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EXPERIMENTAL PAPER

Adaptation Index (KAI) – a new indicator of adaptation and potential antimicrobial resistance

TOMASZ M. KARPIŃSKI

Chair and Department of Medical Microbiology
Poznań University of Medical Sciences
60-806 Poznań, Poland

Correspondence address: e-mail: tkarpin@ump.edu.pl

SUMMARY

Introduction: Many microorganisms are capable of adapting or developing resistance to drugs and natural substances.

Objective: The work aim was to investigate the adaptation development potential in *Staphylococcus aureus* to selected natural and synthetic substances. Additionally, a novel Adaptation Index (KAI) was developed for the assessment of clinical resistance development risk.

Methods: Minimal Inhibitory Concentrations (MIC) assay and adaptation of *Staphylococcus aureus* to selected compounds were performed.

Results: Octenidine (OCT) exhibited the best activity against *Staphylococcus aureus*, while sodium hypochlorite (NaOCl), ethacridine lactate (ET), and curcumin (CU) showed moderate activity. The weakest activity was observed for salicylic acid (SA), chrysin (CH), astaxanthin (AST), and boric acid (BA). Adaptation equal to the MIC value was seen for CH and NaOCl, weak adaptation for AST, SA, and CU, and fair adaptation for OCT and ET. CU, CH, and OCT had very low KAI values and a low risk of clinical resistance, while BA had a low risk, ET a moderate, and NaOCl a very high. *S. aureus* can quickly develop resistance to clinical concentrations of NaOCl. Assessing the risk for AST and SA is challenging due to varying suggested doses.

Conclusions: The studies confirmed the possibility of adaptation development in the *S. aureus* strain under the influence of both natural and synthetic compounds. The Karpinski Adaptation Index (KAI) allows for the assessment of the risk of microorganism adaptation and resistance development to drugs or substances of natural origin.

Key words: ***natural compounds, antimicrobials, antiseptics, Adaptation Index, drug resistance***

Słowa kluczowe: ***substancje naturalne, związki przeciwdrobnoustrojowe, antyseptyki, Wskaźnik Adaptacji, lekooporność***

INTRODUCTION

Many natural substances exhibit antimicrobial activity. The first commercially used antibiotics also originated from nature. These include penicillin, isolated from cultures of the fungus *Penicillium notatum* by Alexander Fleming, streptomycin, the first aminoglycoside compound isolated from actinomycete bacteria by Selman Waksman, and cephalosporins, obtained from the fungus *Cephalosporium acremonium* by Giuseppe Brotzu [1]. Plants also contain antimicrobial compounds. The most common antibacterial phytochemicals belong to phenols, terpenoids, flavonoids, alkaloids, coumarins, and essential oil [1, 2]. Many of these substances are still being studied, and their activity is at various level [3–5].

Currently, a major clinical and epidemiological problem is the increasing drug resistance, particularly to antibiotics. This process affects most antimicrobial compounds used in medicine and veterinary practice. According to the World Health Organization, antimicrobial resistance was directly responsible for 1.14 million global deaths in 2021 and contributed to 4.71 million deaths. Projections indicate that by 2050, the number of deaths due to antimicrobial resistance will rise to approximately 46.5 million [6]. A significant factor in the development of resistance is adaptation, which is common among microorganisms. Drug tolerance can emerge during prolonged exposure to subtherapeutic drug doses or slowly increasing drug concentrations. During this process, cells with increased tolerance are selected, which may be associated with mutations or changes in cell structure [7]. Adaptation is the process of adjusting to new conditions to improve survival or function. Resistance, on the other hand, is the ability of an organism to survive or withstand the action of a chemical substance, often due to genetic adaptations or acquired mechanisms that reduce the substance's effectiveness. According to Windels *et al.* [7] drug adaptation can eventually lead to resistance (fig. 1).

The aim of this paper was to investigate the potential for the development of adaptation in *Staphylococcus aureus* bacteria to selected natural and synthetic substances. Additionally, based on the obtained results, an Adaptation Index (KAI) was developed, which may have practical significance in assessing the relationship between adaptation and resistance.

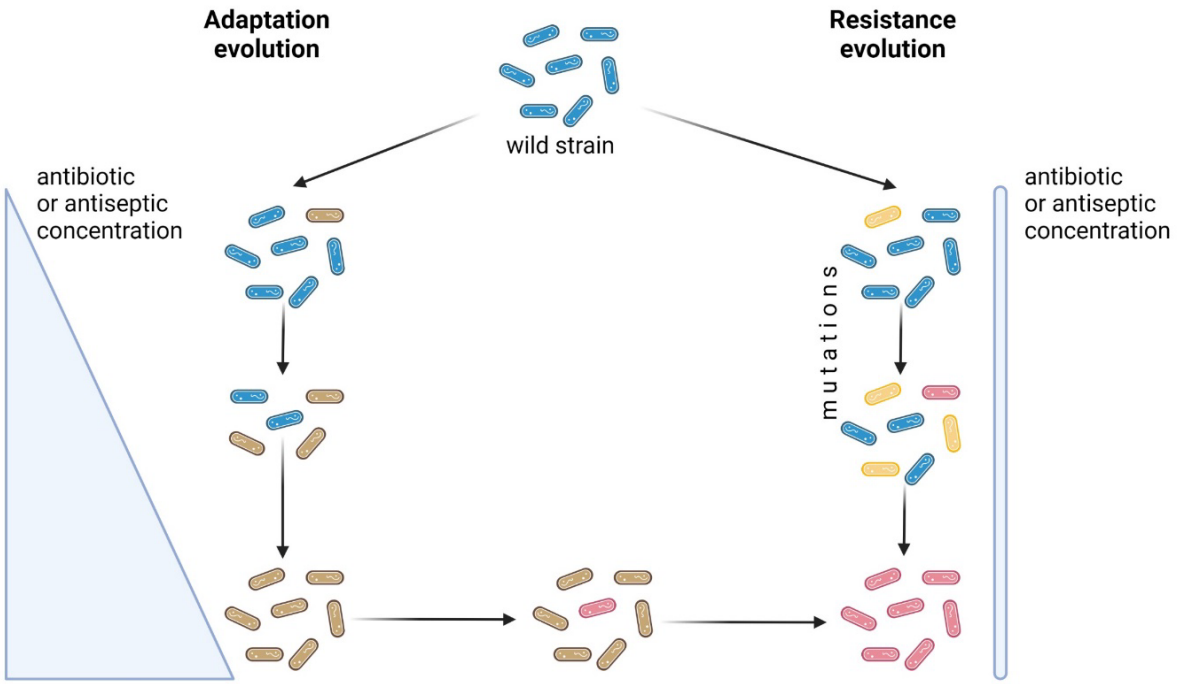

Figure 1.

The relationship between adaptation and drug resistance (based on [7])

MATERIALS AND METHODS

Studied compounds and *Staphylococcus aureus* strain

The effects of four natural substances and four synthetic antiseptics on a clinical strain of *Staphylococcus aureus* were investigated. For the study, compounds with varying antimicrobial activity were selected. Among the natural compounds, the availability of suggested doses in the literature was additionally taken into account. These were adopted as doses equivalent to clinical concentrations. The substances tested included curcumin (Sigma-Aldrich, Poland), astaxanthin (Sigma-Aldrich, Poland), salicylic acid (Warchem, Poland), chrysin (Sigma-Aldrich, Poland), octenidine dihydrochloride (OCT; Schülke & Mayr GmbH, Germany), sodium hypochlorite (NaOCl; Cerkamed, Poland), boric acid (BA; Herbapol Poznań, Poland), and ethacridine lactate (ET; Herbapol Poznań, Poland). The initial concentrations of the plant substances were 10 mg/ml. For the antiseptics, the initial concentrations matched those in commercial products: 0.5 mg/ml for OCT, 0.1 mg/ml for NaOCl, 30 mg/ml for BA, and 1 mg/ml for ET. The *Staphylococcus aureus* strain was cultured on Chapman mannitol salt agar (Graso Biotech, Poland).

Minimal Inhibitory Concentrations (MIC)

The minimal inhibitory concentrations (MIC) is the lowest concentration of an antimicrobial agent that noticeably inhibits the growth of microorganisms. The MIC of the tested compounds were assessed using the microdilution technique with 96-well plates (Nest Scientific Biotechnology, China) in tryptic soy broth (Graso Biotech, Starogard Gdański, Poland). A detailed description is available in our previous publications [5, 8, 9]. Based on the MIC results, the initial concentrations for the adaptation study were established.

Adaptation of *Staphylococcus aureus* to selected compounds

The *S. aureus* strain was initially grown at 50% of the determined MIC values, followed by a 25% increase in compound concentration. The tests were conducted in 96-well plates containing 200 µl of TSB. Every two days, 2 µl of each culture were transferred to a fresh well with TSB and a higher concentration of the compound. The study continued until no bacterial growth was observed at increasing substance concentrations or in case of antiseptics up to commercial concentration (fig. 2).

Ethical approval: The research conducted is not related to either human or animal use.

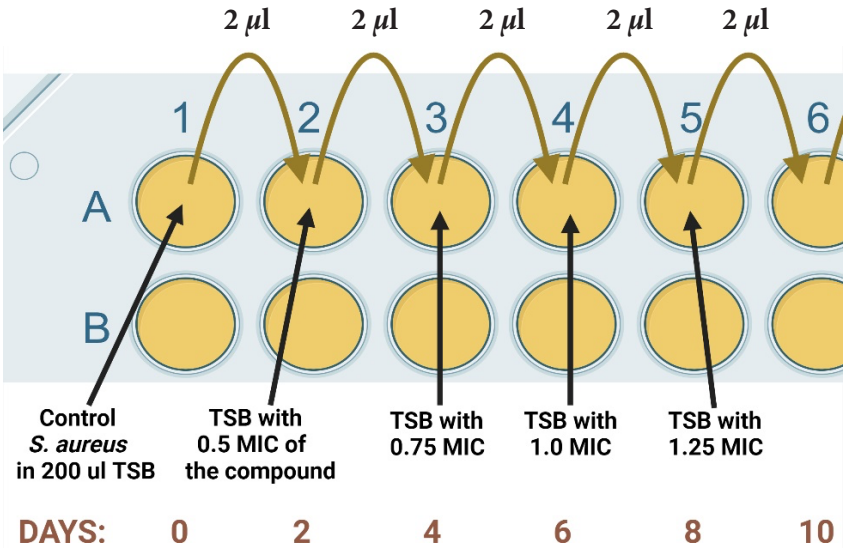

Figure 2.

The initial stages of the *Staphylococcus aureus* adaptation study. The tests were conducted on a 96-well plate. Every two days, 2 µl of the culture was transferred to the next well with a higher concentration of compound

RESULTS

MIC and adaptation study

In this study, it was found that OCT exhibited the best activity against *Staphylococcus aureus*, with an MIC of 2 μg/ml. Moderate activity was shown by NaOCl, ET, and curcumin, with their MIC levels below 1000 μg/ml. The weakest activity against the tested strain was exhibited by salicylic acid, chrysin, astaxanthin, and boric acid with MIC values greater than 1000 μg/ml. At the same time, it was noted that the obtained MIC value for NaOCl is equal to the concentration used in commercial products, which is 100 μg/ml.

For all compounds, adaptation was demonstrated at the level of MIC or higher. Adaptation equal to the MIC value was observed for chrysin and NaOCl. A development of adaptation amounting to 1.25x or 1.5x the MIC value was observed for astaxanthin, salicylic acid, boric acid and curcumin. The highest level of adaptation, amounting to 2.25x the MIC value, was found for octenidine and ethacridine lactate (fig. 3, tab. 1).

Adaptation Index (KAI)

Comparing the level of adaptation with the commercial concentration, it is evident that the adaptation, particularly for OCT, is much lower than the commercial dose. On the other hand, for NaOCl, the increase in adaptation occurred only up to the MIC value, but at the same time, the level of adaptation is equal to the commercial concentration. Therefore, to avoid the possibility of misinterpreting adaptation results, the Karpinski Adaptation Index (KAI) has been introduced for the first time in this paper. The Adaptation Index (KAI) provides information on the level of adaptation compared to the clinical concentration or concentration of commercial product, allowing for an assessment of the risk of developing clinical resistance. It can be calculated using the following formula:

$$\text{Adaptation Index (KAI)} = \frac{\text{Adaptation}}{\text{Clinical concentration}},$$

where 'Adaptation' means the highest drug concentration at which the given microorganism can still grow, and 'Clinical concentration' refers

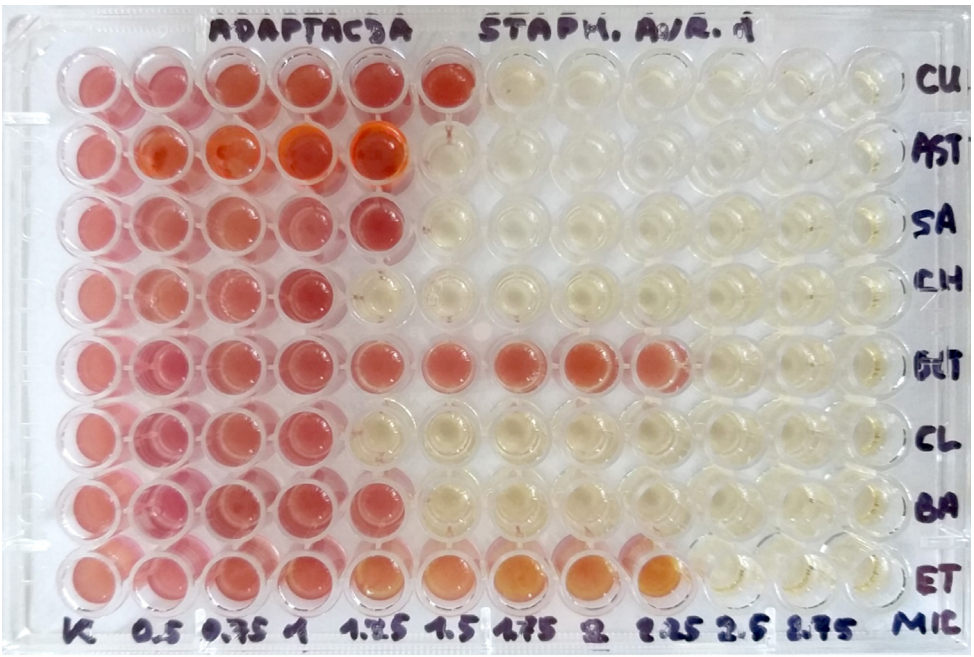

Figure 3.

A picture of a plate showing adaptation testing at concentrations from 0.5 MIC to 3.0 MIC against *Staphylococcus aureus*. The abbreviations mean: CU – curcumin, AST – astaxanthin, SA – salicylic acid, CH – chrysin, OCT – octenidine dihydrochloride, NaOCl – sodium hypochlorite, BA – boric acid, and ET – ethacridine lactate

Table 1.

The obtained values of minimal inhibitory concentration (MIC), levels of adaptation, and Karpinski Adaptation Index (KAI) for *Staphylococcus aureus* under the influence of the tested compounds

Compound	Suggested dose or commercial concentration [μg/ml]	MIC [μg/ml]	Adaptation [μg/ml] (multiple of the MIC value)	Karpinski Adaptation Index (KAI)	Clinical resistance development risk
Curcumin	6,000,000 [10]	625	938 (1.5 MIC)	0.00016	Very low
Astaxanthin	2000–24,000 [11]	2500	3125 (1.25 MIC)	0.13–1.56	Low to very high
Salicylic Acid	5000-100,000 [12]	1250	1563 (1.25 MIC)	0.016–0.31	Very low to moderate
Chrysin	400,000 [13]	1250	1250 (1.0 MIC)	0.003	Very low
Octenidine dihydrochloride	500	2	4.5 (2.25 MIC)	0.009	Very low
Sodium hypochlorite	100	100	100 (1.0 MIC)	1.0	Very high
Boric acid	30,000	3750	4688 (1.0 MIC)	0.156	Low
Ethacridine lactate	1000	125	281 (2.25 MIC)	0.281	Moderate

to the clinical concentration used in treating a given disease, the commercial concentration of the drug, or the suggested dose, for example, for compounds of natural origin. To calculate the Adaptation Index (KAI) for multiple adaptation results at a constant clinical concentration, the average adaptation value $\bar{x}$ can be applied to compute the KAI, according to the following formula:

$$\text{Adaptation Index (KAI)} = \frac{\bar{x}\ \text{Adaptations}}{\text{Clinical concentration}}.$$

Based on the obtained values of the Adaptation Index (KAI), we can determine which levels suggest a probable lack of potential for developing resistance to clinical concentrations, and which may indicate a risk of developing resistance. Considering the obtained results, the following interpretation is suggested:

- KAI≤0.1: Very low risk of clinical resistance. Indicates that the level of adaptation is significantly lower than the clinical concentration. In these situations, the risk of developing resistance is highly unlikely.
- 0.1<KAI<0.2: Low risk of clinical resistance. Suggests that the adaptation level is still well below the clinical concentration, with a low risk of resistance development.
- 0.2<KAI<0.8: Moderate risk of clinical resistance. Moderate values indicating that adaptation is at a medium level compared to the clinical concentration. This may mean that microorganisms could potentially adapt to higher concentrations, which could lead to resistance in the future.
- 0.8<KAI<1.0: High risk of clinical resistance. Suggests similar levels of adaptation and clinical concentration, indicating a higher risk of developing resistance.
- KAI≥1.0: Very high risk of clinical resistance. It indicates that the level of adaptation is equal to or higher than the clinical concentration, which may mean that microorganisms are already fully adapted to the given concentration. This can lead to the development of clinical resistance, or resistance may have already developed.

Taking into account the above interpretation, it can be assumed that curcumin, chrysin and octenidine are characterized by very low values of KAI being more than 100 times lower than the clinical concentration. It can be assumed that the development of clinical resistance for

these compounds is currently not possible. Boric acid has a low risk of resistance development, ethacridine lactate has a moderate risk, while sodium hypochlorite has a very high risk. The presented results even indicate that resistance to clinical/commercial concentrations of NaOCl can develop *in vitro* very quickly in *Staphylococcus aureus*. In the case of astaxanthin and salicylic acid, it is difficult to assess the risk due to the variety of data on the suggested dose. Unfortunately, this is a problem for many natural substances for which clinical doses are not specified.

DISCUSSION

Drug resistance is developed by microorganisms quickly after the introduction of a drug, within a few years or even a few months [14]. It is assumed that after the discovery of penicillin by Alexander Fleming, all *Staphylococcus aureus* strains were susceptible to it. Over the years, bacteria developed resistance mechanisms, and currently, 96% of *S. aureus* strains isolated from humans are penicillin-resistant, which is associated with penicillinase production [15]. Bacteria also develop adaptation and resistance to antiseptics and natural compounds, which unfortunately is poorly studied. Resistance or reduced sensitivity to antiseptics, including chlorhexidine gluconate, benzalkonium chloride [16], hydrogen peroxide and povidone iodine [17]. Adaptation of *Escherichia coli* O157:H7 to organic acids present in juices [18] and increased tolerance of *Saccharomyces cerevisiae* to sorbic acid under low glucose conditions [19] have also been demonstrated. Further studies have shown that flavonoids can change the microorganism's response to stress and induce adaptation to carotenoids, fatty acids, and menaquinones [20]. Cross-adaptation was also found in *Salmonella enterica* serovar Enteritidis, caused by sublethal doses of major essential oil components: cinnamaldehyde, citral, and linalool. Interestingly, bacteria exposed to sublethal doses of linalool developed tolerance to previously lethal concentrations of citral [21]. Similarly, the *S. enterica* serovar Senftenberg isolate was able to develop tolerance to basil oil and linalool. The strain adapted to linalool with at least an 8-fold increase in MIC. Additionally, adaptation to linalool induced cross-adaptation to antibiotics trimethoprim, sulfamethoxazole, piperacillin, chloramphenicol, and tetracycline, increasing their MIC from 2 to 32 times [22]. Cross-adaptation can also result from the use of anticancer agents used in chemotherapy. It was observed that during cancer treatment, *Staphylococcus aureus*, *Mycobacterium vaccae*, *Pseudomonas aeruginosa*, and *Escherichia coli* cells changed metabolism, and a significant increase in the minimum inhibitory concentration (MIC) against several antibiotics occurred [23].

The research presented in this work confirms the possibility of adaptation development in the *S. aureus* strain under the influence of both synthetic compounds and natural compounds. At the same time, the newly introduced Adaptation Index (KAI) can indicate the magnitude of the risk of microorganism adaptation and resistance development to drugs or natural-origin substances.

CONCLUSIONS

Antimicrobial compounds can have varying activity, and microorganisms may adapt to increasing concentrations of these substances.

The Karpinski Adaptation Index (KAI) can be significant in the analysis of adaptation and resistance of microorganisms to natural and synthetic substances. At the same time, it simply indicates the likelihood of developing clinical resistance to drugs.

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Conflict of interest: Authors declare no conflict of interest.

REFERENCES

1. Pancu DF, Scurtu A, Macasoi IG, Marti D, Mioc M, Soica C, et al. Antibiotics: Conventional therapy and natural compounds with antibacterial activity – A pharmaco-toxicological screening. Antibiotics (Basel) 2021;10:401. https://dx.doi.org/10.3390/antibiotics10040401

2. Savoia D. Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiol 2012; 7:979–990. https://dx.doi.org/10.2217/fmb.12.68

3. Wieske LHE, Nchiozem-Ngnitedem VA, Chalo D, Umerevenza D, Ndakala A, Herrebout W, et al. Antimicrobial dihydroflavonols and isoflavans isolated from the root bark of *Dalbergia gloveri*. J Nat Prod 2024; 87. https://dx.doi.org/10.1021/acs.jnatprod.4c00690

4. Karpiński TM, Ożarowski M, Seremak-Mrozikiewicz A, Wolski H. Anti-candida and antibiofilm activity of selected lamiaceae essential oils. Front Biosci (Landmark Ed) 2023; 28:28. https://dx.doi.org/10.31083/j.fbl2802028

5. Adamczak A, Ożarowski M, Karpiński TM. Antibacterial activity of some flavonoids and organic acids widely distributed in plants. J Clin Med 2019; 9:109. https://dx.doi.org/10.3390/jcm9010109

6. GBD 2021 Antimicrobial resistance collaborators. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. Lancet 2024; 404:1199–1226. https://dx.doi.org/10.1016/S0140-6736(24)01867-1

7. Windels EM, Bergh BV den, Michiels J. Bacteria under antibiotic attack: different strategies for evolutionary adaptation. PLOS Pathogens 2020; 16:e1008431. https://dx.doi.org/10.1371/journal.ppat.1008431

8. Korbecka-Paczkowska M, Karpiński TM. *In vitro* assessment of antifungal and antibiofilm efficacy of commercial mouthwashes against *Candida albicans*. Antibiotics (Basel) 2024; 13:117. https://dx.doi.org/10.3390/antibiotics13020117

9. Adamczak A, Ożarowski M, Karpiński TM. Curcumin, a natural antimicrobial agent with strain-specific activity. Pharmaceuticals (Basel) 2020; 13:153. https://dx.doi.org/10.3390/ph13070153

10. Soleimani V, Sahebkar A, Hosseinzadeh H. Turmeric (*Curcuma longa*) and its major constituent (curcumin) as nontoxic and safe substances: Review. Phytother Res 2018; 32:985–995. https://dx.doi.org/10.1002/ptr.6054

11. Karpiński TM, Ożarowski M, Alam R, Łochyńska M, Stasiewicz M. What do we know about antimicrobial activity of astaxanthin and fucoxanthin? Mar Drugs 2021; 20:36. https://dx.doi.org/10.3390/md20010036

12. Salicylic acid (topical route). Mayo Clinic n.d. https://www.mayoclinic.org/drugs-supplements/salicylic-acid-topical-route/description/drg-20066030

13. Walle T, Otake Y, Brubaker JA, Walle UK, Halushka PV. Disposition and metabolism of the flavonoid chrysin in normal volunteers. Br J Clin Pharmacol 2001; 51:143–146. https://dx.doi.org/10.1111/j.1365-2125.2001.01317.x

14. Saini S, Shanbhag C, Saraogi I. Chemical approaches to tackle the silent pandemic of antibiotic resistance. Asia Chem Magazine 2023; 3:164–171. https://dx.doi.org/10.51167/acm00051

15. Kaszanyitzky EJ, Jánosi S, Egyed Z, Agost G, Semjén G. Antibiotic resistance of staphylococci from humans, food and different animal species according to data of the Hungarian resistance monitoring system in 2001. Acta Vet Hung 2003; 51:451–464. https://dx.doi.org/10.1556/AVet.51.2003.4.3

16. Liu WJ, Fu L, Huang M, Zhang JP, Wu Y, Zhou YS, et al. Frequency of antiseptic resistance genes and reduced susceptibility to biocides in carbapenem-resistant *Acinetobacter baumannii*. J Med Microbiol 2017; 66:13–17. https://dx.doi.org/10.1099/jmm.0.000403

17. Merchel Piovesan Pereira B, Wang X, Tagkopoulos I. Biocide-induced emergence of antibiotic resistance in *Escherichia coli*. Front Microbiol 2021; 12:640923. https://dx.doi.org/10.3389/fmicb.2021.640923

18. Koo A, Ghate V, Zhou W. Acid adaptation increased the resistance of *Escherichia coli* O157:H7 in bok choy (*Brassica rapa* Subsp. chinensis) juice to high-pressure processing. Appl Environ Microbiol 2023; 89:e0060223. https://dx.doi.org/10.1128/aem.00602-23

19. Harvey HJ, Hendry AC, Chirico M, Archer DB, Avery SV. Adaptation to sorbic acid in low sugar promotes resistance of yeast to the preservative. Heliyon 2023; 9:e22057. https://dx.doi.org/10.1016/j.heliyon.2023.e22057

20. Linden M, Flegler A, Feuereisen MM, Weber F, Lipski A, Schieber A. Effects of flavonoids on membrane adaptation of food-associated bacteria. Biochim Biophys Acta Biomembr 2023; 1865:184137. https://dx.doi.org/10.1016/j.bbamem.2023.184137

21. Pamplona Pagnossa J, Rocchetti G, Bezerra JDP, Batiha GE-S, El-Masry EA, Mahmoud MH, et al. Untargeted metabolomics approach of cross-adaptation in *Salmonella enterica* induced by major compounds of essential oils. Front Microbiol 2022; 13:769110. https://dx.doi.org/10.3389/fmicb.2022.769110

22. Kalily E, Hollander A, Korin B, Cymerman I, Yaron S. Adaptation of *Salmonella enterica* serovar senftenberg to linalool and its association with antibiotic resistance and environmental persistence. Appl Environ Microbiol 2017; 83:e03398-16. https://dx.doi.org/10.1128/AEM.03398-16

23. de Carvalho CCCR. Adaptation of bacteria to antineoplastic agents involves persister cells and increases resistance to antibiotics. Bioengineering (Basel) 2022; 9:355. https://dx.doi.org/10.3390/bioengineering9080355