Meat industry wastewater: microbiological quality and antimicrobial susceptibility of E. coli and Salmonella sp. isolates, case study in Vojvodina, Serbia

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ABSTRACT

Wastewater from meat processing industries is a fusion of compounds with a high load of organic matter, and pathogen microorganisms like Escherichia coli, and Salmonella sp. The aim of this research was to determine microbiological characteristics of the wastewater discharged from the meat processing industry in order to get a more detailed insight into meat industry wastewater pollution, and to evaluate the resistance of bacterial strains E. coli and Salmonella sp. to antibiotics. The evaluation of the antimicrobial susceptibility was performed on 37 strains of E. coli and eight strains of Salmonella sp. to nine different antibiotics. The number of faecal pollution indicators was very high in all samples. From a total of 37 strains of E. coli, a moderate degree of resistance was shown to tetracycline (37.83%); a low degree of resistance to ampicillin (21.62%), streptomycin (24.32%), trimethoprim-sulfamethoxazol (18.92%) and nalidixic acid (16.22%); and very low to: chloramphenicol (13.51%), ciprofloxacin (2.7%), gentamicin and cefotaxime (0.0%). The results for eight strains of Salmonella sp. show that all eight isolates had some degree of susceptibility to nine tested antimicrobial agents and six strains were fully susceptible to all tested antibiotics.

Key words | antimicrobial susceptibility, meat industry, wastewater

INTRODUCTION

The abattoir and meat production industry are very important components of the industrial production in the Vojvodina region. Wastewater from meat processing industries is a complex fusion of compounds with a high load of organic matter, as well as pathogen microorganisms like Escherichia coli and Salmonella sp. The main problem with wastewater in Serbia is the absence of its treatment and its direct discharge into sewer and in surface receiving waters (Radović et al. 2015). Very often, most of analysed, discharged wastewater samples do not meet the legal set of physico-chemical and microbiological criteria.

The widespread use of antibiotics both in therapy and in prophylaxis has led to a development of resistance in microorganisms. Large scale animal farming provides optimal opportunities for horizontal gene transfer among bacteria, because of the high microbial concentration and selective pressure applied by the routine non-therapeutic use of antibiotics for maximizing the profit. Human and veterinary drugs can be excreted unmetabolized in almost 95% of the administered dose, and discharged into the environment via wastewater, since most of the pharmaceuticals are unaffected by wastewater treatments, and pass into the environment unchanged (Hirsch et al. 1999; Heberer 2002; Calamari et al. 2003; Milic et al. 2012; Radović et al. 2015). Network of Reference Laboratories for Monitoring of Emerging Environmental Pollutants (NORMAN) provided a list of emerging substances of concern (NORMAN 2009). Antibiotics used in this experiment, with the exception of nalidixic acid, as widely used pharmaceuticals in human and veterinary medicine, are present on the NORMAN list of emerging substances of concern, since they are most frequently detected in aquatic environment and could potentially cause an adverse effect on the environment and living organisms, particularly in the aquatic media.

doi: 10.2166/wst.2016.113
Due to their ubiquitous presence in the environment, increasing bacterial resistance to their effects can be expected. *E. coli* is a good indicator organism for monitoring antimicrobial drug resistance in faecal bacteria since it is found in a variety of hosts, it acquires resistance easily and is a reliable indicator of resistance in *Salmonella* sp. (Erb et al. 2007; Womack et al. 2010).

The aim of this research was to determine microbiological characteristics of the wastewater discharged from the meat processing industry in order to get a more detailed insight into meat industry wastewater pollution. Moreover, the resistance of *E. coli* and *Salmonella* sp. bacterial strains to antibiotics was evaluated.

### MATERIALS AND METHODS

During four seasonal sampling campaigns, in the spring, summer and autumn periods in 2013, and the winter period in 2014, four samples were collected each time from three meat processing plants in the Province of Vojvodina (Table 1). The number of samples was limited to four since most of meat processing facilities refused to give permission for wastewater sampling at their location. Due to the fact that we are not permitted to disclose which meat processing industries are in question, they are labelled as MI1, MI2 and MI3.

Two samples in each sampling campaign (sample 1 and sample 4) were effluent waters from two different meat processing facilities, which do not treat their wastewater. The other two samples (sample 2 and sample 3) were taken from the same meat processing facility, but since this facility has wastewater treatment, samples were taken before (sample 2) and after (sample 3) the treatment. Wastewater samples were taken in the peak load time for each meat processing facility, in regard with previous research (Djogo et al. 2015).

Only one of three selected meat processing facilities had wastewater treatment technology. Wastewater treatment in this facility included primary, secondary and tertiary treatment. The primary treatment contained coarse solid separation, flotation and sludge separation. The secondary treatment included pre-oxidation treatment followed by the biological oxidation process. Lastly, tertiary treatment consisted of sedimentation, denitrification and disinfection with sodium hypochlorite.

Microbiological and biochemical analysis included monitoring of seven parameters: number of aerobic mesophilic bacteria, total coliforms, faecal coliforms, *E. coli*, faecal enterococci, presence of *Salmonella* sp. as well as determining the phosphatase activity index (PAI) which is used as a biochemical indicator of heterotrophic activity of aquatic microorganisms, and as an indicator of general organic load. All microbiological parameters were analysed by use of the standard ISO methods: ISO 6222 (aerobic mesophilic bacteria), ISO 9308-2 (total coliforms, faecal coliforms and *E. coli*), ISO 7899-2:2000 (faecal enterococci), ISO 6579-2002/A1 (*Salmonella* sp. presence). PAI was determined by the procedure of Matavulj (p986). High phosphatase activity indicates high organic pollution. PAI values above 15 μmol/s/dm³ pNP classify water samples in the IVb category, the most organically polluted class of surface waters.

*E. coli* strains, tested for antimicrobial resistance, were isolated by spread plating sample dilutions of all samples on ChromoCult® Coliform agar (CCA) plates (Merck, Germany), and randomly selected from plates in order to represent all the samples equally. Typical violet colonies were subcultured on CCA and confirmed by following biochemical tests: Indole production, Methyl Red test, Voges-Proskauer test and Citrate utilization test (Harley & Prescott 2002). *Salmonella* sp. strains were isolated according to ISO 6579:2002/A1 methodology, which consist of five stages: non-selective enrichment, selective enrichment, streaking on xylose lysine deoxycholate (XLD) and *Salmonella* Shigella (SS) agar, biochemical and serological confirmation. Isolated strains were stored at −80 °C until antimicrobial susceptibility testing was performed.

Antibiotic susceptibility testing was performed according to EUCAST Disk Diffusion Test Methodology version 4.0 (EUCAST 2014). Twenty-four hour culture was suspended in saline solution to form a suspension of about 1–2×10⁹ CFU/mL (corresponding to 0.5 McFarland standard and 0.08–0.13 absorbance); a suspension was poured on Mueller-Hinton agar plates (Tololak, Serbia), and antibiotic disks (Becton, Dickinson and Company, USA) were placed on agar surface. Plates were incubated for 16–20 h on 35 °C. Antibiotics, with the amount of each antibiotic on the disc,
used for susceptibility testing were: ampicillin (AMP, 10 μg), cefotaxime (CEF, 30 μg), ciprofloxacin (CIP, 5 μg), chloramphenicol (C, 30 μg), gentamicin (G, 10 μg), nalidixic acid (NA, 50 μg), streptomycin (S, 10 μg), tetracycline (TET, 30 μg), and trimethoprim-sulfamethoxazole (SXT, trimethoprim 1.25 μg/sulfamethoxazole 23.75 μg) (Becton, Dickinson and Company, USA). Tested antibiotics were chosen based on European Food Safety Authority/European Centre for Disease Prevention and Control (EFSA/ECDC) recommendations for Escherichia coli and Salmonella resistance monitoring (EFSA/ECDC 2015). The diameter of inhibition zone was measured, and susceptibility category (susceptible-S, intermediate-I, and resistant-R) was determined according to the antibiotic disk’s manufacturer directions. Bacterial strain Escherichia coli ATCC 25922 was used as a quality control (EFSA/ECDC 2015).

Strains of E. coli tested for antimicrobial resistance were selected randomly from plates of Chromocult Coliform agar used for E. coli enumeration, with regard to represent all of the samples equally. For that reason, comparison of antimicrobial resistance patterns among the tested strains from different locations cannot be inferred. Only a general comparison of resistance of all tested strains from all locations combined together can be made in reference with other literature data.

From every sample positive for the presence of Salmonella sp. one strain of this bacteria was used for antimicrobial susceptibility testing.

The evaluation of the antimicrobial susceptibility was performed on 37 strains of E. coli and eight strains of Salmonella sp. to nine different antibiotics.

RESULTS AND DISCUSSION

Aerobic mesophilic bacteria are reliable microbiological indicators of general organic load in any type of water samples which is why this bacterial group is of great importance in the monitoring of wastewater treatment efficiency. Since all of our samples originated from meat industry facilities, high organic load and significant number of aerobic mesophilic bacteria were expected. All samples taken during four sampling campaigns showed very high counts of aerobic mesophilic bacteria. Sample 1 emerged as the wastewater sample with the highest aerobic mesophilic bacteria count and, therefore, the sample with the most prominent organic pollution, which is in correlation with high values of HPK, BPK₅, and total suspended solids determined by Milanovic et al. (2015). Fluctuations in quality of wastewater are highly dependent on workload and stage of production and can considerably influence the number of present bacteria in the sample.

Unambiguous indicators of the faecal pollution are total coliforms, faecal coliforms, E. coli and faecal enterococci. Domestic animals are often carriers of many pathogenic bacteria which can also be pathogenic to humans (Schwartz et al. 2004). The number of these faecal pollution indicators was very high in all samples, which makes wastewaters from meat industry facilities potentially very hazardous and they should not be discharged in open recipient water without previous treatment, especially when their potential resistance to antibiotics is taken into account. The total coliform bacterial count is a very good example on how the quality of meat industry wastewater samples can fluctuate between two sampling campaigns. The total coliform count in sample 1 decreased from 16.000.000 CFU/mL during spring campaign to 1.700 CFU/mL during summer campaign. Even though the wastewater samples were taken in the peak load time for each meat processing facility, fluctuations in quality of wastewater are evident and highly dependent on work load and stage of production.

Faecal coliforms and E. coli often had similar values in all samples. This means that E. coli is a dominant coliform species present in this type of sample. Enterococci are also used as an indicator of faecal contamination. Their count was, in most cases, 1–2 log10 units lower than the count of aerobic mesophilic bacteria (Figure 1). In regard to the number of the present coliform bacterial group, enterococci values were sometimes significantly lower in comparison to them, but at the other times they were higher, showing the large degree of variability during different sampling campaigns and between the sampling locations. Since coliform bacteria and enterococci are regular microbiomes in the gastrointestinal tracts of all animals, their number in the wastewater sample may vary depending on the type and number on processed animals. Salmonella sp. was always present in samples 2 and 3, which are influent and effluent samples from the same location. On two other locations Salmonella sp. was present at only one time – in sample 1 during the autumn campaign (Table 2).

Only samples from location 3 had IPA values lower than 15 μmol/s/dm³ pNP, and all other samples had values significantly above this limit, making them classified as highly organically polluted waters (Table 2).

Serbian legislation regarding the wastewater quality of the meat processing industry does not regulate any of the microbiological parameters. To evaluate the quality of wastewater samples in this study we used EPA's acceptable
limits (faecal coliforms: 400 MPN/100 mL of effluent wastewater) and limits for treated communal wastewaters in Serbia (total coliforms 10,000/100 mL, faecal coliforms 2,000/100 mL, enterococci 400/100 mL). None of the analysed effluent samples satisfied mentioned criteria.

Particular attention should be drawn to samples 2 and 3 in all sampling campaigns. These are inlet and outlet samples from the same meat processing industry facility, and they show significant decline in all microbiological and biochemical parameters after water treatment. Reduction of all mentioned parameters was from 96.08% to as high as 99.99%. PAI values in outlet samples were significantly lower than in other samples, classifying sample 3 in categories of moderately polluted waters (Matavulj 1986). The only parameter which treatment failed to remove was the presence of Salmonella sp., which is present in every sample before as well as after the water treatment (Table 2). Possible reasons for Salmonella sp. presence in inlet and outlet water could be that animals processed on this location came from the farms which are possible Salmonella sp. hot spots, or their biofilm formed along wastewater flow in the water treatment.

Antimicrobial susceptibility analysis of E. coli and Salmonella sp. bacterial strains showed individual variations (Table 2).

Testing of E. coli resistance on nine antimicrobial agents showed that this bacterium expresses a high degree of susceptibility to ciprofloxacin and cefotaxime as well as to chloramphenicol and trimethoprim-sulfamethoxazole. Lower
degree of susceptibility is detected for nalidixic acid, tetracycline and ampicillin (Table 3).

Testing of *Salmonella* sp. resistance on nine antimicrobial agents showed that eight strains of tested *Salmonella* sp. are susceptible to ciprofloxacin, ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, nalidixic acid, cefotaxime, and tetracycline. Its susceptibility on streptomycin and gentamycin showed a high percentage of intermediate category (Table 3).

From 37 examined strains of *E. coli*, ten strains (18.92%) were multidrug-resistant (MDR). Isolate is considered MDR if it is non-susceptible to at least one antibiotic from three or more different antimicrobial categories as proposed by Magiorakos *et al.* (2012). Five strains of *E. coli* were resistant to three, and two strains to four antimicrobial categories. None of the *Salmonella* isolates was MDR, with only two strains being resistant to tetracycline. Fully susceptible to all tested antibiotics were 54.05% of *E. coli* and 75% of *Salmonella* sp. isolates.

Antimicrobial resistance is almost always detected first with *E. coli* (Mišić *et al.* 2006). Monitoring of *Salmonella* sp. is also of great importance because of their frequent presence in domestic animals processed in the meat industry. These two bacteria among others are encompassed in European monitoring of antimicrobial resistance throughout the food chain: from live animals, farm environment, meat and human clinical isolates conducted yearly by EFSA and ECDC. *Salmonella* sp. are serious human pathogens causing >20 million cases of typhoid infections worldwide annually (Crump *et al.* 2004).

Thirty seven strains of *E. coli* were tested for the resistance on nine different antimicrobial agents, and the results are mostly in correlation with the results of other authors (Table 4).

Monitoring of antimicrobial resistance in *E. coli* is of great importance because this species is commonly present and can often acquire conjugative plasmids from other present enteric bacteria, thus serving as a valuable reservoir of

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### Table 2 | Salmonella sp. presence and PAI values in samples of meat industry wastewater during four sampling campaigns

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
<th>June</th>
<th>July</th>
<th>October</th>
<th>January</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> sp.</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PAI (μmol/s/dm³ pNP)</td>
<td>1</td>
<td>98.04</td>
<td>84.08</td>
<td>65.01</td>
<td>43.10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>65.56</td>
<td>157.60</td>
<td>70.15</td>
<td>98.34</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.53</td>
<td>0.43</td>
<td>10.84</td>
<td>12.03</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>75.19</td>
<td>26.33</td>
<td>55.73</td>
<td>15.43</td>
</tr>
<tr>
<td>COD°</td>
<td>1</td>
<td>3,460</td>
<td>5,680</td>
<td>4,429</td>
<td>1,901</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1,255</td>
<td>3,580</td>
<td>1,630</td>
<td>1,792</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>48.1</td>
<td>19.1</td>
<td>23.4</td>
<td>68.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1,380</td>
<td>840</td>
<td>1,580</td>
<td>526</td>
</tr>
</tbody>
</table>

*Results for COD are taken from Milanovic *et al.* (2015).*

### Table 3 | Antimicrobial susceptibility of *E. coli* and *Salmonella* sp. strains from meat industry wastewaters in Serbia

<table>
<thead>
<tr>
<th>CIP</th>
<th>CEF</th>
<th>C</th>
<th>SXT</th>
<th>NA</th>
<th>TET</th>
<th>AMP</th>
<th>G</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>%Susceptible</td>
<td>97.30</td>
<td>97.30</td>
<td>86.49</td>
<td>81.08</td>
<td>75.68</td>
<td>62.16</td>
<td>51.35</td>
</tr>
<tr>
<td></td>
<td>%Intermediate</td>
<td>0.00</td>
<td>2.70</td>
<td>0.00</td>
<td>0.00</td>
<td>8.11</td>
<td>0.00</td>
<td>27.03</td>
</tr>
<tr>
<td></td>
<td>%Resistant</td>
<td>2.70</td>
<td>0.00</td>
<td>13.51</td>
<td>18.92</td>
<td>16.22</td>
<td>37.84</td>
<td>21.62</td>
</tr>
<tr>
<td><em>Salmonella</em> sp.</td>
<td>%Susceptible</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>%Intermediate</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>%Resistant</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

AMP, ampicillin; C, chloramphenicol; CEF, cefotaxime; CIP, ciprofloxacin; G, gentamicin; NA, nalidixic acid; S, streptomycin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.
resistance genes which can be transferred to other bacteria present in same environment. For these reasons, antimicrobial resistance monitoring of non-pathogenic strains of *E. coli* can serve as an indicator of resistance in the general population (EFSA/ECDC 2018).

As documented in EFSA/ECDC’s report, resistance was lower in meat from pigs than from poultry, which for all tested antimicrobials showed the highest percentage of resistant strains.

The highest rate of resistance in our study was observed for ampicillin and tetracycline (21.6% and 37.8%) which corresponds to EFSA/ECDC data for the same year (2013). Resistance to all used antibiotics is in accordance with the study on antimicrobial resistance of *E. coli* from the pig farms in Germany (Guerra et al. 2003) and in Japan (Kijima-Tanaka et al. 2005).

<table>
<thead>
<tr>
<th>Origin of <em>E. coli</em> isolates</th>
<th>AMP</th>
<th>CEF</th>
<th>C</th>
<th>CIP</th>
<th>G</th>
<th>NA</th>
<th>S</th>
<th>TET</th>
<th>SXT</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe* – meat from bovine animals</td>
<td>17.1</td>
<td>1.2</td>
<td>7.3</td>
<td>1.2</td>
<td>0</td>
<td>1.2</td>
<td>15.9</td>
<td>19.5</td>
<td>–</td>
<td>EFSA/ECDC (2015)</td>
</tr>
<tr>
<td>Europe – meat from broilers</td>
<td>56.6</td>
<td>9.1</td>
<td>12.8</td>
<td>41.8</td>
<td>4.9</td>
<td>39.5</td>
<td>39.2</td>
<td>37.2</td>
<td>–</td>
<td>EFSA/ECDC (2015)</td>
</tr>
<tr>
<td>Europe – meat from pigs</td>
<td>30.7</td>
<td>3.6</td>
<td>8</td>
<td>6.4</td>
<td>1.4</td>
<td>2.9</td>
<td>35.5</td>
<td>32.6</td>
<td>–</td>
<td>EFSA/ECDC (2015)</td>
</tr>
<tr>
<td>Germany – cattle, swine and poultry at farms</td>
<td>30.3</td>
<td>1.3</td>
<td>14.7</td>
<td>6.1</td>
<td>1.8</td>
<td>3.8</td>
<td>47.8</td>
<td>52.8</td>
<td>–</td>
<td>EFSA/ECDC (2015)</td>
</tr>
<tr>
<td>Japan – pig faecal samples from farms</td>
<td>19.0</td>
<td>–</td>
<td>8–11</td>
<td>4.0</td>
<td>2.0</td>
<td>8–11</td>
<td>28/30</td>
<td>28/30</td>
<td>8–11</td>
<td>Guerra et al. (2003)</td>
</tr>
<tr>
<td>Denmark, The Netherlands, Spain, Sweden – pig faecal samples from slaughter</td>
<td>3.4–52.1</td>
<td>0</td>
<td>0.5–52.1</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>10.7–54.2</td>
<td>10.3–59</td>
<td>2.5–52.1</td>
<td>Bywater et al. (2004)</td>
</tr>
<tr>
<td>Thailand – swine, broiler chickens and human workers from farms and abattoirs</td>
<td>61.6</td>
<td>–</td>
<td>–</td>
<td>12.5</td>
<td>–</td>
<td>67.4</td>
<td>–</td>
<td>91.5</td>
<td>–</td>
<td>Hanson et al. (2002)</td>
</tr>
<tr>
<td>Portugal – poultry slaughterhouse wastewater and sludge</td>
<td>56.5</td>
<td>–</td>
<td>15.1</td>
<td>–</td>
<td>5.3</td>
<td>–</td>
<td>39.2</td>
<td>80.7</td>
<td>47.5</td>
<td>da Costa et al. (2008)</td>
</tr>
<tr>
<td>Serbia – meat industry wastewater</td>
<td>21.6</td>
<td>0.0</td>
<td>13.5</td>
<td>2.7</td>
<td>0.0</td>
<td>16.2</td>
<td>24.3</td>
<td>37.8</td>
<td>18.9</td>
<td>This study</td>
</tr>
</tbody>
</table>

*Member states of European Union; AMP, ampicillin; C, chloramphenicol; CEF, cefotaxime; CIP, ciprofloxacin; G, gentamicin; NA, nalidixic acid; S, streptomycin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.*
Resistance of 37.83% *E. coli* strains to tetracycline in the present study is considerably lower compared to the results of Mathew et al. (2007) (98.0%) and Hanson et al. (2002) (91.5%). EFSA/ECDC’s report for 2013 shows similar percentage of resistance in pigs (32.6%) and slightly higher in pig meat (45.9%).

None of *E. coli* isolates in this experiment showed resistance to gentamycin, which is compatible with 0.0% resistance in Bywater et al. (2004) study and 1.5% resistance determined by Kumai et al. (2005). EFSA/ECDC reports overall of 1.8% resistance of *E. coli* isolates from pigs to gentamycin.

*E. coli* isolates in our experiment showed relatively low resistance to streptomycin (24.32%) comparing to the Sunde et al. (1998) study where it was concluded that the greatest degree of *E. coli* resistance is observed with streptomycin. Result of this research, however, is in accordance with the finding that *E. coli* resistance to streptomycin ranges from 10.70 to 54.20% (Bywater et al. 2004).

Resistance to NA in our experiment was 16.22, and 2.70% to CIP. In Germany, Guerra et al. (2003) found that resistance to NA was 8–11%, and 4% to CIP. In Thailand, Hanson et al. (2002) determined higher percentage of *E. coli* isolates resistant to NA (67.4%), and 12.5% to CIP. In Denmark, Holland, Spain, and Sweden, there were no *E. coli* strains derived from pigs which were resistant to CIP (Bywater et al. 2004).

Resistance to trimethoprim-sulfamethoxazole antimicrobial combination was detected in 18.92% of *E. coli* isolates, in our experiment, which is relatively low resistance percentage comparing to 52.10% in Spain (Bywater et al. 2004), but is in correlation with 15.40% *E. coli* strains resistance in Japan (Kumai et al. 2005).

Resistance of *E. coli* strains to cefotaxime in our experiment was 2.70% which is in accordance with EFSA/ECDC report, which states that the lowest cefotaxime resistance was in Denmark (0%), and the highest in Poland (4.6%), with a European average of 1.3% (EFSA/ECDC 2015). In conclusion, the resistance of *E. coli* strains to cefotaxime is very low at the global level.

Resistance to chloramphenicol in our experiment (13.5%), was similar to average European data (14.7%) (EFSA/ECDC 2015).

The largest percentage of veterinary clinical practice data in Serbia is related to *Salmonella* isolated from domestic animals. Mišić et al. (2006) concluded that 40% of tested *Salmonella* showed resistance to various antimicrobial agents, and the remaining 60% is resistant to three out of five antibiotics.

Our study showed that six out of eight tested *Salmonella* isolates were not resistant to any of the tested antimicrobial agents. Two strains were resistant to tetracycline. A large proportion of isolated strains showed to be intermediate to streptomycin (88%) and tetracycline (75%), but none was resistant. This is a very low resistance result considering the fact that all European Union (EU) countries reported high levels of resistance to ampicillin in isolates from meat from pigs (39.7%), sulphonamides (42.3%) and tetracyclines (45.9%) (EFSA/ECDC 2015).

Origin and type of animals processed in abattoirs can influence overall resistance patterns of strains isolated from its wastewater – it had been shown that *E. coli* isolates from wastewater of abattoirs that process free range chicken have a lower level of resistance compared to conventionally grown chicken (da Costa et al. 2008). Resistance patterns of *E. coli* in urban wastewater is in correlation with resistance trends in urban population, making monitoring of antibiotic resistance among faecal bacteria in wastewater a valuable tool for the screening of resistance trends on a population level (Kwak et al. 2015). *E. coli* isolates from poultry slaughterhouses in Portugal had higher levels of resistance compared to pig slaughterhouse isolates from our study (da Costa et al. 2008).

**CONCLUSION**

Microbiological characteristics of the wastewater discharged from the abattoir and meat processing industry were measured in this study in order to get a broader understanding and awareness of the polluting potential of this type of wastewater and its potential hazard to aqueous organisms and human population. Most of the studied parameter values failed to meet the criteria prescribed by Regulations of the Republic of Serbia, so there is an imperative need for pre-treatment of wastewater from the meat industry before it is discharged into the sewer.

From a total of 37 strains of *E. coli*, a moderate degree of resistance was shown to tetracycline (37.83%); a low degree of resistance to ampicillin (21.62%), streptomycin (24.32%), trimethoprim-sulfamethoxazol (18.92%) and nalidixic acid (16.22%); and a very low resistance to: chloramphenicol (13.51%), ciprofloxacin (2.7%), gentamicin and cefotaxime (0.0%).

The results for eight strains of *Salmonella* sp. show that all eight isolates had some degree of susceptibility to nine tested antimicrobial agents and six strains were fully susceptible to all tested antibiotics.
Due to the high public pressure considering the consequences of the mass appearance of multiresistant bacterial strains that have spread from animals to humans, the use of antibiotics is significantly reduced, especially as growth promoters. In the countries of the EU the law prohibiting the use of antibiotics as growth promoters was passed in 2006. Serbia also adopted this EU legislation but more stringent control of law implementation is needed, so that a reduction in number of resistant strains can be achieved.

ACKNOWLEDGEMENTS

This work has been done within the NATO project (Ref. 984087) and has also been financially supported by the Ministry of Education and Science, Republic of Serbia (Project No. 46009).

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First received 30 July 2015; accepted in revised form 15 February 2016. Available online 29 February 2016