Insight into the impacts and mechanisms of ketone stress on the antibiotic resistance in Escherichia coli

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Abstract
Accumulation of toxic organic has posed a substantial pressure on the proliferation of bacterial resistance. While aromatic organics have been demonstrated to enhance the antibiotic resistance in bacteria, no information is yet available on the effects of non-aromatic organics on the variations of bacterial resistance. Here, we investigated the effects of a typical ketone (i.e., methylisobutanone (MIBK)) on the variations of antibiotic resistance in Escherichia coli (E. coli). The results showed that the growth of resistant E. coli under environmental concentration of 50 μg/L MIBK was firstly inhibited as explained by the transient disruption in the cell membrane and then recovered possibly due to the reactive oxygen species. Exposure to 50 μg/L MIBK gradually raised the abundance of representative resistance gene (ampR) in E. coli. In contrast, the high concentration of 50 mg/L MIBK continuously inhibited the growth of resistant E. coli by disrupting cell membrane and notably promoted the proliferation of ampR through enhancing the horizontal transformation and up-regulating the expression of efflux pump gene. These findings provided the first evidence for the evolution of bacterial resistance in response to ketone organics.

Keywords Antibiotic resistance genes (ARGs) · Ketone · Methylisobutanone (MIBK) · Escherichia coli · Transformation

Introduction

According to a review published by UK government, antimicrobial resistance infection may cause 10 million deaths each year and $60–100 trillion economic loss by 2050 (O’Neill 2016). Especially, carbapenem and colistin as the last line of defense against bacterial resistance encounter growing failures (Gogry and Siddiqui 2019; Sheu et al. 2019). More seriously, the inappropriate use of antibiotics and disinfectants during the COVID-19 pandemic may exacerbate the threat of antibiotic resistance (Lu and Guo 2021; Rawson et al. 2020). At present, many countries including the USA (White House 2015), the UK (O’Dowd 2014), China (Xiao and Li 2016), and India (Dutta 2018) have successively issued national action plans to combat antibiotic resistance. Antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) have been regarded as emerging contaminants, which may facilitate the spread of antibiotic resistance in the environments to clinic habitats (Finley et al. 2013; Wang et al. 2017a). Numerous evidences have demonstrated that the propagation of ARGs and ARB in the environments could be promoted by selective factors including antibiotic (Ben et al. 2019; Zhou et al. 2022), disinfectant (Kampf 2018), pesticide (Ramakrishnan et al. 2019), and heavy metals (Poole 2017). More recently, the influences of organic matters (especially polyaromatic hydrocarbons, PAHs) on the evolution of antibiotic resistance in the environments have attracted growing attention (Das et al. 2021; Maurya et al. 2021; Sun et al. 2015; Tan et al. 2019; Wang et al. 2017a). A metagenomic study showed that the abundances of ARGs in
the PAH-contaminated soils collected from a petrochemical plant were approximately 15 times higher than those in the background sites (Chen et al. 2017). Similarly, two typical PAHs (naphthalene and phenanthrene) were reported to dramatically enhance the relative abundance of intI1, sulI, and aadAII in a coastal microbial community by 1–2 orders of magnitude (Wang et al. 2017b). Regarding the resistance phenotype upon exposure to PAHs, benzo(a)pyrene, pyrene, and phenanthrene were observed to confer the tetracycline-resistant Shigella flexneri approximately 20% more sensitive to tetracycline (Rugare et al. 2018), while the minimal inhibitory concentration (MIC50) of Escherichia coli (E. coli) to chloramphenicol and tetracycline increased by tenfold following triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] exposure (Li et al. 2019). Nevertheless, whether the non-PAH organic matters (e.g., non-aromatic ketones), which may coexist with the PAHs contaminated environments, could affect the transmission of bacterial resistance remains largely unknown.

In addition, PAHs have been documented to facilitate the horizontal transfer of ARGs in pure strains or activated sludge. Two PAHs (i.e., naphthalene of > 100 mg/L and phenanthrene of > 10 mg/L) were recorded to increase the conjugative transfer between intI1-positive bacterium Aerococcus sp. and intI1-negative bacterium Pseudoalteromonas sp. by at least threefold as compared to the controls (Wang et al. 2017a). A recent study also showed that 10–100 mg/L of phenolic PAHs including p-nitrophenol, p-aminophenol, and phenol can significantly increase the conjugative transfer frequency of ARGs from E. coli to Acinetobacter or activated sludge (P < 0.05) (Ma et al. 2021). Moreover, PAHs were demonstrated to be involved in the transformation process of ARGs in bacteria. For example, a widely occurring PAHs (e.g., phenanthrene) was found to effectively bind with antibiotic resistant plasmids (pUC19) to form a plasmid-PAH complex through noncovalent interactions, which reduced the transformation frequency of ampicillin resistance gene (Kang et al. 2015). Unlike PAHs, the information regarding the impacts of non-PAHs organics on the horizontal spread of ARGs is still lacking.

To date, the mechanisms of PAHs-mediated bacteria resistance may include (1) inhibiting or killing resistant bacteria by destroying cell membrane (Ma et al. 2021); (2) inducing mutations by stimulating the reactive oxygen species (ROS) production, which enrich ARGs directly by triggering stress/repair systems or changing the DNA composition (Dharmaraja 2017; Guo et al. 2018; Jin et al. 2018; Li et al. 2019); and (3) enhancing the overexpression of synergistic efflux pump for multidrug and PAHs (Pupkou et al. 2020; Wang et al. 2020). However, the underlying mechanisms for the non-PAHs organics influencing the antibiotic resistance in bacteria are not yet clear. As the representative non-aromatic organic contaminants, ketones are ubiquitous in various environments (such as petrochemical wastewater treatment plants and municipal landfill leachates) (Ding et al. 2016; Paxéus 2000) and have been confirmed to impact the activity and growth of microorganisms. For instance, Yang et al. (2008) corroborated that the activity of yeast cell dropped markedly under exposure over 30.0 mmol/L non-aromatic acetophenone. Similarly, non-aromatic trifluoromethyl ketone derivatives have been reported to effectively inhibit the growth of Bacillus megaterium, Corynebacterium, and E. coli (Kawase et al. 2001). Therefore, the potential influencing pathways of ketones on the variation of bacterial resistance deserve further exploration.

In this study, a representative ketone (i.e., methylisobutanone (MIBK)) was chosen to validate its roles in the evolution of antibiotic resistance in E. coli. Herein, MIBK was selected because it dominated (93.11%) in the inlet of petrochemical wastewater treatment plant (Fig. S1), which has been identified as the key reservoir of ARGs (Chen et al. 2017; Jing et al. 2014) and could affect the activity of sludge and pure bacterial strains (Przybulewska and Wieczorek 2008; Wang et al. 2017b). Specifically, the goals of this work were (1) to determine the variations of resistant phenotype and genotype in E. coli upon MIBK exposure, (2) to investigate the transformation of ARGs under MIBK stress, and (3) to elucidate the impacting mechanisms of MIBK on the antibiotic resistance in E. coli based on the changes of cell membrane integrity, ROS production, and ARGs expression. The outcomes would advance our knowledge concerning the roles of ketone compounds in the spread of bacterial resistance.

Materials and methods

Bacteria strain, plasmid, and chemicals

E. coli DH5α and plasmid pUC118 containing ampR (confering resistance to ampicillin) were purchased from Takara Bio Inc. (Dalian, China). The recombinant E. coli DH5α containing pUC118 was obtained by transformation experiment as described in the “Transformation experiments under MIBK exposure” section. Mueller Hinton (MH) agar medium and super optimal broth with catabolite repression (SOC) medium were obtained from Oxide (Basingstoke, UK) and Takara Bio Inc. (Dalian, China), respectively. Luria-Bertani (LB) liquid medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) was prepared according to the guidelines of Molecular Cloning (Green and Sambrook 2012). Ampicillin, MIBK, and other chemicals of reagent-grade were supplied by Macklin Inc. (Shanghai, China).
**MIBK exposure assays**

Prior to exposure tests, 4 mL log-phase cultures of recombinant *E. coli* DH5α suspension were transferred to 500 mL Erlenmeyer flasks. Then, MIBK (final concentrations at 0.05, 50, and 100 mg/L) and sterilized LB liquid medium were added to each flask to give a final volume of 400 mL. Here, the environmental relevantly concentrations of 50 μg/L and high stress concentrations of 50 or 100 mg/L MIBK were adapted to obtain the profiles of the antibiotic resistance in *E. coli* under various degrees of ketone contamination (Lu et al. 2018; Jin et al. 2018; Li et al. 2019; Wang et al. 2017b). After that, all flasks were placed into thermostatic oscillator (37 °C, 180 r/min). Finally, samples were taken at 1, 3, 6, 12, 24, 72, 120, and 168 h and were centrifuged for subsequent analysis. In parallel, *E. coli* cultured in the LB liquid medium without MIBK were served as controls. All tests were performed in triplicate.

**Determination the concentration of resistant *E. coli* and MIC**

The concentration of resistant *E. coli* was determined following the protocols as described previously (Wiegand et al. 2008). In brief, bacterial suspensions were tenfold diluted with 0.85% NaCl. Then, 1 mL of the diluted solution was spread on MH agar plates with or without ampicillin (final concentration at 32 mg/L). The concentration of ampicillin was selected as the breakpoint of antibiotic resistance of common clinical strains suggested by Clinical and Laboratory Standards Institute (Patel 2017). After incubating for 24 h at 37 °C, all plates with colony numbers between 30 colony-forming units (CFU) and 300 CFU were counted to obtain the total number of colonies. The concentration of resistant *E. coli* referred to the number of colonies in the plates amended with ampicillin (CFU/mL).

MICs were determined for the *E. coli* strain against ampicillin before and after MIBK exposure by the broth macrodilution method from Wiegand et al. (2008). Briefly, the *E. coli* strains were firstly inoculated in 14 × 5 mL sterile tubes containing MH broth medium. Subsequently, two-fold dilutions of ampicillin ranging from 0.25 to 2054 μg/mL were amended to each tube. Finally, MIC value was obtained by the lowest concentration exhibiting no visible bacterial growth at 37 °C for 24 h. The OD600 of the bacterial concentration in tube was measured with a spectrophotometer (UV1240, Shimadzu, Tokyo, Japan).

**Quantification of ARGs abundance and expression**

DNA in *E. coli* was isolated with reference to the Molecular Cloning (Green and Sambrook 2012). Bacterial RNA was extracted by RNAprep Pure Cell/Bacteria Kit (TIANGEN Biotech (Beijing) Co., Ltd., Beijing, China) according to the manufacturer’s instructions. Then, reverse transcription of RNA was conducted to generate complementary DNA (cDNA) using PrimeScript RT reagent Kit with genomic DNA (gDNA) Eraser (Takara Bio Inc., Dalian, China). The abundances of *ampR* and efflux pump gene (*acrA*) in DNA and cDNA were quantified by quantitative real-time polymerase chain reaction (qPCR) using SuperReal PreMix (Probe) Kit (TIANGEN Biotech (Beijing) Co., Ltd., Beijing, China) on LightCycler 96 platform (Roche, Basel, Switzerland). Here, multidrug efflux pump AcrAB-TolC encoded gene *acrA* was chosen as it can pump out a broad range of antibiotics from cell compartment and plays a vital role in the development of antibiotic resistance in bacteria (Nolivos et al. 2019; Poole 2005; Xuan et al. 2017). The abundance of ARGs was calculated as the ARGs copies divided by the total concentration of DNA for *ampR* (copies/ng DNA) or RNA for *acrA* (copies/ng RNA) (Hu et al. 2021; Ruiying et al. 2021). Detailed descriptions of DNA extraction, RNA reverse transcription, and qPCR test are presented in the Supporting Information (SI) (S1-S4).

**Transformation experiments under MIBK exposure**

Transformation experiments were conducted in accordance with the instructions from Takara Bio Inc. with minor modifications. Initially, 100 μL of recipient *E. coli* DH5α competent cells was added to each 2-mL round-bottom centrifuge tube. Next, 5 ng pUC118 plasmid and different levels of MIBK (final concentrations at 0, 10, 20, 50, 100, 200, and 300 mg/L) were amended to each tube and mixed well with bacterial suspensions. The mixtures were then placed on ice for 30 min, followed by heat-shock at 42 °C for 45 s and cold treatment in the ice-water bath for 90 s. After that, SOC medium was added to each tube at a final volume of 0.5 mL and was incubated at 37 °C and 180 r/min for 1 h. Finally, 1 mL of tenfold diluted solutions using 0.85% NaCl were spread on MH agar plates with or without of 100 μg/mL ampicillin to determine the numbers of transformants and recipient colonies, respectively. After incubating at 37 °C for 24 h, the colony numbers between 30 and 300 CFU on each plate were counted. The transformation frequency was expressed as the number of transformants divided by the number of total recipients *E. coli* colonies.

**Analyses of ROS and cell membrane permeability**

The ROS was detected using the total DCFH-DA S0033 probe (Beyotime, Beijing, China). The cell membrane permeability was measured by Live/Dead BacLight Bacterial Viability Kits (Invitrogen, Eugene, USA). The protocols for ROS and cell membrane permeability analysis are described in SI S5-S6. To qualitatively analyze the cell morphology,
the bacterial culture was fixed in 2.5% glutaraldehyde solution and was observed by transmission electron microscopy (TEM) (Hitachi H-7650, Tokyo, Japan).

**Statistical analysis**

One-way analysis of variance and independent sample t-tests were conducted on SPSS 19 (IBM-SPSS, USA). A P value less than 0.05 or 0.01 indicated a significant correlation. The fold change of tested parameters (i.e., resistant *E. coli* concentration, ARGs abundance, live cell ratio, ROS level, and transformation frequency) was calculated as the ratio of experimental values in the exposure groups to those in the control group. All error bars represent the standard deviations of triplicate tests.

**Results and discussion**

**The concentration of resistant *E. coli* under MIBK exposure**

To evaluate the effects of ketone on the resistant phenotype in bacteria, we monitored the changes of the ampicillin resistance in *E. coli* under MIBK exposure (Fig. 1a). Upon exposure to the environmentally relevant (50 μg/L) and high concentration (50 mg/L) of MIBK, the concentrations of ampicillin resistant *E. coli* were $1.00 \times 10^4$–$6.95 \times 10^{10}$ and $2.50 \times 10^3$–$7.60 \times 10^9$ CFU/mL, respectively (Fig. 1a). For all tests, the concentration of resistant *E. coli* increased continuously within 24 h, but decreased dramatically at 48 h, and then remained stable until the end of exposure.

Overall, the impacts of MIBK on the distribution of resistant *E. coli* were concentration-dependent. Under the shock of 50 μg/L MIBK, the concentrations of resistant *E. coli* generally changed by $-1.01$–$0.68 \log_{10}$ compared with that in the control (Fig. 1a). During the initial 1 to 3 h, the growth of resistant *E. coli* was significantly inhibited, possibly due to the membrane destruction by the accumulation of MIBK in cell (Lu et al. 2018; Lu et al. 2020; Smet et al. 1978; Yu et al. 2021). At this point, the proportions of living cells were 0.20–0.26-fold lower than that in the control (Fig. 1b). Thereafter, the inhibitory effects of MIBK on resistant *E. coli* diminished and even turned to promote the growth of resistant *E. coli*. Specifically, the concentrations of resistant *E. coli* increased by $0.57$–$0.68 \log_{10}$ compared with the control during 24–72 h (Fig. 1a). In addition, the MIC of ampicillin in *E. coli* was observed to increase by 3-fold under 0.05 mg/L MIBK compared with the control (Fig. S2). Correspondingly, the proportions of living cells increased by 0.59-fold compared to the control at 24–48 h (Fig. 1b). The TEM images showed that *E. coli* cells maintained full shape with smooth surface and no wrinkling deformation (Fig. 2a and b), verifying that they may have gained tolerance to MIBK. Previous studies have confirmed that bacteria could acclimate the toxic organic pollutants through efflux pumps, alterations in the cell membrane composition, and active transport system (Torres et al. 2011). Interestingly,
organic solvent-tolerant bacteria can also confer resistance to antibiotics such as chloramphenicol and tetracycline (Asako et al. 1997), which may be related to the efflux pump genes (Rojas et al. 2001). Additionally, the overproduction of ROS may also explain the variations of resistant E. coli. When exposed to 50 μg/L MIBK, the ROS concentrations were 0.55–0.59-fold higher than that in the control during 24 to 72 h (Fig. 1c). Indeed, the overproduction of ROS was reported to be involved in promoting the conjugative transfer of ARGs under triclosan exposure (Lu et al. 2018). The ROS-mediated mutagenesis in cell membrane, DNA, and protein was also confirmed to induce multi-drug resistance when exposed to antidepressant fluoxetine (Jin et al. 2018). During 120 to 168 h, MIBK had only a slight inhibitory on the growth of resistant E. coli.

On the contrary, MIBK at 50 mg/L continuously inhibited the growth of resistant E. coli by 0.15–2.75 log10 compared to the control throughout the exposure period. In particular, the growth of resistant E. coli was markedly inhibited at 1 to 12 h (Fig. 1a). Fifty milligrams per liter MIBK also leads to the MIC of ampicillin in E. coli decrease by 7-fold than the control (Fig. S2). The proportions of living cells were correspondingly much lower than those in the other groups (except for 1 h) (Fig. 1b). These results strengthened the notion that the alteration of the profiles of resistant E. coli upon 50 mg/L MIBK exposure was largely attributed to the destruction of cell structure (Fig. 2c).

The abundance of ARGs under MIBK exposure

To reveal the influences of ketone exposure on the changes of ARGs level in E. coli, we determined the abundance of representative ARGs (ampR) under different concentrations of MIBK using qPCR (Fig. 3). The abundance of ampR in the control, low concentration (50 μg/L), and high concentration (50 mg/L) group were $3.15 \times 10^1$–$4.66 \times 10^2$, $3.23 \times 10^1$–$7.54 \times 10^3$, and $3.64 \times 10^1$–$5.61 \times 10^3$ copies/ngDNA, respectively. In detail, MIBK at the concentration of 50 μg/L enhanced the proliferation of ampR (0.22–70.40-fold) throughout the culturing process (except for 12 h and 168 h). Especially, the abundance of ampR markedly rose during 24 to 48 h. During this period, the ROS concentrations have increased by 0.58–0.59-fold (Fig. 1c). The overproduction of ROS has been found to induce multi-drug resistance in E. coli and promote the horizontal transfer of ARGs (Jin et al. 2018; Zhang et al. 2021). When exposed to the high concentration of MIBK (50 mg/L), the ampR abundance was enhanced by 0.22–142.39-fold compared to the control (except for 1 h and 12 h) (Fig. 3). In consistent with this result, the relative abundance of the intI1, sul1, and aadA2 genes increased by up to 1–2 orders of magnitude with exposure to PAHs at mg/L level (Wang et al. 2017a). In summary, the ampR abundance may exhibit distinctive behaviors upon...
exposure to different concentrations of MIBK (50 mg/L or 50 μg/L). Further studies are still needed to clarify the pertinent mechanisms for the patterns of ARGs in response to ketone.

The expression of ARGs under MIBK exposure

To further investigate the impacts of ketone on ARGs expression in *E. coli*, the abundance of efflux pump gene *acrA* along the MIBK exposure period was quantified (Fig. 4). Compared to the control, no significant difference in the *acrA* abundance was found under 50 μg/L MIBK exposure, whereas the *acrA* abundance increased by 1.90–253.23-fold at 50 mg/L MIBK exposure (Fig. 4). A similar trend was also observed in *ampR* abundance (Fig. 3). Therefore, we hypothesized that the over-expression of *acrA* may reduce the toxicity of solvents to cells and increase the *ampR* abundance when exposed to 50 mg/L MIBK. Current studies have shown that the changes of gene (especially efflux pump-related genes) expression have been implicated in the development of antibiotic resistance. After exposure to 100 mg/L of fluoxetine for 10 h, the expression amount of multidrug efflux genes (*acrB* and *acrD*) in *E. coli* was reported to increase by 2.50–2.80-fold (Jin et al. 2018). In addition, the multidrug efflux pump gene AcrAB-TolC was found to be preferentially enriched following ionic liquid [BMIm][PF6] exposure (Wang et al. 2020). Actually, AcrAB-TolC in *E. coli* could facilitate the expression of tetA and preserve the tetracycline-resistance acquisition via plasmid transfer (Nolivos et al. 2019). After chlorine exposure, the overproduction of drug efflux pump MexEF-OprN was validated to promote the antibiotic resistance in *Pseudomonas aeruginosa* (Hou et al. 2019), whereas the elevated expression of efflux pump gene norA may potentiate the evolution of antibiotic resistance in *Staphylococcus aureus* (Papkou et al. 2020). These data suggest that the efflux pump may serve as one of the major mechanisms for MIBK shaping the antibiotic resistance in *E. coli*. However, the pathways of gene expression regulating the evolution of antibiotic resistance under ketone exposure still deserve further attention.

The transformation of ARGs after MIBK treatment

Transformation is one of the essential pathways for the horizontal transfer of antibiotic resistance (Chen et al. 2005; Johnston et al. 2014). To explore the potential role of transformation for the spread of ARGs after ketone exposure, we evaluated the frequency of pUC118 plasmid transformed to competent *E. coli DH5α* under different concentrations of MIBK (Fig. 5). The transformation frequency of pUC118 following exposure to MIBK of less than 50 mg/L changed little as compared with the control. When exposed to MIBK of 50 mg/L, the transformation frequency of pUC118 was 5.7% higher than that in the control. Notably, the transformation frequency dramatically increased by 3.01-fold upon 100 mg/L MIBK exposure and remained a high value with 3.50-fold and 3.32-fold upon exposure to 200 mg/L and 300 mg/L MIBK, respectively. Likewise, triclosan at environmental concentrations (0.2–20 μg/L) markedly enhanced the transformation of plasmid-borne ARGs into *E. coli DH5α* for up to 1.40-fold (Lu et al. 2020). In contrast, PAHs such as phenanthrene reduced the transformation frequency of ampicillin resistance gene by 1.07–1.20 times (Kang et al. 2015). This means that the effects of organic matters on the transformation of ARGs may be concentration-dependent and type-dependent.

Considering the remarkable rise in the transformation frequency of pUC118 at the point of 100 mg/L MIBK exposure, the levels of resistant *E. coli, ampR, ROS, acrA*, and membrane permeability were measured to reveal
the underlying molecular mechanisms for the variations of antibiotic resistance in *E. coli* (Fig. 6). The growth of resistant *E. coli* was continuously inhibited and remained stable at a low concentration (1.00 × 10⁰–4.45 × 10¹ CFU/mL) (Fig. 6a). Similarly, the growth of living cells and ROS production was also suppressed (Fig. S3). These results may be explained by the severe disruption of the cell structure under extremely high concentrations (over 100 mg/L) of MIBK exposure. Meanwhile, the *ampR* abundance under 100 mg/L MIBK exposure was 2.42 × 10⁰–1.64 × 10¹ copies/ngDNA (Fig. 6b), which was much lower than that in the control test. In contrast, the efflux pump gene *acrA* was all overexpressed along the 100 mg/L MIBK exposure period (except 1 h) compared to the control (Fig. S3). These data suggest that MIBK over 100 mg/L can improve the transformation of resistance plasmid in *E. coli*, but inevitably inhibit the propagation of resistant *E. coli* and its ARGs.

**The mechanisms for the variations of antibiotic resistance upon MIBK exposure**

The underlying mechanisms for the antibiotic resistance in *E. coli* in respond to different levels of MIBK exposure are shown in Fig. 7. Exposure upon MIBK at environmental concentrations (50 μg/L) caused increased ROS levels, implying ROS production is a critical mechanism of MIBK inducing the growth of resistant *E. coli*. In addition, MIBK under the high concentration of MIBK (50 mg/L) could induce serious damage to cell structure, leading to a decrease in the concentration of resistant *E. coli*. Meanwhile, the up-regulated expression of *acrA* may also explain the proliferation of *ampR*. When exposed to 100 mg/L MIBK, the transformation frequency of pUC118 dramatically increased, whereas the massive death of *E. coli* inhibited the proliferation of *ampR*. In conclusion, the potential influencing mechanisms of MIBK on the antibiotic resistance in *E. coli* are concentration-dependent, and may
Conclusions

The present study demonstrated that MIBK at environmental concentration (50 μg/L) or stress concentration (50 mg/L) can result in an evident variation in the level of antibiotic resistance in E. coli. In addition, when exposed to MIBK greater than or equal 100 mg/L, the transformation frequency markedly elevated, while the amount of resistant E. coli and gene (ampR) decreased. This challenged the widely accepted conception that transformation process always facilitates the spread of ARGs. Overall, our study for the first time demonstrated that the MIBK could affect the spread of drug resistance in E. coli. Despite this, further studies should be conducted to deeply illustrate the conflict for the inconsistence of improving horizontal transformation and decreasing ampR abundance under the shock of high concentration (over 100 mg/L) of MIBK. Beside ROS, cell membrane permeability, and ARG expression, more indexes and advanced technologies (such as omics) are recommended to clarify the core mechanisms for ketone and other non-aromatic organics impacting on the variation of bacterial resistance.

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Data availability  The data that support the findings of this study are available from the corresponding author upon reasonable request.

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