains in 10 mL of physiological solution

were added. The LAB strains diluted with physiological solution were tested as (I) control; (II) with 50 µL of *Thymus vulgaris* EO; (III) with 100 µL of *Thymus vulgaris* EO. Count of LAB was determined after 0 and 24 hours of cultivation at 30°C. The LAB counts were determined on MRS agar (Liofilchem, Roseto degli Abruzzi, Teramo, Italy) using standard plate count techniques (ISO 15214:1998). The plates were incubated at 30°C for 72 h under anaerobic conditions (using an AnaeroGen atmosphere generation system, Oxoid).

RESULTS AND DISCUSSION

Antibiotic Residues in Poultry Meat Samples

Antibiotic residues detected in poultry meat samples are showed in Table 3. Among the different classes of antimicrobials some of them are used for broad applications. For instance, fluoroquinolones and sulphonamides are used as growth promoters (GP) as well as drugs against a broad spectrum of both Gram positive and Gram negative microorganisms (Jiang et al., 2013). In this study, antibiotic residues were found in 3 out of the 20 poultry meat samples analysed: enrofloxacin (0.46 µg/kg) was found in the sample No. 8, enrofloxacin and doxycycline (0.05 and 16.8 µg/kg, respectively) were found in the sample No. 14, and enrofloxacin (2.06 µg/kg) was found in the sample No. 18. Our previous studies showed that 37 out of 40 samples contained residues of enrofloxacin in the concentration range of 3.3 - 1126 ng/kg (Pugajeva et al., 2018). Since finding that ANB can promote the growth of animals, various ANBs have been added to animal feed at sub-therapeutic doses. Although this practice has been beneficial for animal productivity, there is a concern about long term effects or the environment and the public health. The frequent use of ANB in animal feed has led to the dissemination of ANB-resistant strains of poultry pathogens, such as *Salmonella*, *Campylobacter*, and *Escherichia coli* (Gayatri et al., 2018). Also, the use of ANB as a GP in animal feed, which lead to their residues in meat, can cause allergic reactions, as well as technological problems during fermentation of

certain meat products (Pavlov et al., 2005). The European Centre for Disease Prevention and Control (ECDC) states that ANB resistance continues to be a serious public health threat worldwide, and the European Commission (EC) decided in 2006 to ban all commonly used ANB-GP in animal feed due to concerns about the potential for ANB resistant strains of bacteria and ANB residues in meat products. For this reason, there has been considerable interest in alternatives to ANB (Denli and Demirel, 2018). In order to reduce the risk of anti-bacterial resistance, the European Union (EU) applied a "precautionary principle" model by banning certain antimicrobial GP (Kriebel et al., 2001). For those ANB that are not banned, maximum residue limits (MRLs) of ANB have been set by EU countries and the USA to ensure the safety of consumers. According to the definition by EU authorities, the MRL is the maximal legally acceptable amount of pharmacologically active substances and their metabolites in foodstuffs originating from animals. The MRLs are calculated with reference to the Acceptable Daily Intake (ADI), which includes a large safety margin in the calculation, and the ADI for meat is about 500 grams per person (Mungroo and Neethirajan, 2014). The requirements of those regulations can be met by relying on a withdrawal period, which is the time period between the last doses of any pharmacologically active substance administered to the animal and the time at which the residue level in tissues or products must not exceed the MRL. Withdrawal periods promote consumer safety by ensuring that the MRL is not exceeded (MRLs, 2014; MRLs, 2001). Although efforts have been made to harmonize MRLs worldwide under the aegis of World Trade Organization (WTO) and the Codex Alimentarius, MRLs still vary from one geographical location to another. In fact, MRLs in a particular animal product may differ from one country to another depending on the local food safety regulatory agencies and drug usage patterns (APVMA, 2014). Acceptable daily intake (ADI) is also a key requirement that is established on the basis of the No Observable Effect Level (NOEL), as identified from toxicological studies, divided by a safety factor (often 100) (MRLs, 2001). The MRLs for the sum of enrofloxacin and ciprofloxacin and for doxycycline in poultry muscle are 100 μg/kg. According to

the results of this study, the problem with ANB residues is still relevant in the poultry industry of Germany, Poland, and Lithuania. However, in comparison with our previous results, ANB residues were found at lower amounts. These findings can be explained by improved control of food quality and the increased use of alternatives to ANB in the poultry industry.

Lactic Acid Bacteria, Essential Oils and Their Composition Antimicrobial Properties against Salmonella Strains

The inhibition zones (IZ) caused by LAB against the tested *Salmonella* strains, as well as the minimal inhibitory concentrations (MIC) of the tested LAB strains and Eos, and the IZ of their combinations are shown in Tables 4, 5, and 6, respectively.

When comparing the IZ caused by LAB against *Salmonella*, the LAB strains *Leuconostoc mesenteroides* LUHS225, *Lactobacillus curvatus* LUHS51, and *Lactobacillus brevis* LUHS173 did not inhibit the tested *Salmonella* strains. Furthermore, *Lactobacillus farraginis* LUHS206 did not exhibit antimicrobial activity against *Salmonella* K43, while *Pediococcus pentosaceus* LUHS183 and *Pediococcus acidilactici* LUHS29 did not exhibit antimicrobial activity against the *Salmonella* strain K76 (Table 4). However, the other tested LAB strains inhibited all of the tested *Salmonella* strains and the highest IZ was caused by the LAB strains LUHS122, LUHS135, and LUHS245 against the *Salmonella* strain K2 (the average IZ diameter was 14.3 mm), LAB strains LUHS206 and LUHS245 against the *Salmonella* strain K5 (the average IZ diameter was 14.2 mm), LAB strain LUHS245 against the *Salmonella* strain K43 (the average IZ diameter was 14.0 mm), LAB strain LUHS135 against the *Salmonella* strain K72 (the average IZ diameter was 14.0 mm), and LAB strain LUHS245 against the *Salmonella* strain K76 (the average IZ diameter was 14.0 mm).

When comparing the MIC of the LAB strains and EOs against the tested *Salmonella* strains, it was found that all of the tested LAB strains at both test concentrations inhibited *Salmonella*, except for 0.5

mL of LUHS29 + 0.01 mL of *Salmonella* strain K43 suspension (Table 5). Comparing the MICs of the tested EOs, the oregano EO did not inhibit *Salmonella* strains at any of the tested concentrations, while the thyme EO at 0.2% concentration inhibited the *Salmonella* strains K2 and K72, at 0.5% concentration inhibited the *Salmonella* strains K2, K72, and K76, and at 1.0% inhibited all of the tested *Salmonella* strains.

Further experiments were performed with the LAB strains LUHS122, LUHS242, LUHS210, LUHS244, LUHS135, LUHS71, and LUHS245 in combination with different concentrations of thyme EO, which had previous shown the highest antimicrobial activity against *Salmonella* (Table 6). It should be mentioned that it is very important to reduce the necessary concentration of EOs, because EOs possess very intense flavours that may not be palatable for animals and thus negatively affect the feed consumption. When comparing the antimicrobial properties of LAB and EO combination with the effects of LAB alone, the addition of EOs at the concentrations of 0.1 and 0.2% reduced the antimicrobial properties of the mixture (the strains K5, K43, and K76 were not inhibited, while the inhibition of strain K76 remained similar in comparison with pure LAB). However, the addition of EOs at the concentrations of 0.5 and 1.0% enhanced the antimicrobial properties of the LAB mixture, compared to LAB strains alone, and the antimicrobial activity was further improved by increasing the concentration of EO (the IZ diameters resulting from 0.5 and 1.0% of EO in combination with LAB were on average 12.4 and 14.5 mm, respectively). It should be mentioned that the *Salmonella* strain K2 was not inhibited by LAB strains alone or in mixtures with EOs at the concentrations of 0.1 and 0.2%, however, increasing the concentration of EO to 0.5% and 1.0% suppressed this strain (the IZ diameters were 13.0 and 14.2 mm for LAB in combination with 0.5 and 1.0% of EO, respectively).

At the last stage of this experiment, the antimicrobial properties of thyme EO at the selected concentrations were tested against LAB strains (Table **7**). It was established that most of the LAB strains were inhibited by thyme EO at 0.5 and 1.0% concentrations, except for LUHS122, LUHS210,

311 and LUHS245. By using 0.5% of thyme EO, the counts of LAB strains LUHS122, LUHS210, and
312 LUHS245 were reduced by 26.5, 16.7, and 27.8%, respectively. When using 1.0% of thyme EO, the
313 counts of LAB strains LUHS122, LUHS210, and LUHS245 were reduced by 29.2, 44.7, and 43.2%,
314 respectively. Finally, it could be assumed *Salmonella* inhibition was not caused directly by the the
315 viable cells of LAB strains, but rather their metabolites and further studies will be needed to identify
316 which metabolites are the most important.

317 The desirable properties of probiotics (PRO) in poultry have been recognized since Rantala and
318 Nurmi (1973), who observed that the bacteria from the gut of mature birds can be used for the
319 protection of young chicks from infection. Baba et al. (1991) published their findings that the
320 composition of several PRO strains is more effective at reducing *Salmonella* colonization in chicks
321 than any individual PRO strain. Later it was published that PRO comprised of 29 bacterial strains also
322 reduced the amount of recoverable *Salmonella* from chicks (Corrier et al., 1990). Furthermore,
323 anaerobic PRO extracted from caeca suppressed *Salmonella* (Impey et al., 1984) or *Salmonella* and
324 *Campylobacter* (Blankenship et al., 1993; Stern et al., 2001; Higgins et al., 2007).

325 Thomas et al. (2019) published that culture supernatants from *Lactobacillus ingluviei* strain
326 UMNPBX19 and *Lactobacillus salivarius* strain UMNPBX2 exhibited antimicrobial activity
327 against *Salmonella*. A study by Adetoye et al. (2018) demonstrated *in vitro* suppression of *Salmonella*
328 by intestinal LAB from cattle (*Lactobacillus amylovorus* C94 and *Lactobacillus salivarius* C86). The
329 data published by Burkholder et al. (2019) suggested a protective effect of *L. acidophilus*, *L.*
330 *rhamnosus*, and *L. casei* against *Salmonella enterica Javiana*. Ahmed et al. (2019) concluded that
331 *Lactobacillus* species with PRO properties can be used in poultry feed formulation for their health
332 benefits to combat gastrointestinal infections. In their study, 6 out of 21 *Lactobacillus* strains showed
333 good antimicrobial activities against *S. aureus*, *S. typhimurium*, and *E. coli*. Our results are in
334 agreement with the aforementioned studies that demonstrated the ability of some LAB strains to

suppress *Salmonella*. However, the antimicrobial activity mechanisms of LAB can be explained in different ways. The data published by Zhu et al. (2019) indicate that the main mechanism of LAB activity against *Salmonella* infection is mediated by short-chain fatty acids (SCFA) excreted by the *Lactobacillus johnsonii* L531 strain used. Other authors have described how the surface proteins of *Lactobacillus kefiri* strains 8321 and 83113 and *Lactobacillus plantarum* strain 83114 can be used as alternative means for the control of *Salmonella* biofilm formation in the poultry industry (Lina Merino et al., 2019). Also, LAB can produce various inhibitory compounds such as bacteriocins, organic acids, hydrogen peroxide, diacetyl, and carbon dioxide that are known to inhibit pathogenic microorganisms (Vieco-Saiz et al., 2019). Enzymes excreted by LAB improve the rates of nutrient absorption, as well as stimulate the immune system of animals. It was demonstrated that nisin and beta-lactams excreted by LAB can inhibit the *Salmonella enterica serovar Typhimurium* (Rishi et al., 2014; Singh et al., 2014). It should be mentioned that the heterofermentative LAB can produce other metabolites: organic acids, ethanol, diacetyl, hydrogen peroxide (H_2O_2) etc. (Schnürer and Magnusson, 2005; Elshaghabee et al., 2016). Results of this study showed that not the viable LAB strains but their metabolites were the most important in *Salmonella* inhibition, and further studies are needed to identify which metabolites are the most important.

Organic acids excreted by LAB reduce pH, creating unfavorable local microenvironment for pathogens, resulting in their inhibition and death (Surendran Nair et al., 2017; Zhitnitsky et al., 2017; Dittoe et al., 2018). As demonstrated by Wang et al. (2015) lactic acid concentrations of 0.5% (v/v) could completely inhibit the growth of *Salmonella* spp. However, these acids do not affect animal epithelial cells (Allen and Flemström, 2005). The presence of ethanol excreted from LAB was shown to result in bacterial cell death due to plasma membrane leakage (Ingram, 1989). It was described that *Lb. plantarum*, *Lb. helveticus*, *Lb. bulgaricus*, *Ent. faecalis*, and mainly *Leuc. mesenteroides* and *Lc. lactis biovar diacetylactis* are the most common LAB species producing diacetyl (García-Quintáns et

359 al., 2008; Singh, 2018), which interferes with arginine utilization by reacting with the arginine-binding
360 protein of Gram-negative bacteria (Lindgren and Dobrogosz, 1990). Also, LAB can create anaerobic
361 environment by excreting CO_2, and aerobic bacteria cannot propagate in such environment (Singh,
362 2018). Some strains of LAB are able to produce hydrogen peroxide (H_2O_2), which can inhibit
363 pathogens devoid of catalase at low quantities via superoxide anion chain reaction enhancing toxic
364 oxidation (Mitchell et al., 2015). However, the antibacterial activity of H_2O_2 depends on its
365 concentration, pH, temperature, and other factors (Surendran Nair et al., 2017).
366 According to Sadia Ashraf et al. (2018), phytochemicals also can provide alternative options for the
367 treatment of antibiotic-resistant *Salmonella*, and it was concluded that *N. sativa* has the necessary *in-*
368 *vitro* activity against *S. enetrica* and thus can be used as a therapeutic agent. In a study with extracts of
369 natural compounds it was shown that some phenolic type natural products possessed evident
370 antibacterial ability against pathogenic bacteria, but not against LAB. The most common phenolic
371 compounds (carvacrol, *trans*-cinnamaldehyde, *p*-coumaric acid, eugenol, gallic acid, and rosmarinic
372 acid) exhibit strong antibacterial effects against pathogenic bacteria that are mainly responsible for the
373 antibacterial activity of EOs (Chak-LunChan et al., 2018). It was reported that a combination of EOs
374 obtained from *S. aromaticum* and *C. zeylanicum* inhibited both *S. Enteritidis* and *S. Typhimurium*
375 isolates. Such antimicrobial activity has been attributed to the main EO compounds: cinnamaldehyde
376 and eugenol (Ismail et al., 2017). Cinnamaldehyde and eugenol are able to inhibit the production of
377 essential bacterial enzymes due to the presence of a carbonyl group that binds and inactivates them
378 and/or causes damage to the bacterial cell wall (Di Pasqua et al., 2007). The presence of
379 cinnamaldehyde and eugenol may enhance the antibacterial effect, as suggested by Burt (2004). EOs
380 from *A. triphylla*, *C. citratus*, *L. cubeba*, and *M. piperita* showed no relevant activity against
381 *Salmonella*, however, other authors have described *in vitro* antibacterial activity of EOs from *S.*
382 *aromaticum* and *C. zeylanicum* against paratyphoid *Salmonella* strains (Thanissery et al., 2014;

Simitzis et al., 2014; Abbes et al., 2018). It has been reported that the EOs of cinnamon (*Cinnamomum zeylanicum*) and thyme (*Thymus vulgaris*) produced the highest activity, with 22.5–38.5 mm inhibition zones against five *Salmonella* serotypes (Olaimat et al., 2019). In a different application, the EO of thyme in combination with cold plasma treatment led to a higher antibacterial activity of plasma-treated nanofibers (Lin et al., 2019). EOs could be applied for the purposes of facility disinfection, as well as added to chicken feed to prevent intestinal colonization with pathogens (Ebani et al., 2019). The antimicrobial activity data for EOs showed that thymol, eugenol, and carvacrol exhibit strong antimicrobial activity against both *Escherichia coli* and *Salmonella typhimurium* (Bassole and Juliani, 2012; Franz et al., 2010; Hippenstiel et al., 2011). Thymol, eugenol, and carvacrol have similar chemical structures and exert synergic antimicrobial effects (Bassole and Juliani, 2012), but it is necessary to optimize their formulation (Zhai et al., 2018). In conclusion, it must be pointed out that although there are several viable approaches for pathogen control on meat and eggs in the conventional poultry industry, the selection of acceptable antibacterials is much more limited for organic poultry producers (Arsi et al., 2019). The findings of this study provide useful data regarding effective strategies for pathogen control at organic farms.

CONCLUSIONS

The problem with ANB residues still is highly relevant in the poultry industries of Germany, Poland, and Lithuania, despite the fact that only low ANB concentrations were established (0.46 µg/kg of enrofloxacin in sample No.8, 0.05 and 16.8 µg/kg of enrofloxacin and doxycycline, respectively, in sample No.14, and 2.06 µg/kg of enrofloxacin in sample No.18). For this reason, there is a ongoing search for new alternatives to ANB in the poultry industry. The most effective composition for the control of *Salmonella* tested in this study consists of thyme EO (1.0%) with the following LAB strains:

LUHS122, LUHS242, LUHS210, LUHS244, LUHS135, LUHS71, and LUHS245. However, it should be mentioned that most of the tested LAB strains were inhibited by thyme EO at the concentrations of 0.5 and 1.0%, except for LUHS122, LUHS210, and LUHS245. Finally, it can be noted that further studies are needed to identify the particular metabolites of LAB that are the most effective agents for the control of *Salmonella spp.*.

Compliance with Ethical Standards

Conflict of Interest. The authors declare that they have no conflicts of interest.

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674 **Tables**

675

676 **Table 1.** Poultry meat samples.

No.	Type of poultry	Country of origin	The country of retail purchase
1	Chicken	Germany	Germany
2			
3			
4			
5			
6			
7			
8			
9			
10			
11		Latvia	Lithuania
12		Lithuania	
13		Poland	
14		Poland	
15		Lithuania	
16		Lithuania	
17		Lithuania	
18		Lithuania	
19		Lithuania	
20		France	

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679 **Table 2.** The antibiotic – resistance profile of *Salmonella*.

Salmonella strains	Antibiotics
Salmonela K2	AMP, KAN, NEO, TET, DOXY, CIP
Salmonela K5	AMP, KAN, NEO, GEN, DOXY
Salmonela K43	AMP, DOXY, CIP, SXT, FUR
Salmonela K72	FUR
Salmonela K76	DOXY, FUR

AMP – ampicillin; KAN – kanamycin; NEO – neomycin; GEN – gentamicin; DOXY – doxycycline; CIP – ciprofloxacin; SXT – sulfamethoxazole/trimethoprim; FUR – nitrofurantoin

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683 **Table 3.** Antibiotic residues in poultry meat samples.

No.	Type of poultry	Country of origin	The country of retail purchase	Enrofloxacin	Doxycycline
				µg/kg	
8	Chicken	Germany	Germany	0.46±0.03	nd
14		Poland	Lithuania	0.05±0.01	16.80±0.13
18		Lithuania		2.06±0.05	nd

Values are mean ± SD (standard deviation) of three replicate analyses (n=3).

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688 **Table 4.** The inhibition zones (mm) caused by lactic acid bacteria (LAB) against the tested *Salmonella*

689 strains.

Salmo-nella strains	Diameter of inhibition zone, mm												
	LAB strains												
	225	122	242	210	51	206	183	29	244	135	71	173	245
K2	nd	14.3 ±1.2b	12.3 ±0.3a	10.3 ±0.5a	nd	10.2 ±0.6a	11.0 ±0.9a	12.1 ±0.6a	11.3 ±0.3a	14.2 ±0.2b,c	11.0 ±0.5a	nd	14.3 ±0.5b
K5	nd	12.1 ±0.9a	12.0 ±0.3a	12.0 ±1.0a	nd	14.3 ±0.7c	11.0 ±0.4a	12.0 ±0.3a	12.1 ±0.5a	13.3 ±0.3b	11.0 ±0.3a	nd	14.0 ±0.3b
K43	nd	13.2 ±0.4a	13.3 ±0.2b	11.2 ±0.9a	nd	nd	11.0 ±0.6a	12.3 ±0.5a	13.2 ±0.3b	12.4 ±0.5a	12.3 ±0.2b	nd	14.0 ±0.5b
K72	nd	13.3 ±0.5a	11.3 ±0.9a	10.0 ±0.7a	nd	12.3 ±1.0b	12.3 ±0.9a	12.0 ±0.3a	13.3 ±0.3b	14.0 ±0.6b	11.5 ±0.3a	nd	12.3 ±0.6a
K76	nd	12.1 ±1.1a	11.0 ±0.7a	11.3 ±1.2a	nd	12.0 ±0.7b	nd	nd	11.0 ±0.3a	13.1 ±0.3b	12.3 ±0.3b	nd	14.0 ±0.4b

225 - *Leuconostoc mesenteroides* LUHS225*;* 122- *Lactobacillus plantrum* LUHS122*;* 242 - *Enteroccocus pseudoavium* LUHS242; 210 - *Lactobacillus casei* LUHS210*;* 51 - *Lactobacillus curvatus* LUHS51*;* 206 - *Lactobacillus farraginis* LUHS206*;* 183 - *Pediococcus pentosaceus* LUHS183*;* 29 - *Pediococcus acidilactici* LUHS29*;* 244 - *Lactobacillus paracasei* LUHS244*;* 135 - *Lactobacillus plantarum* LUHS135*;* 71 - *Lactobacillus coryniformins* LUHS71*;* 173 - *Lactobacillus brevis* LUHS173*;* 245 - *Lactobacillus uvarum* LUHS245.

Values are mean ± SD (standard deviation) of three replicate analyses (n=3).

[a-c] Mean values with different letters are significantly different ($p \leq 0.05$).

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693 **Table 5.** The minimal inhibitory concentrations (MIC) of the lactic acid bacteria (LAB) strains and

694 essential oils (EOs) against the tested *Salmonella* strains.

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Salmonella strains	MIC												
	Lactic acid bacteria strains												
	0.5 mL LAB + 0.01 mL pathogen												
	225	122	242	210	51	206	183	29	244	135	71	173	245
K2	-	-	-	-	-	-	-	-	-	-	-	-	-
K5	-	-	-	-	-	-	-	-	-	-	-	-	-
K43	-	-	-	-	-	-	-	+	-	-	-	-	-
K72	-	-	-	-	-	-	-	-	-	-	-	-	-
K76	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.5 mL LAB + 0.1 mL pathogen												
K2	-	-	-	-	-	-	-	-	-	-	-	-	-
K5	-	-	-	-	-	-	-	-	-	-	-	-	-
K43	-	-	-	-	-	-	-	-	-	-	-	-	-
K72	-	-	-	-	-	-	-	-	-	-	-	-	-
K76	-	-	-	-	-	-	-	-	-	-	-	-	-
	EOs												
	0.1% Eos + 0.01 mL pathogen		0.2% Eos + 0.01 mL pathogen		0.5% Eos + 0.01 mL pathogen		1% Eos + 0.01 mL pathogen						
	Thy	Ore	Thy	Ore	Thy	Ore	Thy	Ore					
K2	+	+	+	+	+	+	-	+					
K5	+	+	-	+	-	+	-	+					
K43	+	+	-	+	-	+	-	+					
K72	+	+	+	+	+	+	-	+					
K76	+	+	-	+	+	+	-	+					

225 - *Leuconostoc mesenteroides* LUHS225*;* 122- *Lactobacillus plantrum* LUHS122*;* 242 - *Enteroccocus pseudoavium* LUHS242; 210 - *Lactobacillus casei* LUHS210*;* 51 - *Lactobacillus curvatus* LUHS51*;* 206 - *Lactobacillus farraginis* LUHS206*;* 183 - *Pediococcus pentosaceus* LUHS183*;* 29 - *Pediococcus acidilactici* LUHS29*;* 244 - *Lactobacillus paracasei* LUHS244*;* 135 - *Lactobacillus plantarum* LUHS135*;* 71 - *Lactobacillus coryniformins* LUHS71*;* 173 - *Lactobacillus brevis* LUHS173*;* 245 - *Lactobacillus uvarum* LUHS245; Thy - *Thymus vulgaris;* Ore - *Origanum vulgare L.*
Values are mean ± SD (standard deviation) of three replicate analyses (n=3).
MIC – minimal inhibitory concentration.
(-) – the pathogens did not grew, (+) – the pathogens grow.

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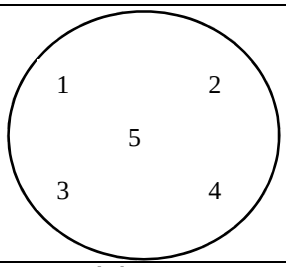
700 **Table 6.** The inhibition zones (mm) of the lactic acid bacteria (LAB) strains and thyme (Thy) essential

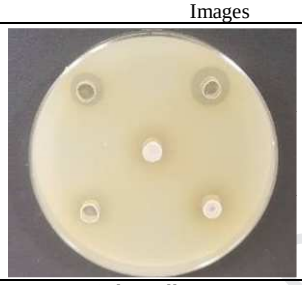
701 oil (EO) compositions against the tested *Salmonella* strains.

Salmonella strains	Inhibition zone, mm				
	LAB strains composition	LAB strains and Thy EO composition (0.1 % EOs)	LAB strains and Thy EO composition (0.2 % EOs)	LAB strains and Thy EO composition (0.5 % EOs)	LAB strains and Thy EO composition (1 % EOs)
K2	nd	nd	nd	13.0±0.2	14.2±0.3
K5	10.0±0.3	nd	nd	12.5±0.3	15.0±0.2
K43	11.0±0.1	nd	nd	11.2±0.1	15.4±0.5
K72	10.5±0.4	nd	nd	12.0±0.3	14.1±0.3
K76	10.0±0.2	10.0±0.1	10.0±0.3	13.5±0.2	14.0±0.4

Images

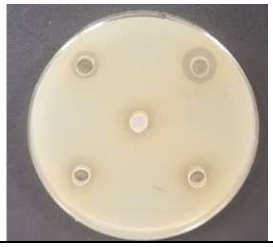


1 – LAB strains and Thy EO composition (0.5 % EOs); 2 – LAB strains and Thy EO composition (1.0 % EOs); 3 – LAB strains and Thy EO composition (0.2% EOs); 4 – LAB strains and Thy EO composition (0.1 % EOs); 5 – LAB strains composition



Salmonella K2

Salmonella K5



Salmonella K43

Salmonella K72

Salmonella K76

LAB - Lactic acid bacteria; EO – essential oil; Thy – Thymus vulgaris.
Lactic acid bacteria composition consists of LUHS122, LUHS242, LUHS210, LUHS244, LUHS135, LUHS71, LUHS245 strains (122- *Lactobacillus plantarum* LUHS122*;* 242 - *Enteroccocus pseudoavium* LUHS242; 210 - *Lactobacillus casei* LUHS210*;* 244 - *Lactobacillus paracasei* LUHS244*;* 135 - *Lactobacillus plantarum* LUHS135*;* 71 - *Lactobacillus coryniformins* LUHS71*;* 245 - *Lactobacillus uvarum* LUHS245).
Values are mean ± SD (standard deviation) of three replicate analyses (n=3).

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704 **Table 7.** The effect of *Thymus vulgaris* essential oil (EO) influence on lactic acid bacteria (LAB)

705 inhibition.

	Lactic acid bacteria strains												
	LUHS 122	LUHS 244	LUHS 210	LUHS 242	LUHS 245	LUHS 135	LUHS 71	LUHS 183	LUHS 51	LUHS 29	LUHS 225	LUHS 206	LUHS 173
	log_{10} cfu mL^{-1}												
0.5 mL LAB	8.26 ± 0.03	8.32 ± 0.04	7.47 ± 0.02	7.99 ± 0.07	7.30 ± 0.06	7.09 ± 0.05	7.35 ± 0.04	7.59 ± 0.01	7.62 ± 0.06	7.50 ± 0.02	7.61 ± 0.03	6.22 ± 0.02	7.93 ± 0.04
0.5 mL LAB + Thy EO composition (0.5 % EOs)	6.07 ± 0.6	nd	6.22 ± 0.06	nd	5.27 ± 0.01	nd	nd	nd	nd	nd	nd	nd	nd
0.5 mL LAB + Thy EO composition (1.0 % EOs)	5.85 ± 0.06	nd	4.13 ± 0.04	nd	4.15 ± 0.03	nd	nd	nd	nd	nd	nd	nd	nd

LAB - Lactic acid bacteria; EO – essential oil; Thy – *Thymus vulgaris*.
LUHS122 - *Lactobacillus plantrum;* LUHS244 - *Lactobacillus paracasei;* LUHS210 - *Lactobacillus casei;* LUHS242 - *Enteroccocus pseudoavium;* LUHS245 - *Lactobacillus uvarum;* LUHS135- *Lactobacillus plantarum;* LUHS71 - *Lactobacillus coryniformins;* LUHS206 - *Lactobacillus farraginis;* LUHS29 - *Pediococcus acidilactici;* LUHS183 - *Pediococcus pentosaceus;* LUHS225 - *Leuconostoc mesenteroides;* LUHS173 - *Lactobacillus brevis;* LUHS51 - *Lactobacillus curvatus*

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708 **Table S1.** The characteristic data for mass spectrometric detection of antibiotics.

No	Compound	Antibiotic class	Retention time (min)	SRM1 (*m/z*)	CE1 (eV)	SRM2 (*m/z*)	CE2 (eV)
1	Amoxicillin	Penicillins	1.3	366→349	11	366→114	20
2	Ampicillin	Penicillins	6.4	350→106	20	350→160	15
3	Bacitracin	Peptides	10.1	712→199	35	475.2→199	25
4	Cefacetrile	Cephalosporins	2.6	357→156	13	357→280	20
5	Cefalexin	Cephalosporins	7.1	380→198	18	380→106	18
6	Cefalonium	Cephalosporins	3.3	459→152	30	459→337	14
7	Cefapirim	Cephalosporins	2.1	424→124	35	424→292	20
8	Cefazolin	Cephalosporins	6.3	455→156	30	455→323	18
9	Cefoperazone	Cephalosporins	7.5	646→143	40	646→530	20
10	Cefquinome	Cephalosporins	4.3	529→134	25	265→134	25
11	Ceftiofur	Cephalosporins	9.3	524→210	25	524→241	20
12	Chlortetracycline	Tetracyclines	7.8	479→444	21	479→462	20
13	Ciprofloxacin	Quinolones	6.2	332→288	22	332→314	15
14	Cloxacillin	Penicillins	10.4	468→160	25	468→436	20
15	Danofloxacin	Quinolones	6.6	358→255	42	358→340	20
16	Dicloxacillin	Penicillins	10.4	470→160	20	470→311	25
17	Difloxacin	Quinolones	7.0	400→356	23	400→382	23
18	Doxycycline	Tetracyclines	9.2	445→321	45	445→428	20
19	Enrofloxacin	Quinolones	6.5	360→245	24	360→316	20
20	Erythromycin	Macrolides	10.2	734.4→158	20	734.4→576	33
21	Flumequine	Quinolones	9.8	262→202	10	262→244	20
22	Josamycin	Macrolides	10.5	828→174	30	861→109	34
23	Kitasamycin	Macrolides	10.2	805→109	45	805→174	40
24	Lincomycin	Lincosamide	3.7	407→126	25	407→359	16
25	Marbofloxacin	Quinolones	4.7	363→320	14	363→276	14
26	Nafcillin	Penicillins	10.6	415→199	20	415→171	40
27	Nalidixic acid	Quinolones	9.6	233→187	26	233→215	15
28	Neospiramycin	Macrolides	8.5	366→174	20	350→174	20
29	Norfloxacin	Quinolones	5.9	320→276	20	320→302	15
30	Novobiocin	Other antibiotics	11.6	635→418	20	613.5→189	20
31	Orbifloxacin	Quinolones	6.8	396→295	22	396→352	27
32	Oxacillin	Penicillins	10.3	402→160	20	402→243	30
33	Oxolinic acid	Quinolones	8.6	263→217	35	263→245	25
34	Oxytetracycline	Tetracyclines	5.4	461→426	20	461→443	20
35	Penicillin G	Penicillins	6.0	335→128	32	335→176	30
36	Penicillin V	Penicillins	8.5	351→114	40	351→160	20
37	Pirlimycin	Lincosamide	9.3	411→112	35	411→363	26
38	Rifaximin	Rifamycins	10.9	786.5→754	22	787.5→755	50
39	Sarafloxacin	Quinolones	9.0	386→342	22	386→299	28
40	Spiramycin	Macrolides	8.0	422→174	30	422→350	12
41	Sulfachloropyridazine	Sulphonamides	5.1	285→156	16	285→92	33
42	Sulfadiazine	Macrolides	1.4	251→92	30	251→156	18
43	Sulfadimethoxine	Sulphonamides	8.4	311→156	25	311→108	35
44	Sulfadimidine	Sulphonamides	4.0	279→124	23	279→186	20
45	Sulfadoxine	Sulphonamides	6.3	311→108	27	311→156	20
46	Sulfamerazine	Sulphonamides	2.4	265→156	20	265→172	18
47	Sulfamethiazole	Sulphonamides	4.0	271→156	14	271→92	28
48	Sulfamonomethoxine	Sulphonamides	5.8	281→108	25	281→156	20
49	Sulfanilamide	Sulphonamides	6.1	172→156	10	172→108	15
50	Sulfathiazole	Sulphonamides	1.9	256→92	30	256→156	15
51	Tetracycline	Tetracyclines	5.4	445→154	30	445→410	20
52	Thiamphenicol	Amphenicols	3.6	356→229	30	356→308	20
53	Tiamulin	Pleuromutilins	10.1	494→192	20	494→119	35
54	Tildipirosin	Macrolides	4.5	637.6→174	35	637.6→464	35
55	Tilmicosin	Macrolides	9.6	435→696.5	20	435.5→99	25
56	Trimethoprim	Other antibiotics	4.3	291→110	30	291→123	30
57	Tulothromycin A	Macrolides	7.3	806→420	35	806→577	20
58	Tylosin	Macrolides	10.1	917→174	35	917→772.6	26
59	Tylvalosin	Other antibiotics	10.7	1043→174	40	1043→109	45
60	Valnemulin	Other antibiotics	10.6	565→147	40	565→263	20

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