Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Artificial Sweeteners Induce Bacterial Drug Resistance and Modulate Gene Expression

Xin Jin[1], Juan Xu[2], Yu Cao[1], Zhen Dai[1], Ruirong Wang[1], Xiaoming Ge[1], Rui Cai[3], Ziyan Hu[1], Xiaojie Sun[1], Jing Chen[4], Ling Zhang[1]

[1]Nanjing Institute for Food and Drug Control, Jiangsu, Nanjing, People's Republic of China; [2]Nanjing Institute of Technology, Jiangsu, Nanjing, People's Republic of China; [3]Jiangsu Province Hospital of Chinese Medicine, Jiangsu, Nanjing, People's Republic of China; [4]China Pharmaceutical University, Jiangsu, Nanjing, People's Republic of China

Correspondence: Xin Jin; Ling Zhang, Nanjing Institute for Food and Drug Control, No. 199, Wenfang Road, Jianye District, Nanjing, Jiangsu, People's Republic of China, Email xjin14@fudan.edu.cn; 2459437556@qq.com

Objective: To explore the effects of artificial sweeteners on reduced antibiotic susceptibility and expression of antibiotic resistance and virulence-related genes in bacteria exposed to permitted daily intake levels.

Methods: We first evaluated the antibacterial effects of five commonly used artificial sweeteners (saccharin, cyclamate, aspartame, acesulfame potassium, and sucralose) on *Escherichia coli* and *Bacillus subtilis*, and detected changes in reactive oxygen species (ROS) production. We investigated the expression of genes associated with resistance, oxidative stress, and virulence using transcriptome sequencing after 2 h of exposure.

Results: The minimum inhibitory concentration (MIC) of artificial sweeteners for *E. coli* and *B. subtilis* were higher than the Codex Alimentarius Commission (CAC) defined daily intake limits. Except for saccharin, these sweeteners did not significantly affect the bacterial growth within 48 h. However, at these limiting concentrations, artificial sweeteners are associated with reduced antibiotic susceptibility and upregulation of resistance-related genes and enhance ROS production. Transcriptome analysis at 2 h revealed that artificial sweeteners upregulated genes associated with resistance, oxidative stress, and virulence compared to the glucose control. Additionally, we observed the effects of artificial sweeteners on iron uptake-related genes in *E. coli*, suggesting potential implications for bacterial ferroptosis that require further validation.

Conclusions: Exposure to artificial sweeteners at CAC-permitted doses is associated with reduced antibiotic susceptibility and may affect bacterial function. Therefore, the safety of artificial sweeteners as substitutes for natural sugars requires careful consideration and further in vivo validation.

Keywords: antibiotic resistance, artificial sweeteners, gene expression, safety

Introduction

Artificial sweeteners are a class of food additives that are produced by humans using chemical methods. Because they are high in sweetness and mostly non-caloric, they are considered an alternative to sugar in a wide variety of foods.[1] Currently, natural sweeteners fall far short of the demand for their use because of cost and commercialization, and artificial sweeteners continue to be major sweeteners in the food industry.[2] Its consumption has increased significantly over the past few years and its demand in the global consumer market is very strong.[3,4] Artificial sweeteners were once considered the best option for obese people and those with diabetes, and some children's foods have started to promote artificial sweeteners over natural ones.[5] Non-caloric artificial sweeteners have long been assumed to be metabolically inert, similar to natural sweeteners, with no significant side effects.[6] However, a growing body of evidence suggests that long-term consumption of artificial sweeteners may affect the immune system, leading to an increased risk of type 2 diabetes, cardiovascular disease, and even cancer.[7–10] Therefore, whether artificial sweeteners are safe alternatives to natural sweeteners requires further study.

Approximately 700,000 people worldwide die annually from infections caused by drug-resistant bacteria. It is estimated that ten million people will die from AMR infections by 2050, if no action is taken now.[11] The emergence and spread of antibiotic

Infection and Drug Resistance downloaded from https://www.dovepress.com/
For personal use only.

https://doi.org/10.2147/IDR.S578529
Received: 2 November 2025
Accepted: 26 February 2026
Published: 8 May 2026

© 2026 Jin et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v4.0) License (http://creativecommons.org/licenses/by-nc/4.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

Graphical Abstract

Safety of artificial sweeteners as natural sugar substitutes?

resistance genes (ARGs) are usually attributed to the misuse or overuse of antibiotics.[12] These resistance genes can also be transferred and expressed in the gut microbiome, further increasing the risk of drug resistance.[13] Some commonly used artificial sweeteners such as saccharin and acesulfame potassium also contain an antibiotic-like sulfonyl group, which is a functional group of sulfonamides. Artificial sweeteners are more likely to cause oxidative stress than natural sweeteners, which typically leads to bacterial resistance to antibiotics.[14,15] Overall, there has not been much research on the effects of artificial sweeteners on bacterial resistance and their associated genes, especially on the differences between artificial and natural sweeteners at daily doses.

Recent evidence indicates that artificial sweeteners can reshape gut microbial composition and function through mechanisms beyond simple metabolic inertness, potentially creating selective pressure for antimicrobial resistance (AMR) evolution.[16] The gut microbiome represents a crucial interface where sweetener-induced metabolic shifts may drive bacterial adaptation strategies, including enhanced efflux pump activity and oxidative stress tolerance, thereby facilitating AMR development.[16,17] Therefore, we selected five commonly used artificial sweeteners (saccharin (SAC), cyclamate (CYC), sucralose (SUC), aspartame (ASP), and acesulfame potassium (ACE-K)) and two strains (*E. coli* and *B. subtilis*) for validation, covering both gram-negative and gram-positive bacteria. We first investigated whether each artificial sweetener had a significant antibacterial effect and changes in bacterial susceptibility to antibiotics and ROS production before and after the addition of artificial sweeteners. Bacterial models were subsequently constructed and transcriptome analysis was performed to investigate differences in the expression of genes associated with resistance, oxidative stress, and virulence in bacteria treated with different artificial sweeteners and glucose (GLC). This article also discusses the safety risks of using artificial sweeteners in daily life as substitutes for natural sweeteners.

Materials and Methods

Bacterial Strains and Chemicals

Gram-negative *E. coli* ATCC 25922 and Gram-positive *B. subtilis* ATCC 6633 were used in this study. *E. coli* and *B. subtilis* are representative commensal bacteria in the gut. Both *E. coli* and *B. subtilis* were susceptible to ampicillin (Amp). Five analytical chemicals of artificial sweeteners (SAC, CYC, SUC, ASP, and ACE-K) were ordered from

Altascientific Technology Co., Ltd. (Tianjin, China) and dissolved in Milli-Q water as stock solutions for further use. Antibiotics were purchased from the National Institutes for Food and Drug Control (Beijing, China), dissolved in Milli-Q water at an initial concentration of 1024 μg/mL, and filtered before use.

Growth Curve Determination

A single bacterial colony of *E. coli* or *B. subtilis* was inoculated aseptically into LB broth supplemented with artificial sweeteners and GLC at various concentrations (SAC, 2500 mg/L; CYC, 2000 mg/L; ASP, 5000 mg/L; ACE-K, 5000 mg/L; SUC, 1800 mg/L; GLC, 5000 mg/L), and allowed to grow for up to 48 h. Growth was recorded as absorbance at 600 nm (OD600) using a Multimode plate reader.

Minimum Inhibitory Concentrations Determination

According to the Clinical and Laboratory Standards Institute (CLSI, 2019) standard, the MIC is defined as the minimum concentration of the antibiotic ampicillin (Amp) and sweetener (CYC, SAC, SUC, ASP, and ACE-K) at which no bacteria (*E. coli* and *B. subtilis*) are visibly growing. Antibiotics were prepared using 2-fold serial dilutions in LB broth, starting from 1024 μg/mL down to 0.5 μg/mL, following CLSI 2019 guidelines. For each artificial sweetener, 2-fold serial dilutions were similarly prepared in LB broth, with the CAC-defined maximum concentration serving as the starting point. Initially, each bacterial strain was incubated overnight in LB medium and washed thrice with LB broth. The cell pellets were resuspended in fresh LB medium and uploaded to a 96-well plate at approximately 10^5 CFU/mL. Each well consisted of a total volume of 200 μL containing various concentrations of artificial sweeteners or antibiotics. The plate was incubated overnight at 37 °C and was scanned using a multimode plate reader (Molecular Devices, USA) at a wavelength of 600 nm (OD600). Each trial was repeated three times. LB culture medium with antibiotics was used as a positive control and culture medium without antibiotics (LB broth-only wells) was used as a negative control in all experiments.

Measurement of ROS Production

To determine whether artificial sweeteners influence oxidative stress, intracellular ROS concentrations were measured using a 2′,7′-dichlorofluorescein diacetate (DCFH-DA) cellular ROS detection assay kit (Solarbio, Beijing, China). Initially, the cell suspensions (initial concentration of approximately 10^6 CFU/mL in PBS solution) were incubated with DCFH-DA at 10 μM for 30 min at 37 °C in the dark. Sweeteners were added and incubated at room temperature in the dark for 2 h. The suspensions were analyzed using a fluorescence microplate (Molecular Devices, USA) at excitation and emission wavelengths of 488 and 525 nm, respectively. Both positive (3% hydrogen peroxide, final concentration) and negative (Milli-Q water) suspensions were used as controls for the ROS analysis.

Transcriptome Sequencing

Both bacterial strains were cultured at 10^6 CFU/mL in the LB medium. They were then treated with artificial sweeteners at CAC-defined concentrations (SAC: 2500 mg/L; CYC: 2000 mg/L; ASP:5000 mg/L; ACE-K:5000 mg/L; SUC:1800 mg/L; GLC: 5000 mg/L) and incubated at 37 °C for 2 h (the exposure time selected to capture early transcriptional stress responses before growth phase alterations). Samples were prepared with three biological replicates per condition. Bacterial cells were collected by centrifugation (8000 × g, 3 min), rapidly frozen in liquid nitrogen, and stored at −80°C. RNA quality was assessed using Agilent 2100 Bioanalyzer (RIN > 7.0), and libraries were constructed using the TruSeq RNA Sample Preparation Kit. Sequencing was performed on an Illumina NovaSeq platform with paired-end 150 bp reads. After identification by 16S rRNA sequencing, samples were subjected to RNA-seq analysis. Library construction was performed once sequencing requirements were met. An Agilent 2100 Bioanalyzer was used to measure the library size and concentration for RNA-seq. The procedures for sample determination, sequencing, and analysis were conducted using PersonalBio. Sequencing was performed using an Illumina platform.

Bioinformatics Analysis

The gene read count was calculated using HTSeq (v0.9.1) as the original expression level of the gene. To make the gene expression levels comparable across samples, Fragments Per Kilobase of transcript per Million mapped reads (FPKM)

were used for normalization for visualization purposes. In transcriptome sequencing, genes with an FPKM >1 are generally considered to be expressed. For exploratory data presentation, fold-change was calculated as FPKM treatment/FPKM control, representing the relative level of gene upregulation or downregulation. However, for rigorous statistical identification of differentially expressed genes (DEGs), DESeq2 (v1.34.0) was employed to account for library size variations and RNA composition bias. Significantly differentially expressed genes were defined as those meeting two criteria: |log2FoldChange| >1 and Benjamini-Hochberg adjusted p-value (padj) <0.05. Genes with log2FoldChange >0 were designated as upregulated, and those with log2FoldChange <0 were designated as downregulated.

Statistical Analysis

All data are presented as mean ± standard deviation (SD), and the results were analyzed using analysis of variance (ANOVA) and independent-sample *t*-test methods, with the Benjamini–Hochberg correction. The corrected p-values were less than 0.05 and were statistically significant.

Results

Effect of Artificial Sweeteners on Bacterial Growth Curves

The growth of *E. coli* and *B. subtilis* was measured every 6–12 hours after exposure to various concentrations of artificial sweeteners (CYC, SAC, SUC, ASP, and ACE-K) for 48 h. In *E. coli*, growth was not significantly affected by the different artificial sweetener groups compared with the control group supplemented with GLC over the measured time, except for the SAC group (Figure 1A). Similarly, there was no significant effect of artificial sweeteners on the growth of *B. subtilis* at any time point compared with that in the GLC group (Figure 1B). In addition, the growth rates of *E. coli* and *B. subtilis* slowed or even decreased after 24 h of exposure to all sweeteners. However, *E. coli* and *B. subtilis* continued to grow after 24 h of exposure to GLC.

Minimum Inhibitory Concentration of Artificial Sweeteners

The maximum concentration of each sweetener used in this study was determined by the CAC, which is the maximum limit for additives in food and beverages (SAC, 2500 mg/L; CYC, 2000 mg/L; ASP, 5000 mg/L; ACE-K, 5000 mg/L; SUC, 1800 mg/L). Of these, the maximum dose of ASP recommended by the CAC was 10,000 mg/L, but it was experimentally found that ASP could not be completely dissolved at this concentration, which was reduced to 5000 mg/L to avoid compromising the results of the experiment. In this study, the MIC of each sweetener against both strains were higher than the maximum concentration in the experiment, which proved that the antibacterial properties of artificial sweeteners at the daily usage concentrations were not obvious (Table 1). Notably, both strains were sensitive to Amp (CLSI 2019) before exposure to artificial sweeteners, with MIC values of 2μg/mL and 1μg/mL, respectively. However, 48 h after exposure to each artificial sweetener, the MIC values for Amp increased for both strains, indicating reduced antibiotic susceptibility (ranging from 2-fold to 4-fold increases) rather than stable clinical resistance, whereas the MIC values for Amp did not change for either strain after exposure to GLC (Table 2). These modest effect sizes are consistent with adaptive tolerance rather than the development of stable resistance.

Expression of Drug Resistance-Related Genes

To investigate early transcriptional stress responses and avoid confounding by growth-phase alterations, transcriptome analysis was performed on *E. coli* and *B. subtilis* after 2 h exposure to each artificial sweetener. Differentially expressed genes (DEGs) were identified using DESeq2 with |log2FoldChange| >1 and Benjamini–Hochberg adjusted p-value <0.05. Based on gene expression values of each sample PCA principal component analysis, the result shows that the largest principal component of *E. coli* (PC1:68.5%) and *B. subtilis* (PC1:61.5%) was better able to distinguish between the artificial sweetener and GLC groups, while the artificial sweetener group clustered together (Figure 1C and D).

To test whether artificial sweeteners influenced the expression of resistance-related genes, we compared the expression of genes highly associated with resistance in two species of bacteria, most of which were efflux pump genes. They are involved in the active efflux of drugs and enhance bacterial survival under antibiotic pressure. The results showed that

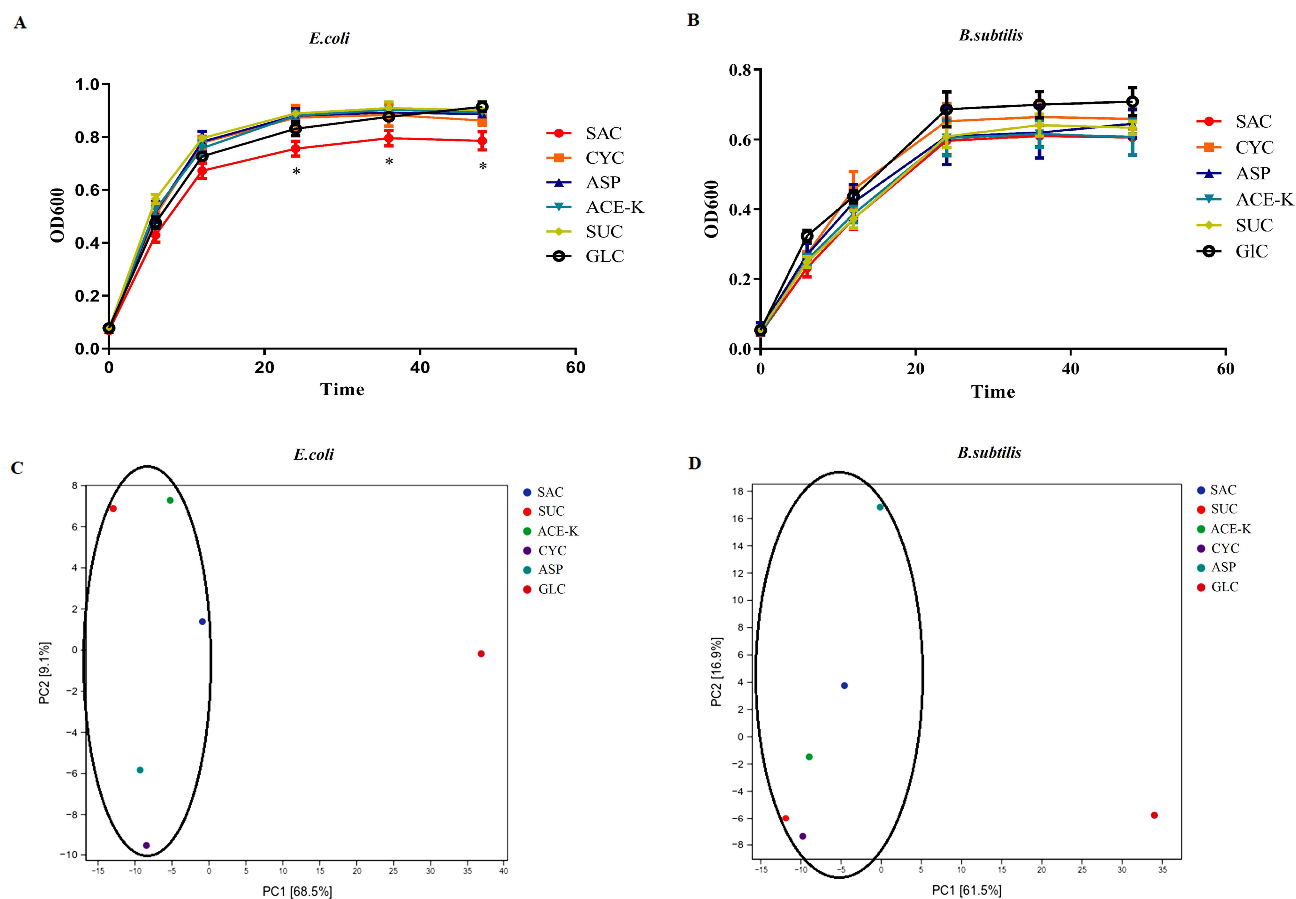

Figure 1 Growth curves of (**A**) *E. coli* and (**B**) *B. subtilis* following artificial sweetener treatment (n=3). PCA of artificial sweetener and glucose gene expression profiles in (**C**) *E. coli* and (**D**) *B. subtilis*. Data were presented as mean ± standard error mean. Significant differences between individual sweetener-treated groups and the control (GLC) were tested with Independent-sample *t* test, **p* < 0.05.

in *E. coli* and *B. subtilis*, the expression of resistance-related genes was mostly upregulated in the sweetener-treated group compared to the control GLC group. Especially in *E. coli*, this phenomenon is particularly pronounced in *E. coli* because of the abundance of efflux pump genes (Figure 2). For example, DESeq2 analysis revealed 1.6-, 2.5-, and 2-fold increases in *acrA, acrB* and *tolC* were detected in CYC-treated *E. coli* compared with the control, whereas in *E. coli* treated with SAC, 1.7-, 5.2-, and 8.2-fold increases in *ompA, ompC*, and *ompF*, respectively, were detected in *E. coli* treated with SUC, 2.0 and 2.3-fold increases in *evgA* and *mdtJ* were detected, respectively. In *E. coli* treated with ACE-K,

Table 1 MIC (mg/Ml) Data of 5 Artificial Sweeteners to 2 Bacterial Based on Antibiotic Susceptibility Testing

Species	SAC	CYC	ASP	ACE-K	SUC	Ampicillin (μg/mL)
E. coli	>2.5	>2	>5	>5	>1.8	2/S
B. subtilis	>2.5	>2	>5	>5	>1.8	1/S

Note: S - Susceptible.

Table 2 MIC (μg/Ml) Data of Amp After the Treatment of Artificial Sweeteners to 2 Bacteria Based on Antibiotic Susceptibility Testing

NO.	SAC	CYC	ASP	ACE-K	SUC	GLU
Ampicillin (*E. coli*)	4	8	4	4	4	2
Ampicillin (*B. subtilis*)	2	2	4	2	2	1

https://doi.org/10.2147/IDR.S578529

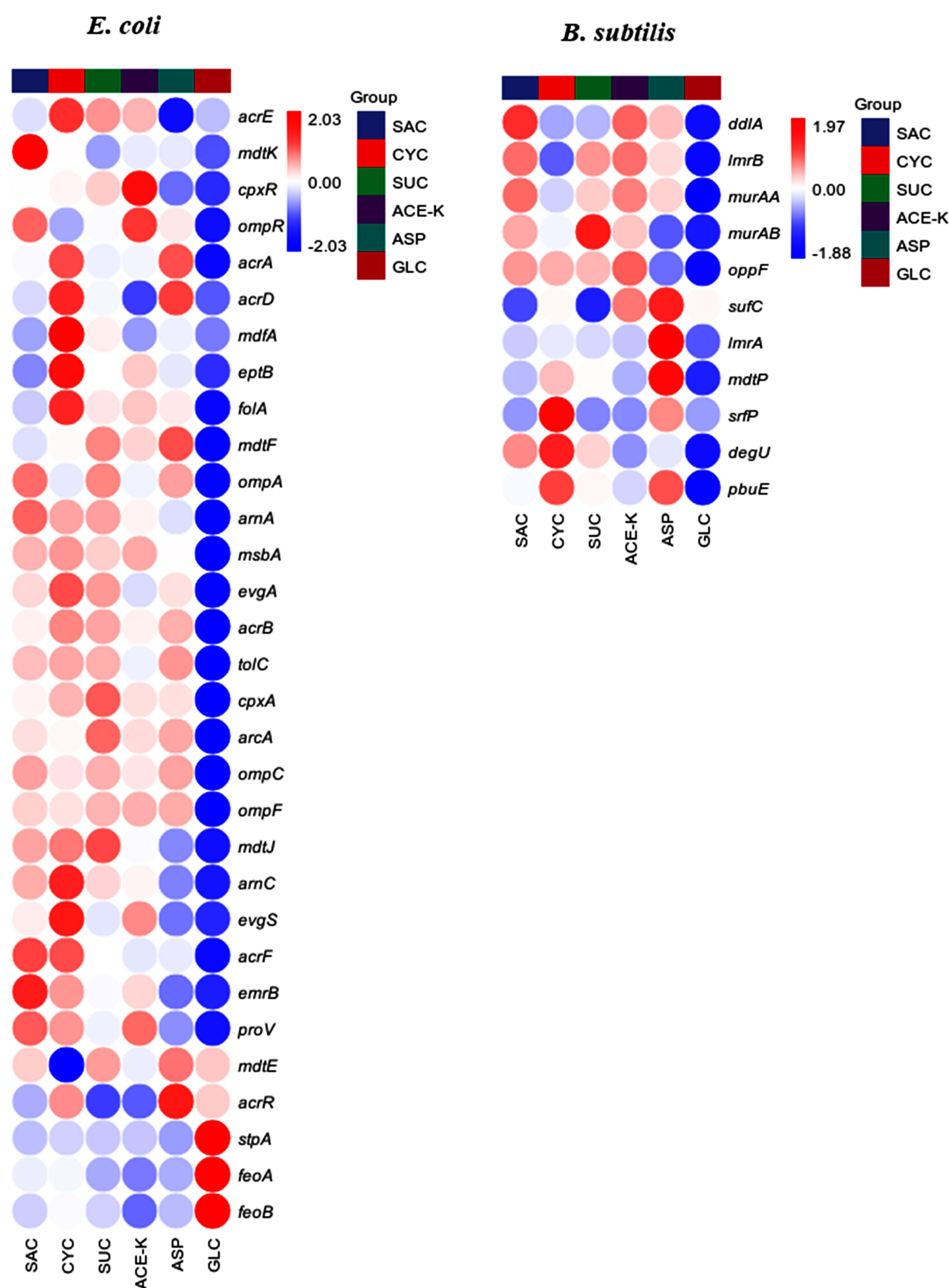

Figure 2 Heatmap showing the expression of resistance-associated genes in *E. coli* and *B. subtilis* following artificial sweetener treatment.

9.3-fold and 3.7-fold increases in *msbA* and *proV*, respectively, were detected. A 2.7-fold increase in *arcA* expression was detected in ASP-treated *E. coli* (Tables S1 and S2).

Effects of Artificial Sweeteners on ROS and Expression of Related Genes

As intracellular reactive signaling molecules associated with various bacterial physiological activities.[18,19] Once exposed to adverse conditions, reactive oxygen species can accumulate in bacterial cells. Excessive ROS can lead to oxidative stress and cell damage, which are also mechanisms of action of common antimicrobial drugs.[20] Therefore, we measured the ROS production in *E. coli* and *B. subtilis* treated with artificial sweeteners for 2 h. We found a significant increase in intracellular ROS levels for both bacterial species in the treated group compared to the control group, and this was positively correlated with concentration (Figure 3A and C). For example, at 300 mg/L, CYC, ASP, ACE-K, and SUC induced 1.53-, 1.58-, 1.62- and 1.58-times higher

https://doi.org/10.2147/IDR.S578529

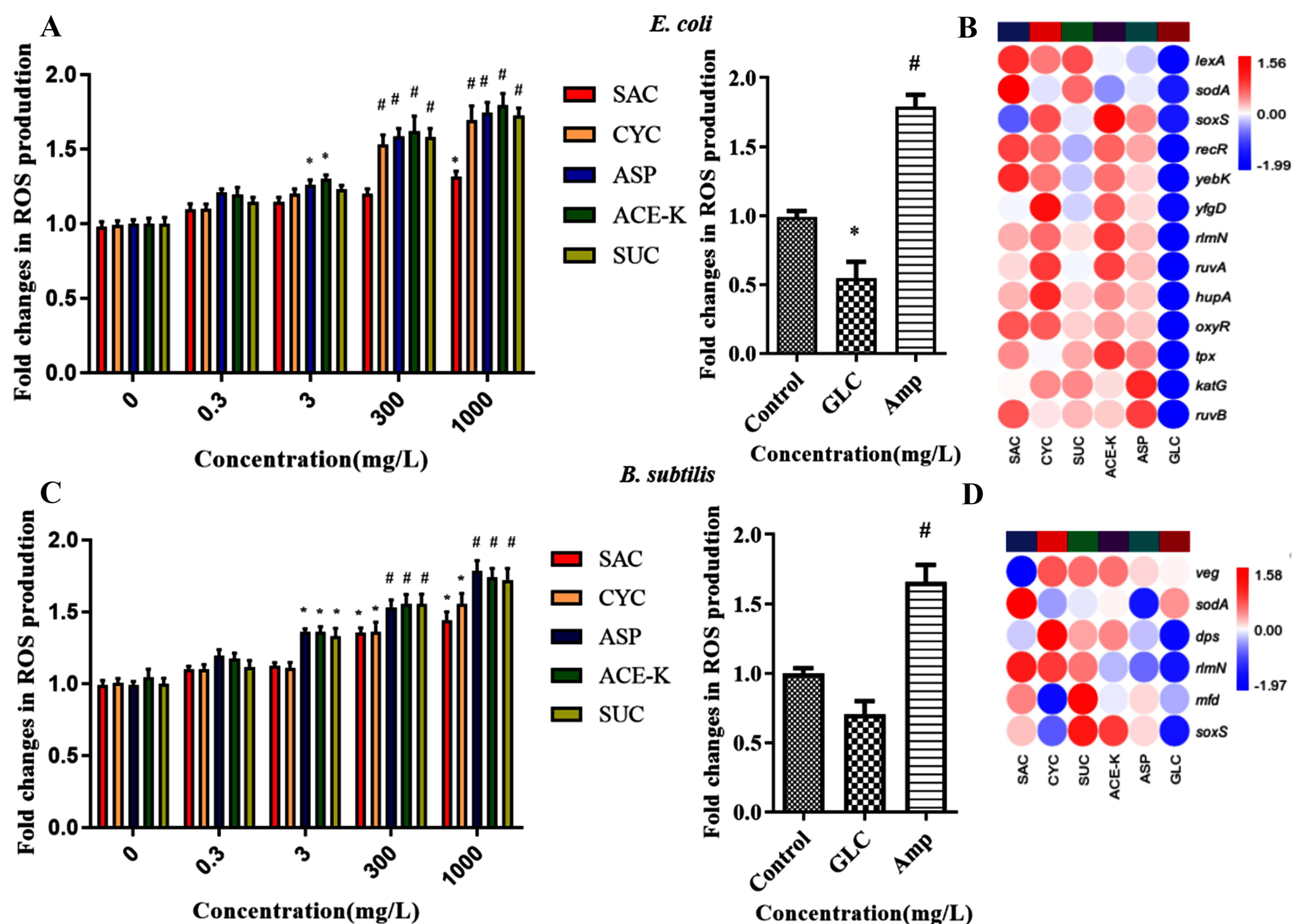

Figure 3 Generation of reactive oxygen species (ROS) induced by treatments with artificial sweeteners at different concentrations. Effects of artificial sweeteners (n=3, ANOVA, $p < 0.05$) on intracellular ROS production in *E. coli* (**A**). Heatmap depicting the expression profiles of oxidative stress-associated genes in *E. coli* (**B**). Effects of artificial sweeteners (n=3, ANOVA, $p < 0.05$) on intracellular ROS production in *B. subtilis* (**C**). Heatmap showing the expression profiles of oxidative stress-associated genes in *B. subtilis* (**D**). Significant differences between individual sweetener-treated groups and the control (0 mg/L of sweeteners) were assessed using an independent-sample *t*-test. Statistical significance is indicated by symbols (* $p < 0.05$ and # $p < 0.01$).

ROS production in *E. coli*, respectively, whereas at 1000 mg/L, ROS production increased 1.69-, 1.74-, 1.79 and 1.72 times, respectively. However, the SAC group shows 1.32 times increase at the highest concentration. The antibiotic Amp group also showed significantly increased intracellular ROS production in both the bacterial species. In contrast, GLC treatment did not increase ROS production in either the bacterial cell line or significantly decreased ROS production in *E. coli*.

Regarding gene expression at the 2 h exposure timepoint, we focused on changes in the expression of genes associated with oxidative stress in both bacteria after each sweetener was used. These genes were generally highly expressed in the artificial sweetener group compared to the GLC control group, and this was more pronounced in *E. coli* (Figure 3B and D). For example, in SAC treated *E. coli*, 5.3-, 4.8-, and 5.5-fold increases in *recR, oxyR* and *yfgD*, respectively, while the CYC-treated group showed 14.4-, 4.7-, and 4.7-fold increases in *soxS, hupA* and *oxyR*, respectively, while the SUC-treated group showed 9.4-, 4.4-, and 4.4-fold increases in *soxS* and *lexA*, respectively. 18.1-, 5.1-, and 4.3-fold increases in *soxS, recR* and *tpx* were detected in ACE-K-treated cells. 12.6, 2.2 and 2.1-fold increases in *soxS, katG* and *ruvB* were detected in the ASP treated group, respectively (Tables S3 and S4). Taken together, these results further confirmed that artificial sweeteners are more likely than GLC to promote ROS overproduction in bacterial cells at early exposure timepoints (2 h).

Effect of Artificial Sweeteners on the Expression of Virulence-Related Genes

To assess early transcriptional changes in bacterial pathogenicity at 2 h post-exposure, we focused on the expression of virulence factor-related genes in two bacterial strains treated with artificial sweeteners. Similar to the above results, in

 https://doi.org/10.2147/IDR.S578529

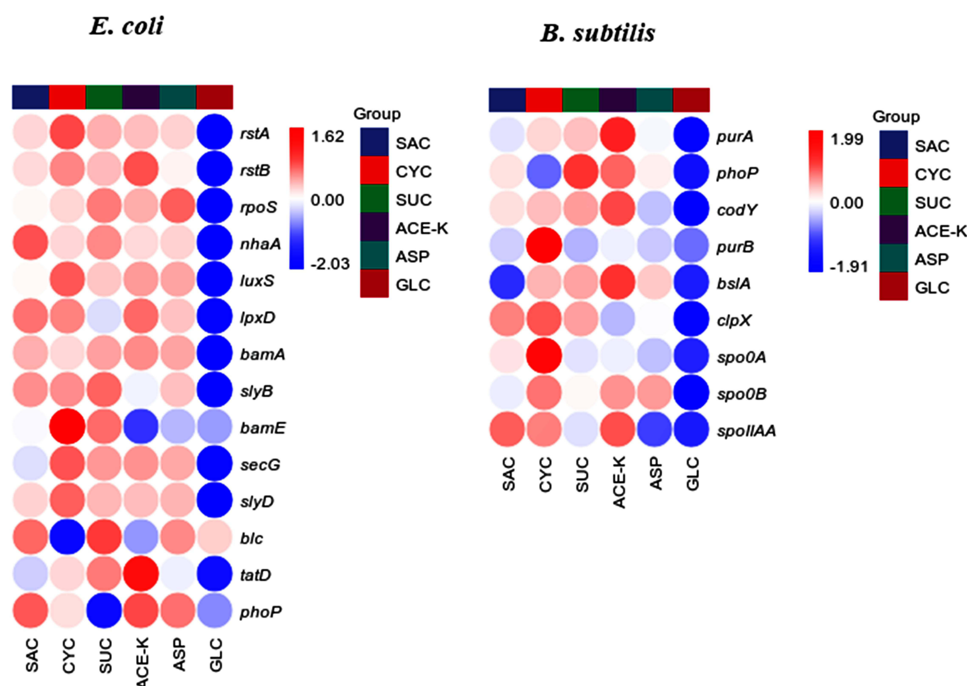

Figure 4 Heatmap showing the expression of virulence-associated genes in *E. coli* and *B. subtilis* following sweetener treatment.

E. coli, virulence factor-related genes were upregulated in almost all artificial sweetener-treated groups compared with the GLC controls (Figure 4). The expression of the *rstA* gene increased 13-fold in all five artificial sweetener-treated groups compared with that in the control group, and the expression of the *rstA* gene was up to 16.7-fold higher in the CYC group. In avian pathogenic *E. coli* (APEC) and uropathogenic *E. coli* (UPEC), deletion of the *rstA* gene significantly reduces the virulence of the bacteria.[21] In addition, the expression of *slyD* increased more than 5-fold in all five artificial sweetener-treated groups (Table S5). In Helicobacter pylori, *SlyD* has been identified as a new virulence factor that promotes intestinal metaplasia of the gastric mucosa, a precancerous lesion in gastric cancer, by regulating signaling pathways in host cells.[22]

Similar results were obtained for *B. subtilis* (Figure 4). Notably, the expression of several genes involved in sporulation was significantly upregulated in some of the artificial sweetener-treated groups compared to the controls, with *spo0A, spo0B* and *spoIIAA* all of which are key regulators of sporulation.[23] In particular, the expression of *spoIIAA* was upregulated by 4.4-, 8.1-, 4.9-, 9.0-, and 2.2-fold in the SAC, CYC, SUC, ACE-K, and ASP treatment groups, respectively, compared to the control group (Table S6). This suggests that the addition of artificial sweeteners causes a degree of stress to bacteria, which may promote spore formation.

Discussion

Although artificial sweeteners have been one of the most important achievements of the food industry to date, their impact on human health is controversial.[24] A recent study found that high intake of sucralose reduced the activity of T cells in mice, which had some effect on the immune system.[25] It is essential to note that the first digestive organ that artificial sweeteners contact after entering the body is the gastrointestinal tract, which is in contact with the intestinal flora for 18–36 h and may affect the functions of many bacteria, including intestinal probiotics.[26] Recent evidence indicates that artificial sweeteners, once considered metabolically inert, function as active microbiota-modulating agents that can reshape gut microbial composition and function, thereby creating selective pressure for antimicrobial resistance (AMR) evolution beyond simple caloric replacement.[16] The gut microbiome serves as both a reservoir for resistance genes and a potential therapeutic target for metabolic interventions, where sweetener-induced oxidative stress and efflux pump activation may inadvertently drive bacterial adaptation strategies.[17] Studies have shown that exposure to these sweeteners increases cell envelope permeability,

upregulates genes encoding DNA uptake and translocation machinery, and may affect the balance of the gut microbial community via quorum sensing inhibition.[27,28] Therefore, we hypothesized that at CAC-permitted dietary concentrations, artificial sweeteners induce oxidative stress, leading to upregulation of efflux pump expression and subsequent reduction in antibiotic susceptibility, without exerting significant bactericidal effects.

Our study focused on whether artificial sweeteners affect the resistance of gut bacteria and the expression of related genes. CAC allowed eight types of artificial sweeteners, including alitame, aspartame, cyclamate, neotame, sucralose, advantame, saccharin, and acesulfame potassium, and established the corresponding upper limits for each sweetener. In our study, the results showed that under the experimental concentrations applied, except for SAC, which had a significant impact on the growth of *E. coli*, the artificial sweeteners exhibited no significant inhibitory effects on the growth of the two bacterial strains tested. This finding is consistent with previously reported results.[29] In this study, the MIC values of all artificial sweeteners exceeded the maximum experimental concentration. Nonetheless, after 48 h of exposure to artificial sweeteners, bacterial susceptibility to antibiotics decreased (adaptive tolerance), whereas in the GLC group, antibiotic sensitivity was maintained. Similar findings have been reported previously, suggesting that certain artificial sweeteners may induce the evolution of bacterial antibiotic resistance. The MIC values of the four artificial sweeteners used in a previous study against the three strains also exceeded the maximum experimental concentration of 1500 mg/L.[14]

However, it is crucial to note that these effects are concentration-dependent, distinguishing between environmental/lethal concentrations and sublethal daily intake levels. At higher concentrations (beyond CAC limits), artificial sweeteners may exhibit bactericidal properties or potentiate antibiotic activity through membrane disruption, as reported in recent studies.[17] This paradoxical effect underscores the translational relevance of our findings: while artificial sweeteners may increase antibiotic sensitivity at high concentrations, their effects at daily exposure levels (CAC-permitted) appear to be the opposite, inducing adaptive tolerance through efflux pump upregulation and ROS-mediated stress responses rather than stable clinical resistance.

Other studies have demonstrated that ACE-K and SAC exhibit stronger inhibitory effects on bacterial strains,[30,31] ACE-K has a strong inhibitory effect on *A. baumannii* and *P. aeruginosa* but has no effect on *S. aureus*, showing that the antibacterial efficacy of artificial sweeteners varies significantly among different bacteria. The concentration of artificial sweeteners is also a key factor determining their inhibitory effects on bacterial growth. Similar to our results, previous studies have shown that low concentrations of sucralose (28.7 mM=11.4 mg/mL, with a concentration of 1.8 mg/mL in our experiment) had no inhibitory effect on all six bacterial strains. 55.7 mM sucralose showed a slight inhibitory effect on the isolates, with a significant difference between the two isolates.[32] Notably, these findings also revealed that sublethal concentrations of ACE-K enhanced the sensitivity of *A. baumannii* to multiple antibiotics, particularly carbapenems. In contrast, our experimental findings showed a different trend. This discrepancy may stem from differences in the concentrations of artificial sweeteners used in our experiment compared to those used in previous studies. Specifically, our study focused on the concentrations of artificial sweeteners recommended daily by CAC, which are far below the lethal levels for bacteria. At these concentrations, only the bacterial efflux pump gene was activated, reducing antibiotic sensitivity, whereas damage to the bacterial cell membrane was minimal and insufficient to enhance the penetration of carbapenem drugs. These experimental results suggest that, while artificial sweeteners may increase antibiotic sensitivity at high concentrations, their effects on daily exposure levels may be the opposite. Additionally, our experiments corroborated previous findings[14] showing that ROS production in bacteria correlated positively with artificial sweetener concentration. Although the concentration range in our study was broad, the overall trends remained consistent.

Therefore, we speculated that daily intake of artificial sweeteners within this concentration range is more likely to reduce antibiotic susceptibility compared to GLC. This hypothesis was supported by gene expression analysis at 2 h post-exposure, in which genes associated with antibiotic resistance in *E. coli* and *B. subtilis* were significantly upregulated in the artificial sweetener group compared with those in the GLC group. Among them, the AcrAB-TolC complex can significantly reduce intracellular antibiotic accumulation by efflux of drug molecules, and high expression of this efflux pump is one of the important mechanisms of bacterial adaptation and survival under antibiotic pressure.[33] These metabolic and stress-related pathways induced by sweeteners represent relevant targets in combination therapy strategies to overcome reduced susceptibility.[17] The excessive production of ROS may activate the bacterial stress response mechanism and then upregulate the expression of the AcrAB-TolC efflux pump,[34] consistent with our hypothesis linking ROS induction to reduced antibiotic

https://doi.org/10.2147/IDR.S578529

susceptibility via efflux pump activation. We observed that *arcA* expression in the sweetener group was more than 2.5 times higher in the artificial sweetener group than in the GLC group. Upregulation of *arcA* reduces ROS production by regulating metabolic pathways and oxidative stress responses, thereby enhancing bacterial tolerance to oxidative stress.[35]

In addition, msbA is highly expressed in all artificial sweeteners (seven times higher than GLC), and the MsbA protein is an ATP-binding cassette (ABC) transporter in gram-negative bacteria, such as *E. coli* which is responsible for transporting lipooligosaccharides (LOS), the precursor of lipopolysaccharide (LPS), from the cytoplasm to the periplasmic side of the inner membrane. This process is critical for LPS biosynthesis, as LPS is a major component of the outer membrane of gram-negative bacteria and provides a barrier against antibiotics and environmental stresses.[36] The expression of the *ompA, ompC*, and *ompF*, which encode extracellular proteins that play important roles in bacterial extracellular permeability, resistance, and virulence, respectively, is upregulated in almost all artificial sweeteners. These proteins enhance bacterial resistance by regulating the permeability of the outer membrane and affecting the entry of antibiotics. Therefore, these proteins play an indispensable role in biofilm formation and stabilization, which is one of the most influential mechanisms of bacterial resistance.[37]

Notably, in *E. coli, stpA* was significantly downregulated in all artificial sweetener groups compared to that in the GLC group. StpA, a homolog of H-NS, can activate the CRISPR-Cas system in *E. coli* to defend it from natural transformations. This defence mechanism can prevent the integration of foreign DNA, including resistance plasmids, and thus reduce the spread of resistance genes.[38] In addition, the expression of *feoA* and *feoB*, which are key components of the ferrous ion transport system (Feo system) in gram-negative bacteria, was downregulated at 2 h post-exposure. The Feo system plays an important role in bacterial oxidative stress protection and virulence, and its loss of function may lead to an increased tolerance of bacteria to oxidative stress and certain antibiotics.[39] While this pattern suggests potential implications for ferroptosis-like mechanisms, we acknowledge that without direct lipid peroxidation assays (eg, malondialdehyde measurement), iron-dependent cell death validation, or ferroptosis inhibitor experiments, this interpretation remains speculative and requires further investigation.

The expression of related genes is also upregulated in *B. subtilis*. For example, *murAA* and *murAB*, which play key roles in bacterial peptidoglycan biosynthesis, were significantly upregulated in almost all artificial sweetener groups. In *Enterococcus faecalis*, the gram-positive bacterium MurAB regulates the stability of MurAA. This regulatory mechanism is activated under cell wall stress (such as cephalosporin exposure), leading to the accumulation of MurAA. Thus, the enhanced bacterial resistance to cephalosporins.[40]

Similar results were observed for ROS gene expression, such as a significant upregulation of *rlmN* gene expression (both more than seven times higher than that in the GLC group). Recent studies have shown that the *rlmN* acts as a stress sensor that directly senses reactive ROS and triggers a bacterial stress response. Upregulation of *rlmN* gene may lead to the production of stress response proteins, improve tolerance to oxidative stress, and thus affect the sensitivity of bacteria to drugs.[41] In *B. subtilis*, changes in ROS in the SAC and CYC groups were not as pronounced as those in the other artificial sweetener groups. It is suggested that other artificial sweeteners are stronger than SAC in increasing bacterial oxygen-containing free radicals, especially at low concentrations; however, this difference decreases at higher concentrations.[42]

These results indicate that artificial sweeteners can exert pressure on bacterial survival, and this pressure stimulates bacterial stress response and activation of efflux pumps, which is key to the formation of tolerant cells in bacteria and a universal mechanism observed in response to antibiotics.[43,44] No such phenomena were observed in the GLC group.

Notably, the expression of *rstA*, which encodes RstA protein, an important regulator of iron uptake in *E. coli*, was significantly elevated in the artificial sweetener groups. Together, *rstA* and *rstB* form a two-component system (RstA/RstB) that regulates the expression of iron-uptake-related genes by sensing the concentration of iron in the environment. Studies have shown that deletion of *rstA* reduces the ability of *E. coli* to take up iron and affects the expression of virulence factors.[21] The upregulation of virulence-associated genes, including *rstA* and *slyD*, aligns with the concept that resistance and virulence are frequently co-regulated traits in bacterial adaptation, suggesting that artificial sweeteners may inadvertently promote a more virulent phenotype alongside reduced antibiotic susceptibility.[45] Iron overload is a key factor in ferroptosis. Recent studies have shown that high concentrations of sucralose induce cell ferroptosis.[46] Based on the current experimental results, we found for the first time that some artificial sweeteners affect the expression of genes responsible for iron ion absorption in bacteria, and their ability to induce ferroptosis needs to be further validated in follow-up studies.

In addition, our recent study revealed that probiotics in commercially available probiotic preparations are commonly resistant to multiple antibiotics[47] and that some of these probiotic preparations are supplemented with artificial sweeteners to improve taste; however, whether there is a causal relationship requires further research.

Several limitations should be acknowledged. The 2 h exposure captures early transcriptional responses but does not reflect long-term resistance development. The observed MIC increases (2–4 fold) represent adaptive tolerance rather than stable clinical resistance. Furthermore, single-species bacterial cultures cannot recapitulate the complex ecological interactions of the gut microbiome, limiting extrapolation to human microbiota. In vivo validation using complex models is essential. Finally, the ferroptosis hypothesis requires validation through lipid peroxidation assays.

Conclusion

The results indicate that the MIC values of five commonly employed artificial sweeteners (saccharin, cyclamate, sucralose, aspartame, and acesulfame potassium) for *E. coli* and *B. subtilis* exceeded the CAC-determined daily limit concentration. Except for SAC, the artificial sweeteners did not significantly affect the growth of *E. coli* or *B. subtilis* within 48 h. However, at this limiting concentration, they were associated with reduced antibiotic susceptibility and increased ROS production at 2 h post-exposure. Transcriptome analysis at 2 h revealed that the artificial sweetener group generally had upregulated genes associated with resistance mechanisms, oxidative stress, and virulence compared to the GLC group. This transcriptional response is consistent with the induction of adaptive tolerance rather than stable clinical resistance. In addition, artificial sweeteners affected the expression of iron uptake-related genes in *E. coli*, though the implications for ferroptosis remain speculative and require validation through lipid peroxidation assays.

These findings derive from in vitro single-species bacterial cultures and should be interpreted with caution, as they cannot fully recapitulate the complexity of the human gut microbiome. While our results highlight the need for careful consideration of artificial sweetener safety profiles at permitted daily intake levels, they do not replace clinical or in vivo studies. Future research should include longitudinal in vivo studies using complex microbiome models to assess whether chronic low-dose exposure selects for stable resistance, and to validate the ferroptosis hypothesis through iron metabolism studies. Considering the widespread use of artificial sweeteners in food and medicine worldwide, their potential impact on bacterial antibiotic susceptibility warrants further in vivo investigation under physiologically relevant conditions.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

Funding

This study was supported by the Science and Technology Program of the China Market Regulation Administration (2023MK160), Scientific Research Program of Jiangsu Drug Administration (202350), and Jiangsu Agricultural Science and Technology Innovation Fund (CX (24) 3060).

Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this study.

References

1. Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil A. Effects of sweeteners on the gut microbiota: a review of experimental studies and clinical trials. *Adv Nutr*. 2019;10:S31–S48. doi:10.1093/advances/nmy037
2. Iizuka K. Is the use of artificial sweeteners beneficial for patients with diabetes mellitus? The advantages and disadvantages of artificial sweeteners. *Nutrients*. 2022;14:4446. doi:10.3390/nu14214446
3. Haalck I, Székely A, Ramne S, et al. Are we using more sugar substitutes? Wastewater analysis reveals differences and rising trends in artificial sweetener usage in Swedish urban catchments. *Environ Int*. 2024;190:108814. doi:10.1016/j.envint.2024.108814

4. Yang G, Cao JM, Cui HL, Zhan XM, Duan G, Zhu YG. Artificial sweetener enhances the spread of antibiotic resistance genes during anaerobic digestion. *Environ Sci Technol*. 2023;57:10919–10928. doi:10.1021/acs.est.2c08673
5. Espinosa A, Mendoza K, Laviada-Molina H, et al. Effects of non-nutritive sweeteners on the BMI of children and adolescents: a systematic review and meta-analysis of randomised controlled trials and prospective cohort studies. *Lancet Glob Health*. 2023;11(1):S8. doi:10.1016/S2214-109X(23)00093-1
6. Ghusn W, Naik R, Yibirin M. The impact of artificial sweeteners on human health and cancer association: a comprehensive clinical review. *Cureus*. 2023;29:e51299. doi:10.7759/cureus.51299
7. Diaz C, Rezende LFM, Sabag A, et al. Artificially sweetened beverages and health outcomes: an umbrella review. *Adv Nutr*. 2023;14:710–717. doi:10.1016/j.advnut.2023.05.010
8. Suez J, Cohen Y, Valdés-Mas R, et al. Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell*. 2022;185:3307–3328.e19. doi:10.1016/j.cell.2022.07.016
9. Posta E, Fekete I, Gyarmati E, Stündl L, Zold E, Barta Z. The effects of artificial sweeteners on intestinal nutrient-sensing receptors: dr. Jekyll or mr. Hyde? *Life*. 2023;14:10. doi:10.3390/life14010010
10. Debras C, Chazelas E, Srour B, et al. Artificial sweeteners and cancer risk: results from the NutriNet-Santé population-based cohort study. *PLoS Med*. 2022;19:e1003950. doi:10.1371/journal.pmed.1003950
11. Stange C, Yin D, Xu T, Guo X, Schäfer C, Tiehm A. Distribution of clinically relevant antibiotic resistance genes in Lake Tai, China. *Sci Total Environ*. 2019;10(655):337–346. doi:10.1016/j.scitotenv.2018.11.211
12. Morales-Durán N, León-Buitimea A, Álvarez Martínez R, Morones-Ramírez JR. Deciphering common genetic pathways to antibiotic resistance in Escherichia coli using a MEGA-plate evolution system. *Antibiotics*. 2025;14:841. doi:10.3390/antibiotics14080841
13. Wang X, Zhang H, Yu S, et al. Inter-plasmid transfer of antibiotic resistance genes accelerates antibiotic resistance in bacterial pathogens. *ISME J*. 2024;18:wrad032. doi:10.1093/ismejo/wrad032
14. Yu Z, Guo J. Non-caloric artificial sweeteners exhibit antimicrobial activity against bacteria and promote bacterial evolution of antibiotic tolerance. *J Hazard Mater*. 2022;433:128840. doi:10.1016/j.jhazmat.2022.128840
15. Lin XL, Guo F, Rillig MC, Chen C, Duan GL, Zhu YG. Effects of common artificial sweeteners at environmentally relevant concentrations on soil springtails and their gut microbiota. *Environ Int*. 2024;185(108496):108496. doi:10.1016/j.envint.2024.108496
16. Hetta HF, Sirag N, Elfadil H, et al. Artificial sweeteners: a double-edged sword for gut microbiome. *Diseases*. 2025;13(4):115. doi:10.3390/diseases13040115
17. Hetta HF, Ramadan YN, Al-Kadmy IMS. Editorial for special issue "antibiotic combination therapy: a strategy to overcome bacterial resistance". *Biomedicines*. 2025;13(1):129. doi:10.3390/biomedicines13010129
18. D'Autréaux B, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol*. 2007;8:813–824. doi:10.1038/nrm2256
19. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat Rev Immunol*. 2013;13:349–361. doi:10.1038/nri3423
20. Van Acker H, Coenye T. The role of reactive oxygen species in antibiotic-mediated killing of bacteria. *Trends Microbiol*. 2017;25:456–466. doi:10.1016/j.tim.2016.12.008
21. Gao Q, Su S, Li X, Wang H, Liu J, Gao S. Transcriptional analysis of RstA/RstB in avian pathogenic Escherichia coli identifies its role in the regulation of hdeD-mediated virulence and survival in chicken macrophages. *Vet Microbiol*. 2020;241:108555. doi:10.1016/j.vetmic.2019.108555
22. Zheng S, Wang Y, Ni C, et al. Helicobacter pylori SlyD stabilizes TPT1 via hnRNPK and enhances OCT1-mediated CDX2 transcriptional activation to drive gastric intestinal metaplasia. *BMC Med*. 2025;23:71. doi:10.1186/s12916-025-03911-8
23. Brunsing RL, La Clair C, Tang S, et al. Characterization of sporulation histidine kinases of Bacillus anthracis. *J Bacteriol*. 2005;187:6972–6981. doi:10.1128/JB.187.20.6972-6981
24. Carocho M, Morales P, Ferreira ICFR. Sweeteners as food additives in the XXI century: a review of what is known, and what is to come. *Food Chem Toxicol*. 2017;107:302–317. doi:10.1016/j.fct.2017.06.046
25. Zani F, Blagih J, Gruber T, et al. The dietary sweetener sucralose is a negative modulator of T cell-mediated responses. *Nature*. 2023;615:705–711. doi:10.1038/s41586-023-05801-6
26. Sun Y, Xu B. A critical review on effects of artificial sweeteners on gut microbiota and gastrointestinal health. *J Sci Food Agric*. 2025;105:2737–2747. doi:10.1002/jsfa.14148
27. Yu Z, Wang Y, Henderson IR, Guo J. Artificial sweeteners stimulate horizontal transfer of extracellular antibiotic resistance genes through natural transformation. *ISME J*. 2022;16(2):543–554. doi:10.1038/s41396-021-01095-6
28. Markus V, Share O, Shagan M, et al. Inhibitory Effects of Artificial Sweeteners on Bacterial Quorum Sensing. *Int J Mol Sci*. 2021;22(18):9863. doi:10.3390/ijms22189863
29. Shil A, Chichger H. Artificial sweeteners negatively regulate pathogenic characteristics of two model gut bacteria, E. coli and E. faecalis. *Int J Mol Sci*. 2021;22(10):5228. doi:10.3390/ijms22105228
30. de Dios R, Proctor CR, Maslova E, Dzalbe S, Rudolph CJ, McCarthy RR. Artificial sweeteners inhibit multidrug-resistant pathogen growth and potentiate antibiotic activity. *EMBO Mol Med*. 2023;15(1):e16397. doi:10.15252/emmm.202216397
31. de Dios R, Gadar K, Proctor CR, et al. Saccharin disrupts bacterial cell envelope stability and interferes with DNA replication dynamics. *EMBO Mol Med*. 2025;17(5):993–1017. doi:10.1038/s44321-025-00219-1
32. Omran A, Ahearn G, Bowers D, Swenson J, Coughlin C. Metabolic effects of sucralose on environmental bacteria. *J Toxicol*. 2013;2013:372986. doi:10.1155/2013/372986
33. Alenazy R. Identification of potential therapeutics of mentha essential oil content as antibacterial MDR agents against AcrAB-TolC multidrug efflux pump from Escherichia coli: an in silico exploration. *Life*. 2024;14:610. doi:10.3390/life14050610
34. Weston N, Sharma P, Ricci V, Piddock LJV. Regulation of the AcrAB-TolC efflux pump in Enterobacteriaceae. *Res Microbiol*. 2018;169:425–431. doi:10.1016/j.resmic.2017.10.005
35. Brown AN, Anderson MT, Bachman MA, Mobley HLT. The ArcAB Two-component system: function in metabolism, redox control, and infection. *Microbiol Mol Biol Rev*. 2022;86:e0011021. doi:10.1128/mmbr.00110-21

36. Chang G, Roth CB. Structure of MsbA from E. coli: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters. *Science*. 2001;293:1793–1800. doi:10.1126/science.293.5536.1793
37. Bianchi M, Winterhalter M, Harbig TA, et al. Fosfomycin uptake in escherichia coli is mediated by the outer-membrane porins OmpF, OmpC, and LamB. *ACS Infect Dis*. 2024;10:127–137. doi:10.1021/acsinfecdis.3c00367
38. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. *Clin Microbiol Rev*. 2018;31: e00088–17. doi:10.1128/CMR.00088-17
39. Lau CK, Krewulak KD, Vogel HJ. Bacterial ferrous iron transport: the Feo system. *FEMS Microbiol Rev*. 2016;40:273–298. doi:10.1093/femsre/fuv049
40. Mascari CA, Little JL, Kristich CJ. PASTA-kinase-mediated signaling drives accumulation of the peptidoglycan synthesis protein MurAA to promote cephalosporin resistance in Enterococcus faecalis. *Mol Microbiol*. 2023;120:811–829. doi:10.1111/mmi.15150
41. Khadka R, Maravich B, Demarest N, et al. Stressosome-independent but RsbT-dependent environmental stress sensing in Bacillus subtilis. *Nat Commun*. 2025;16:1591. doi:10.1038/s41467-025-56871-1
42. Zhu W, Xiao L, Hong S, et al. Exogenous glucose interferes with antimicrobial-mediated ROS accumulation and bacterial death. *ACS Infect Dis*. 2024;10:1896–1903. doi:10.1021/acsinfecdis.4c00167
43. Gaurav A, Bakht P, Saini M, Pandey S, Pathania R. Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. *Microbiology*. 2023;169:001333. doi:10.1099/mic.0.001333
44. Lories B, Roberfroid S, Dieltjens L, De Coster D, Foster KR, Steenackers HP. Biofilm bacteria use stress responses to detect and respond to competitors. *Curr Biol*. 2020;30:1231–1244.e4. doi:10.1016/j.cub.2020.01.065
45. Hetta HF, Alanazi FE, Ali MAS, et al. Hypervirulent *Klebsiella pneumoniae*: insights into virulence, antibiotic resistance, and fight strategies against a superbug. *Pharmaceuticals*. 2025;18:724. doi:10.3390/ph18050724
46. Hacioglu C. Long-term exposure of sucralose induces neuroinflammation and ferroptosis in human microglia cells via SIRT1/NLRP3/IL-1β/GPx4 signaling pathways. *Food Sci Nutr*. 2024;12:9094–9107. doi:10.1002/fsn3.4488
47. Jin X, Zhang L, Cao Y, et al. Antibiotic resistance characterization, virulence factors and molecular characteristics of Bacillus species isolated from probiotic preparations in China. *J Glob Antimicrob Resist*. 2025;43:35–39. doi:10.1016/j.jgar.2024.06.015

Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/infection-and-drug-resistance-journal