

**Evaluation by Conductance Assay of Shiga Toxin Producing *Escherichia coli* (STEC) O157 and O26 and their Sensitivity to Selected Disinfectants**

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**Abstract**

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The sensitivity of field isolates of STEC O157, O26 and reference strains from collections to different concentrations of sodium hypochlorite, sodium benzensulfochloramid (chloramine B), glutaraldehyde with glyoxal, peracetic acid and lactic acid was verified. The most effective disinfectant was peracetic acid at the concentration of 0.02%, followed by chloramine B and lactic acid at the concentration of 0.5%. The field isolate of STEC O157 in comparison with the other tested strains of *E. coli* was more resistant to the activity of peracetic both and lactic acid and to chlorine releasing disinfectants. Its resistance was comparable to that of reference *E. coli* strain CNCTC 301/60, which is the reference micro-organism for efficacy testing of disinfectants. Statistically significant difference among the individual tested strains was not found. Differences between the tested STEC strains and reference strains in evaluation of growth curves were shown. STEC O157 and O26, more than the other tested strains, showed a higher resistance to acid environment in this characteristic. The reference *E. coli* strain CNCTC 301/60 and field isolate STEC *E. coli* O157 were the most resistant of all the tested strains, reference non toxigenic *E. coli* O157 strain was the most resistant to aldehydes.

STEC, VTEC, EHEC, disinfectants, sanitation, HACCP

Shiga toxin producing *E. coli* strains (STEC), especially serotypes O157 and O26 are etiological agents of infections, which endanger lives of people in a number of advanced countries in the world. Among the main sources of these alimentary infections are insufficiently heat processed foods of bovine origin (Chapman et al. 1993; Morgan et al. 1993; Bell et al. 1994), contaminated water (Swerdlow et al. 1992; Dev et al. 1991; Danon-Schaffer 2001) and direct contact with animals in farms (Trevena et al. 1996; Bielaszewska et al. 2000). Quite a number of results of the studies pointed out the frequent occurrence of STEC O157 in faeces of healthy feedlot and dairy cattle, seasonal dynamics of excretion with culmination in summer months and different incidence of germ-carrier in herds depending on the age of animals, kind of feedstuff and intensity of farming (Dargatz et al. 1997; Hancock et al. 1997; Čížek et al. 1999). Germ-carrying and excretion of STEC in faeces of healthy animals is irregular and is dangerous especially with regard to the possibility of direct milk contamination during milking and meat contamination in slaughter process of animals. Different processes of sanitation of technological part of cattle-shed and manufacturing industry (e.g. disinfection of milking equipment, treatment of slaughtered cattle carcasses with sanitation solutions etc.) are used to prevent and reduce contamination of raw milk and meat. These sanitation procedures use the disinfection effect of different chemical substances, whose activity to STEC has only been tested in a limited number of cases (Oie et al. 1999; Castlilii et al. 1999; Rice et al. 1999; Taylor et al. 1999).

Methods of testing efficacy of disinfectant are based on suspension quantitative test used for determining bactericide, fungicide and sporicide efficacy in tested substances or

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products. Another phase when testing efficacy is the procedure based again on suspension test, which is applied to simulate practical conditions of the use of disinfectants or antiseptic product. The third phase of efficacy verifying is carrying out the test in the field with the influence of natural conditions.

Exploiting culture medium changes caused by bacterial growth, when the conductance changes ( $\mu\text{S/t}$ ) are statistically significant, is an alternative method to the suspension test. Conductance changes are in this case extrapolated as detection time of micro-organisms (DT). Conductance is measured in a cell with two electrodes whose resistance depends on the characteristics of a solution, area and distance of these electrodes. This is a procedure of evaluation of antibacterial efficacy, which comprises appropriate culture medium inoculated with the selected suspension of micro-organisms of adequate same density in the presence of concentration gradient of disinfectant, to obtain complete inhibition of growth of microbial culture and to determine the minimum inhibitory concentration (MIC).

The main aim of this study was to establish the level of resistance of the field isolates of *E. coli* STEC O157, O26 and non toxigenic control strains of *E. coli* to selected disinfectants by the determination of their MIC via the measuring of conductance on growth analyser MALTHUS AT (conductance assay) and minimal bactericidal concentrations (MBC) via the subculturing examination.

#### Materials and Methods

##### Disinfectants

The following disinfectants were used in this study:

|                             |   |
|-----------------------------|---|
| sodium hypochlorite         | (SAVO, by BOCHEMIE, Bohumín, CZ),   |
| sodium benzensulfochloramid | (CHLORAMINE B with min. 25.0% of active chlor, by BOCHEMIE, Bohumín, CZ),                                     |
| glutaraldehyde with glyoxal | (INCIDUR 50% glutaraldehyd and 40% glyoxal, by FARMAK a.s., Olomouc, CZ in co-operation with Henkel, Germany) |
| peracetic acid              | (PERSTERIL with 32-36% peracetic acid, made by Chemická závody, Sokolovo, CZ)                                 |
| lactic acid p.a.            | (Sigma Aldrich)   |

All tested *E. coli* strains were exposed to effect of selected disinfection products in following concentrations [%]:

|                             |   |
|-----------------------------|---|
| sodium hypochlorite         | 2.0; 1.0; 0.5; 0.2; 0.1; 0.05; 0.02; 0.01       |
| sodium benzensulfochloramid | 0.2; 0.1; 0.05; 0.02; 0.01; 0.005; 0.002; 0.001 |
| glutaraldehyde with glyoxal | 2.0; 1.0; 0.5; 0.2; 0.1; 0.05; 0.02; 0.01       |
| peracetic acid              | 0.2; 0.1; 0.05; 0.02; 0.01; 0.005; 0.002; 0.001 |
| lactic acid p.a.            | 2.0; 1.0; 0.5; 0.2; 0.1; 0.05; 0.02; 0.01       |

##### Bacterial strains and their cultivation

The field isolates of Shiga toxin producing *E. coli* O157 EC 81 (STEC O157 T),

*E. coli* O26 EH 97 (STEC O26 T), reference non toxigenic *E. coli* strain O157 NCTC 12900 (*E. coli* O157 NT) and reference control *E. coli* strain CNCTC 301/60, reference strain for disinfectant efficacy testing and *E. coli* ATCC 25922 reference strain for antibiotic susceptibility testing. Strains were collected and stored in cryoprotective medium at -80 °C. Biochemical activity of tested strains (production of tryptophanase and beta-glukuronidase by Coli test, Pliva - Lachema, Brno, CZ) and the presence of genes *st1*, *st2*, *eaeA* and *hlyA* multiplex PCR (Paton and Paton 1998) were tested before trial with disinfectants.

All tested *E. coli* strains were cultivated over night on Columbia Blood Agar at 37 °C and from the culture obtained was suspension was made in 3 ml of culture medium (BC 7 - enzymatic casein hydrolysate 1.25 g, yeast autolysate 1.25 g, NaCl 0.625 g, glukose 0.25 g ad 250 ml distilled water). Optical density of suspension was detected on photometer (Spekol 11, Carl Zeiss, Jena, Germany) and the number of bacteria was quantified from calibration curve. This suspension was used for dilution to achieve the final concentration of tested strain  $2 \cdot 10^5$  CFU/ml. Prepared suspension in quantity of 0.1 ml then was inoculated into 1.8 ml of culture medium (BC 7) in Malthus cells and after adding of 0.1 ml of solution of known concentration of tested disinfectant, the content of the cells was carefully mixed. The control cells without disinfectant, used for standardisation of measured growth of tested strains, were simultaneously inoculated. The conductance tests for estimating bacterial growth were carried out on MALTHUS AT growth analyser (Malthus Instruments, England). The detection times of the tested strains were measured during analysis and the number of viable bacteria and minimum inhibitory concentration

(MIC) of disinfectants used were established by reading from calibration growth curve of standard reference strains. The bacterial effect confirmation (i.e. estimating minimum bactericide concentration - MBC) was made by inoculation of culture medium (line made with a calibrated sterile bacteriological loop 10 µl on Blood Agar) using suspension from the cells containing disinfectants, where the growth of tested strains was not detected (detection time 0), and from the last cell, where the growth of tested strains was detected.

#### Presentation of the results

Growth of the tested strains was determined before their exposure to the effect of disinfectants. The initial number of bacteria was determined by reading from calibration growth curve of standard reference strain (*E. coli* CNCTC 301/60). The mean detection time (DT) of growth of every tested strain of *E. coli* (STEC O157 T, O157 NT, STEC O26 T, CNCTC 301/60, ATCC 25 922) was further determined. Detected data value are presented in Table 1.

Table 1  
Growth of tested strains before their exposure to the effect of disinfectants

| Tested strain               | Number CFU/ml       | Detection growth (DT) in hours | Standard mean deviation | Variation coefficient |
|-----------------------------|---------------------|--------------------------------|-------------------------|-----------------------|
| STEC O157 T                 | 4.5.10 <sup>4</sup> | 5.21                           | 0.1215                  | 2.33                  |
| <i>E. coli</i> O157 NT      | 1.5.10 <sup>4</sup> | 6.07                           | 0.2765                  | 4.55                  |
| STEC O26 T                  | 2.5.10 <sup>4</sup> | 5.53                           | 0.0744                  | 1.34                  |
| <i>E. coli</i> CNCTC 301/60 | 4.0.10 <sup>4</sup> | 5.30                           | 0.1188                  | 2.35                  |
| <i>E. coli</i> ATCC 25922   | 1.0.10 <sup>4</sup> | 6.13                           | 0.0744                  | 1.21                  |

The quantitative test of disinfectant efficacy is expressed as the minimum inhibitory concentration (MIC), log<sub>10</sub> of number of surviving cells of tested strains allows to compare quantitatively the effect of individual concentration of disinfectants. Minimum bactericide concentration (MBC) is marked + (plus), when the strain grows and marked – (minus), when the strain does not grow on blood agar. Index of effectiveness is determined as MBC and MIC ratio.

The percentage of number of killed microorganisms from initial inoculum of tested strains was used to express the efficacy of selected concentrations and to interpret more transparently received MIC and MBC data values as well as to compare efficacy of individual assessed disinfectants.

The concentration where bactericidity (or growth suppression) of tested micro-organisms was more than 50%, was marked as LC 50 and the concentration where bactericidity (or growth suppression) was 100%, was marked as LC 100. The values LC 50 and LC 100 were used with every disinfectant to show the tested strains resistance, from the most resistance strain (denomination 1) to the least resistant (denomination 5). The sums of individual orders were used for evaluation of the most resistant and the least resistant strain from the strains tested in this study.

#### Statistical processing

The number of surviving cells of tested strains in individual concentrations of disinfectants was used for determination of decreasing number of cells in comparison with the control group without disinfectants.

## Results and Discussion

The present methods used in Europe for testing of antibacterial active substances are being auditioned and harmonised with the EU Biocides Directive 98/8/EC. The standard suspension test is the most widespread method used for quantitative determination of the number of surviving micro-organism to define the biocide efficacy of the checked antibacterial substances. It is necessary to take into account the impact of various factors, which can influence the result of antimicrobial efficacy and thus the final efficacy evaluation. Most authors drew attention to this fact (e.g. Evans et al. 1990; Russel et al. 1993; Brown et al. 1994; Bloofield et al. 1995; Langsrud and Sundheim 1998). Specific growth demands of individual tested strains should be taken in consideration in terms of methodical procedures used. In opposite of the fact that the conductance assays have not been common used so far and we have not come across them yet in official recommended methods of micro-organism counting, undoubtedly they have advantages: besides their rapidity and accuracy there is also a possibility of monitoring the course of growth curve of microbial strain during the action of various concentrations of antimicrobial substances

Table 2  
MIC and MBC data determined in individual disinfectants to tested strains

| Tested strains              | Mode of evaluation | Concentration of sodium hypochlorite in % |     |     |                     |                     |                     |                     |                     |
|-----------------------------|--------------------|---|-----|-----|---------------------|---------------------|---------------------|---------------------|---------------------|
|                             |                    | 2.0                                       | 1.0 | 0.5 | 0.2                 | 0.1                 | 0.05                | 0.02                | 0.01                |
| STEC O157 T                 | MIC                | 0   | 0   | 0   | 1.10 <sup>2</sup>   | 4.5.10 <sup>4</sup> | 4.5.10 <sup>4</sup> | 4.5.10 <sup>4</sup> | 4.5.10 <sup>4</sup> |
|                             | MBC                | -   | -   | +   | +                   | +                   | +                   | +                   | +                   |
| <i>E. coli</i> O157 NT      | MIC                | 0   | 0   | 0   | 9.4.10 <sup>3</sup> | 1.0.10 <sup>4</sup> | 1.5.10 <sup>4</sup> | 1.5.10 <sup>4</sup> | 1.5.10 <sup>4</sup> |
|                             | MBC                | -   | -   | +   | +                   | +                   | +                   | +                   | +                   |
| STEC O26 T                  | MIC                | 0   | 0   | 0   | 9.5.10 <sup>3</sup> | 2.0.10 <sup>4</sup> | 2.0.10 <sup>4</sup> | 2.0.10 <sup>4</sup> | 2.0.10 <sup>4</sup> |
|                             | MBC                | -   | -   | +   | +                   | +                   | +                   | +                   | +                   |
| <i>E. coli</i> CNCTC 301/60 | MIC                | 0   | 0   | 0   | 2.10 <sup>2</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   |
|                             | MBC                | -   | -   | +   | +                   | +                   | +                   | +                   | +                   |
| <i>E. coli</i> ATCC 25922   | MIC                | 0   | 0   | 0   | 6.10 <sup>3</sup>   | 9.10 <sup>3</sup>   | 1.10 <sup>4</sup>   | 1.10 <sup>4</sup>   | 1.10 <sup>4</sup>   |
|                             | MBC                | -   | -   | -   | +                   | +                   | +                   | +                   | +                   |

tested. For that reason, the method of counting the surviving micro-organisms based on the change of conductivity of medium was used in our study for detection of susceptibility, let us say for resistance of the STEC strains. This method was used by Ogden (1993) for *E. coli* counting in foods, by MacRae et al. (1997) for evaluation of susceptibility of *E. coli* O157 to antibacterial substances and for comparison of results obtained both by conductance method and by conventional suspension test. The results of their work demonstrate, that merits of the electrometer system are not only in a much shorter period of time needed for carrying out the tests, but also in the fact, that from the course of changes of impedance growth curve observed continuously it is possibly to read whether the concentration of antibacterial substance had an inhibitory effect or not.

The MIC and MBC data determined in individual disinfectants to tested strains are shown in Tables 2 and 6. All tested strains were susceptible to selected range of concentration of evaluated disinfectants. Peracetic acid was already effective in concentration 0.02%, then followed sodium benzensulfochloramid and lactic acid in concentration 0.1%, sodium hypochlorid and glutaraldehyd with glyoxal in concentration 0.5%.

The results of evaluation of tested strains susceptibility according to values LC 50 and LC 100 are listed in Tables 7 and 8. The sequence of tested strains according to their resistance was in LC 50: *E. coli* ATCC 25 922, *E. coli* STEC O26 T, *E. coli* CNCTC 301/60, *E. coli*

Table 3  
MIC and MBC data determined in individual disinfectants to tested strains

| Tested strain               | Mode of evaluation | Concentration of sodium benzensulfochloramid % |     |                     |                     |                     |                     |                     |                     |
|-----------------------------|--------------------|--|-----|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|                             |                    | 0.2  | 0.1 | 0.05                | 0.02                | 0.01                | 0.005               | 0.002               | 0.001               |
| STEC O157 T                 | MIC                | 0  | 0   | 1.10 <sup>1</sup>   | 4.5.10 <sup>4</sup> | 4.5.10 <sup>4</sup> | 4.5.10 <sup>4</sup> | 4.5.10 <sup>4</sup> | 4.5.10 <sup>4</sup> |
|                             | MBC                | -  | -   | +                   | +                   | +                   | +                   | +                   | +                   |
| <i>E. coli</i> O157 NT      | MIC                | 0  | 0   | 0                   | 9.0.10 <sup>3</sup> | 9.5.10 <sup>3</sup> | 9.5.10 <sup>3</sup> | 1.5.10 <sup>4</sup> | 1.5.10 <sup>4</sup> |
|                             | MBC                | -  | -   | +                   | +                   | +                   | +                   | +                   | +                   |
| STEC O26 T                  | MIC                | 0  | 0   | 5.0.10 <sup>1</sup> | 1.0.10 <sup>4</sup> | 2.5.10 <sup>4</sup> | 2.5.10 <sup>4</sup> | 2.5.10 <sup>4</sup> | 2.5.10 <sup>4</sup> |
|                             | MBC                | -  | -   | +                   | +                   | +                   | +                   | +                   | +                   |
| <i>E. coli</i> CNCTC 301/60 | MIC                | 0  | 0   | 2.10 <sup>2</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   |
|                             | MBC                | -  | -   | +                   | +                   | +                   | +                   | +                   | +                   |
| <i>E. coli</i> ATCC 25922   | MIC                | 0  | 0   | 6.10 <sup>1</sup>   | 9.5.10 <sup>3</sup> | 1.10 <sup>4</sup>   | 1.10 <sup>4</sup>   | 1.10 <sup>4</sup>   | 1.10 <sup>4</sup>   |
|                             | MBC                | -  | -   | +                   | +                   | +                   | +                   | +                   | +                   |

Table 4  
MIC and MBC data determined in individual disinfectants to tested strains

| Tested strain               | Mode of evaluation | Concentration of glutaraldehyde with glyoxal in % |     |     |                     |                     |                     |                     |                     |
|-----------------------------|--------------------|---|-----|-----|---------------------|---------------------|---------------------|---------------------|---------------------|
|                             |                    | 2.0   | 1.0 | 0.5 | 0.2                 | 0.1                 | 0.05                | 0.02                | 0.01                |
| STEC O157 T                 | MIC                | 0   | 0   | 0   | 4.5.10 <sup>1</sup> | 5.10 <sup>3</sup>   | 9.5.10 <sup>3</sup> | 4.10 <sup>4</sup>   | 4.5.10 <sup>4</sup> |
|                             | MBC                | -   | -   | -   | +                   | +                   | +                   | +                   | +                   |
| <i>E. coli</i> O157 NT      | MIC                | 0   | 0   | 0   | 9.0.10 <sup>2</sup> | 6.0.10 <sup>3</sup> | 9.0.10 <sup>3</sup> | 9.8.10 <sup>3</sup> | 9.8.10 <sup>3</sup> |
|                             | MBC                | -   | -   | +   | +                   | +                   | +                   | +                   | +                   |
| STEC O26 T                  | MIC                | 0   | 0   | 0   | 2.5.10 <sup>3</sup> | 9.0.10 <sup>3</sup> | 2.0.10 <sup>4</sup> | 2.0.10 <sup>4</sup> | 2.0.10 <sup>4</sup> |
|                             | MBC                | -   | -   | -   | +                   | +                   | +                   | +                   | +                   |
| <i>E. coli</i> CNCTC 301/60 | MIC                | 0   | 0   | 0   | 2.10 <sup>2</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   |
|                             | MBC                | -   | -   | -   | +                   | +                   | +                   | +                   | +                   |
| <i>E. coli</i> ATCC 25922   | MIC                | 0   | 0   | 0   | 9.10 <sup>3</sup>   | 9.10 <sup>3</sup>   | 1.10 <sup>4</sup>   | 1.10 <sup>4</sup>   | 1.10 <sup>4</sup>   |
|                             | MBC                | -   | -   | -   | +                   | +                   | +                   | +                   | +                   |

Table 5  
MIC and MBC data determined in individual disinfectants to tested strains

| Tested strain               | Mode of evaluation | Concentration of peracetic acid in % |     |      |      |                     |                     |                     |                     |
|-----------------------------|--------------------|--------------------------------------|-----|------|------|---------------------|---------------------|---------------------|---------------------|
|                             |                    | 0.2                                  | 0.1 | 0.05 | 0.02 | 0.01                | 0.005               | 0.002               | 0.001               |
| STEC O157 T                 | MIC                | 0                                    | 0   | 0    | 0    | 8.10 <sup>2</sup>   | 9,5.10 <sup>3</sup> | 4,5.10 <sup>4</sup> | 4,5.10 <sup>4</sup> |
|                             | MBC                | -                                    | -   | -    | -    | +                   | +                   | +                   | +                   |
| <i>E. coli</i> O157 NT      | MIC                | 0                                    | 0   | 0    | 0    | 9.5.10 <sup>2</sup> | 5.0.10 <sup>3</sup> | 7.5.10 <sup>3</sup> | 7.5.10 <sup>3</sup> |
|                             | MBC                | -                                    | -   | -    | -    | +                   | +                   | +                   | +                   |
| STEC O26 T                  | MIC                | 0                                    | 0   | 0    | 0    | 1.5.10 <sup>3</sup> | 9.5.10 <sup>3</sup> | 1.0.10 <sup>4</sup> | 2.2.10 <sup>4</sup> |
|                             | MBC                | -                                    | -   | -    | -    | +                   | +                   | +                   | +                   |
| <i>E. coli</i> CNCTC 301/60 | MIC                | 0                                    | 0   | 0    | 0    | 2.10 <sup>3</sup>   | 1.10 <sup>4</sup>   | 5.10 <sup>4</sup>   | 5.10 <sup>4</sup>   |
|                             | MBC                | -                                    | -   | -    | -    | +                   | +                   | +                   | +                   |
| <i>E. coli</i> ATCC 25922   | MIC                | 0                                    | 0   | 0    | 0    | 1.10 <sup>2</sup>   | 4.10 <sup>2</sup>   | 8.10 <sup>3</sup>   | 8.10 <sup>3</sup>   |
|                             | MBC                | -                                    | -   | -    | -    | +                   | +                   | +                   | +                   |

Table 6  
MIC and MBC data determined in individual disinfectants to tested strains

| Tested strain               | Evaluation | Concentration of lactic acid in % |     |     |     |     |                     |                     |                     |
|-----------------------------|------------|-----------------------------------|-----|-----|-----|-----|---------------------|---------------------|---------------------|
|                             |            | 2.0                               | 1.0 | 0.5 | 0.2 | 0.1 | 0.05                | 0.02                | 0.01                |
| STEC O157 T                 | MIC        | 0                                 | 0   | 0   | 0   | 0   | 2.0.10 <sup>3</sup> | 1.0.10 <sup>4</sup> | 1.0.10 <sup>4</sup> |
|                             | MBC        | -                                 | -   | -   | -   | -   | +                   | +                   | +                   |
| <i>E. coli</i> O157 NT      | MIC        | 0                                 | 0   | 0   | 0   | 0   | 5.0.10 <sup>1</sup> | 1.5.10 <sup>3</sup> | 4.0.10 <sup>3</sup> |
|                             | MBC        | -                                 | -   | -   | -   | -   | +                   | +                   | +                   |
| STEC O26 T                  | MIC        | 0                                 | 0   | 0   | 0   | 0   | 9.5.10 <sup>1</sup> | 7.5.10 <sup>3</sup> | 8.0.10 <sup>3</sup> |
|                             | MBC        | -                                 | -   | -   | -   | -   | +                   | +                   | +                   |
| <i>E. coli</i> CNCTC 301/60 | MIC        | 0                                 | 0   | 0   | 0   | 0   | 6.0.10 <sup>2</sup> | 9.0.10 <sup>3</sup> | 1.0.10 <sup>4</sup> |
|                             | MBC        | -                                 | -   | -   | -   | +   | +                   | +                   | +                   |
| <i>E. coli</i> ATCC 25922   | MIC        | 0                                 | 0   | 0   | 0   | 0   | 2.2.10 <sup>2</sup> | 7.5.10 <sup>3</sup> | 7.5.10 <sup>3</sup> |
|                             | MBC        | -                                 | -   | -   | -   | -   | +                   | +                   | +                   |

Legend: - 0 tested strain do not growth ( qualitative parameter),  
 - + tested strain growth  
 - x.10<sup>x</sup> the number of survival (viable) bacteria: log (quantitative parameter)

Table 7  
Results of evaluation of tested strains susceptibility according to values LC 50

| Tested strain               | The sequence of resistance against disinfectant products in LC 50 |           |                                    |                |             | Total score | Total sequence |
|-----------------------------|---|-----------|------------------------------------|----------------|-------------|-------------|----------------|
|                             | Sodium hypochlorite   | Chloramin | Aldehyds (glutaraldehyde, glyoxal) | Peracetic acid | Lactic acid |             |                |
| STEC O157 T                 | 4   | 4         | 5                                  | 3              | 1           | 17          | 4              |
| <i>E. coli</i> O157 NT      | 3   | 5         | 3                                  | 5              | 5           | 21          | 5              |
| STEC O26 T                  | 2   | 3         | 2                                  | 1              | 4           | 12          | 2              |
| <i>E. coli</i> CNCTC 301/60 | 5   | 1         | 4                                  | 2              | 2           | 14          | 3              |
| <i>E. coli</i> ATCC 25922   | 1   | 2         | 1                                  | 4              | 3           | 11          | 1              |

Legend:

1 –the most resistant tested strain, 5 – the least resistant tested strain

STEC O157 T and *E. coli* O157 NT and in LC 100 *E. coli* CNCTC 301/60, STEC O26T, STEC O157 T, *E. coli* ATCC 25 922 and *E. coli* O157 NT.

It is necessary to be aware of lactic acid efficacy in very low concentrations in evaluation of their action against every tested *E. coli* strains. LC 50 was the lowest in *E. coli* O157 NT, then sequentially with increasing LC 50 *E. coli* STEC O26 T, *E. coli* ATCC 25 922, *E. coli* CNCTC 301/60 and *E. coli* O157 T. LC 100 was the same for all tested strains except *E. coli* CNCTC 301/60. The *E. coli* 301/60 was ten times more resistant than the other strains, LLC 100 of this strain was 0.2% compared to 0.1% of concentration effective on the other strains, whereas the highest concentration effect was bacteriostatic and MIC did not corresponded to MBC as in the other strains.

No statistically significant variations in growth and number of tested bacterial strains used in study were detected.

There were no significant differences in susceptibility among individual tested strains, only in evaluation of growth dynamics it is possible to observe certain tendencies to different

Table 8  
Results of evaluation of tested strains susceptibility according to values LC 100

| Tested strain               | The sequence of resistance against disinfectant products in LC 100 |           |                                   |                |             | Total score | Total sequence |
|-----------------------------|--|-----------|-----------------------------------|----------------|-------------|-------------|----------------|
|                             | Sodium hypochlorite  | Chloramin | Aldehyds (glutaraldehyd, glyoxal) | Peracetic acid | Lactic acid |             |                |
| STEC O157 T                 | 2  | 4         | 5                                 | 3              | 2           | 16          | 3              |
| <i>E. coli</i> O157 NT      | 2  | 5         | 1                                 | 5              | 5           | 18          | 4              |
| STEC O26 T                  | 3  | 3         | 3                                 | 1              | 4           | 14          | 2              |
| <i>E. coli</i> CNCTC 301/60 | 1  | 1         | 4                                 | 2              | 1           | 9           | 1              |
| <i>E. coli</i> ATCC 25922   | 5  | 2         | 2                                 | 4              | 3           | 16          | 3              |

Legend:

1 –the most resistant tested strain, 5 – the least resistant tested strain

behaviour of individual strains. On the basis of these evaluation it is possible to observe certain difference among the susceptibility of pathogenic STEC O157 and O26 in comparison with non toxigenic *E. coli* strains. Higher susceptibility of wild strains in comparison with depository strains was not unambiguously demonstrated, even though the field isolate STEC O157 was highly resistant to acids than the other strains. Bactericidal concentration is however both in peracetic acid, and in lactic acid the same for every tested strain with the exception of *E. coli* CNCTC 301/60 resistance. In this respect, it is possible to agree with Gonzalez et al. (1998), who describes enhanced resistance of STEC O157 to acids. Variability of our results acquired in the study, apparent resistance of STEC O157 and O26 and in the contrary considerable sensitivity in a non toxigenic strain O157, requires cautiousness in interpreting this results unambiguously.

Another important finding was the fact that non toxigenic *E. coli* O157 strain was the most resistant strain against aldehydes, in contrary to STEC O157 and control strain *E. coli* CNCTC 301/60, which were the most sensitive of all the tested strains.

Dependence of efficacy on different initial numbers of tested strains bacteria (vary from  $1.10^4$  to  $4.5.10^4$  CFU/ml) was not explicitly demonstrated. The higher initial number of bacteria corresponds with the higher resistance only in the case of acids both peracetic and lactic. In the other cases it is different and actually *E. coli* strain ATCC 25922, with the lowest initial number of bacterial cells, shows, with the exception of peracetic acid efficacy higher resistance against the other disinfectants than the other tested strains.

MIC in most disinfectants, with the exception of chlorine products matched with MBC. MIC of sodium hypochlorite, in all of the tested strains except *E. coli* ATCC 25 922, was always lower than MBC, it means that this kind of disinfectant shows bacteriostatic efficacy and the efficacy index is 2.5. The course of growth inhibition (metabolic activity) of bacteria was very sharp in all strains, which is in accordance with McKenn and Davies (1988).

Bacteriostatic efficacy was not demonstrated in chloramine except the activity against *E. coli* O157 ATCC 25 922, when MBC was 0.05% as in the other tested strains, but MIC of this strain was 0.02%, efficacy index being 2.5.

As an interesting fact appears the great difference in resistance of tested strains against sodium hypochlorite in evaluation of LC 50 and LC 100, in evaluation of LC 50 low resistant strains of STEC O157 and *E. coli* CNCTC 301/60 were, however, in evaluation of LC 100, detected as the most resistant. The contrary is true in *E. coli* ATCC 25 922, which was the most resistant strain in evaluation of LC 50 and in LC 100 the least resistant one. This situation was not so obvious in evaluation of the activity of the other disinfectants, nevertheless it influenced the total evaluation of tested strains resistance, see Tables 7 and 8. The most resistant strain in our study was *E. coli* CNCTC 301/60, the laboratory reference strain for testing efficacy of disinfectants, and both the STEC O157 and O26 strains. This fact makes us consider the reasons of this phenomenon. In accordance with the references (Jatzwauk 1984; Heinzl 1998; Russell et al. 1998; McDonnell et al. 1999) we can expect the fact, that resistant strains of microbes have in their population a much higher number of cells, that are very sensitive and very resistant to the prejudice of the part of individuals with the average resistance. This phenomenon may be related to the different course of growth curves and the different number of starving cells in inoculum. This presumption was partially confirmed by findings of Lislíe et al. (1998), which demonstrated that starvation of *E. coli* O157 cells increases the resistance to free chlorine.

#### **Studie odolnosti *E. coli* O157 k dezinfekčnímu účinku vybraných dezinfekčních přípravků**

V našem ověření bylo prokázáno, že všechny testované kmeny jsou dostatečně citlivé na zvolené koncentrace vybraných dezinfekčních přípravků. Nejúčinnějším dezinfekčním

přípravkem je kyselina peroxooctová v 0,02 % koncentraci, pak následuje chloramin a kyselina mléčná v 0,1 % koncentraci a dále chlornan sodný a glutaraldehyd v kombinaci s glyoxalem v 0,5 % koncentraci. Patogenní verotoxigenní kmen *Escherichia coli* O157 izolovaný z terénních podmínek je velmi odolným kmenem proti působení kyseliny peroxooctové a mléčné i proti vlivu chlor uvolňujících dezinfekčních přípravků. Jeho odolnost je srovnatelná s odolností sbírkového kmene *Escherichia coli* Eck 301, používaného pro testování účinnosti dezinfekční látek.

Nebyl zaznamenán žádný výrazný rozdíl v citlivosti mezi jednotlivými testovanými kmeny, pouze při hodnocení dynamiky růstu lze sledovat určité tendence k rozdílnému chování u jednotlivých kmenů. Na základě těchto hodnocení lze pozorovat určitý rozdíl mezi citlivostí patogenních verotoxigenních kmenů *Escherichia coli* O157 a O26 proti netoxigenním kmenům *E. coli*. Proti působení kyselin vykazují verotoxigenní kmeny *Escherichia coli* O157 a O26 větší odolnost než ostatní kmeny s výjimkou odolnosti sbírkového kmene *Escherichia coli* Eck 301.

U testovaných kmenů nebyla pozorována žádná závislost mezi citlivostí, respektive odolností k testovaným dezinfekčním látkám a k antibiotikům.

Sbírkový kmen *E. coli* 301 a toxigenní kmen *E. coli* O157 byly nejodolnějšími ze všech testovaných kmenů, netoxigenní kmen *E. coli* O157 byl však nejodolnějším kmenem proti aldehydům.

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