Environment International 114 (2018) 280–287

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Petrol and diesel exhaust particles accelerate the horizontal transfer of plasmid-mediated antimicrobial resistance genes

Ye Zhang[a], April Z. Gu[b], Tianyu Cen[a], Xiangyang Li[a], Dan Li[a,*], Jianmin Chen[a]

[a] Shanghai Key Laboratory of Atmospheric Particle Pollution and Prevention (LAP^3), Department of Environmental Science and Engineering, Fudan University, Shanghai 200433, China

[b] School of Civil and Environmental Engineering, Cornell University, Ithaca, NY 14853, United States.

ARTICLE INFO

Keywords:
Petrol and diesel exhaust particles
Antimicrobial resistance
Conjugative transfer
ROS formation

ABSTRACT

Particles exhausted from petrol and diesel consumptions are major components of urban air pollution that can be exposed to human via direct inhalation or other routes due to atmospheric deposition into water and soil. Antimicrobial resistance is one of the most serious threats to modern health care. However, how the petrol and diesel exhaust particles affect the development and spread of antimicrobial resistance genes (ARGs) in various environments remain largely unknown. This study investigated the effects and potential mechanisms of four representative petrol and diesel exhaust particles, namely 97 octane petrol, 93 octane petrol, light diesel oil, and marine heavy diesel oil, on the horizontal transfer of ARGs between two opportunistic *Escherichia coli* (*E. coli*) strains, *E. coli* S17-1 (donor) and *E. coli* K12 (recipient). The results demonstrated that these four representative types of nano-scale particles induced concentration-dependent increases in conjugative transfer rates compared with the controls. The underlying mechanisms involved in the accelerated transfer of ARGs were also identified, including the generation of intracellular reactive oxygen species (ROS) and the consequent induction of oxidative stress, SOS response, changes in cell morphology, and the altered mRNA expression of membrane protein genes and those involved in the promotion of conjugative transfer. The findings provide new evidences and mechanistic insights into the antimicrobial resistance risks posed by petrol and diesel exhaust particles, and highlight the implications and need for stringent strategies on alternative fuels to mitigate air pollution and health risks.

1. Introduction

Particles exhausted from petrol and diesel consumptions in vehicle engines and stationary sources are major components of urban air pollution in China and worldwide (Xu et al., 2013; Hesterberg et al., 2010; MacIntyre et al., 2014; Jin et al., 2017). Nearly all these particles have dimensions < 1 μm ($PM_{1.0}$), and the vast majority of these are known as ultrafine particles (UFPs) with dimensions < 100 nm ($PM_{0.1}$) (Hesterberg et al., 2010; Durga et al., 2014). Numerous epidemiological studies and historical data indicate the severe health consequences of exposure to petrol and diesel exhaust particles, which are associated with the prevalence of respiratory tract infections, decreased lung function, and exacerbation of cardiovascular and cerebrovascular diseases (MacIntyre et al., 2014; Aguilera et al., 2013). Particularly, multidrug resistant pathogenic bacteria (also known as “superbugs”) are increasingly isolated from patients with respiratory tract infections during the haze weather, which can escalate the risks of worse clinical outcomes and even death (MacIntyre et al., 2014; WHO, 2014). In addition to the inhalation by human and animals, most of the petrol and diesel exhaust particles eventually deposit in the water and soil environments (He et al., 2014), which contain a high abundance and diversity of ARGs and antimicrobial resistant bacteria (ARB) (WHO, 2014; Andersson and Hughes, 2014; Bengtsson-Palme and Larsson, 2015; Chen et al., 2016; Zhu et al., 2013). However, how the petrol and diesel exhaust particles affects the development and dissemination of ARGs in various environments remain unknown at present.

Antimicrobial resistance is one of the most serious threats to modern health care (WHO, 2014; Q. Chen et al., 2015) and has been estimated to cause > 700,000 deaths yearly (O'Neill, 2014). The development and spread of antimicrobial resistance have been accelerated by the recruitment of ARGs into bacteria via de novo mutation (Andersson and Hughes, 2014; Lv et al., 2014) and by the horizontal transfer of mobile genetic elements (MGEs) (Andersson and Hughes, 2014; Bengtsson-Palme and Larsson, 2015), such as plasmids, transposons and integrons. Specifically, the horizontal transfer of ARGs was acknowledged as an important pathway to acquire and spread ARGs in various

* Corresponding author.
E-mail address: lidanfudan@fudan.edu.cn (D. Li).

https://doi.org/10.1016/j.envint.2018.02.038
Received 7 November 2017; Received in revised form 22 February 2018; Accepted 22 February 2018
Available online 08 March 2018
0160-4120/

environments (Bengtsson-Palme and Larsson, 2015; Alekshun and Levy, 2007). Previous evidences suggested that the enhanced intracellular ROS production and the SOS response induced by antibiotics, disinfectants and disinfection by-products (DBPs) can be important mechanisms for horizontal transfer of ARGs (Andersson and Hughes, 2014; Beaber et al., 2004; Zhang et al., 2017). Nanomaterials (Qiu et al., 2012), ionic liquids (Wang et al., 2015), and disinfectants (Zhang et al., 2017) were previously found to promote conjugative transfer by increasing cell membrane permeability and altering the expression of conjugation-related genes.

The physical and chemical properties of petrol and diesel exhaust particles, which are major contributors to the ultrafine particles in urban smog, have been extensively studied (Yadav et al., 2010; Zhang et al., 2015; Durga et al., 2014; Wu et al., 2017). Previous findings showed that petrol and diesel exhaust particles had nano-scale structures and consisted of a carbon core coated with organic chemicals and metals (Yadav et al., 2010; Zhang et al., 2015; Wu et al., 2017), which could induce the ROS-mediated cytotoxicity and genetic toxicity at varying degrees in both prokaryotic and eukaryotic cells (Wu et al., 2017; Verma et al., 2015; Gerlofs-Nijland et al., 2013; Durga et al., 2014). Therefore, we hypothesize that petrol and diesel exhaust particles can accelerate the horizontal transfer of ARGs among bacteria via generating the intracellular ROS, triggering oxidative stress and the SOS response, damaging cell membrane structures, and altering the expression of genes involved in the conjugative transfer of ARGs.

To test this hypothesis, this study systematically investigated the effects of particles exhausted from four different types of petrol and diesel oils on the conjugative ARGs transfer between *Escherichia coli* (*E. coli*) strains, which are important opportunistic pathogens and can cause intestinal and respiratory tract diseases (WHO, 2014; Chmielarczyk et al., 2014; Katouli, 2010). The underlying mechanisms responsible for the accelerated transfer of ARGs were also identified, including the changes of the intracellular ROS, cell morphology, cell membrane permeability, oxidative stress, SOS response, and the mRNA expression of conjugative transfer-related genes. The findings provide evidence and mechanistic insights into the antimicrobial resistance risks posed by petrol and diesel exhaust particles, and highlight the implications and the need for stringent strategies on utilizing alternative fuels to reduce air pollution.

2. Material and methods

2.1. Chemicals and reagents

Petrol and diesel fuels used in this study were bought from petrol and diesel station in Shanghai, China. All the chemicals used in present study were reagent-grade. Tween 80, tryptone, yeast extract, and sodium chloride (NaCl) were purchased from Sigma-Aldrich (Sigma-Aldrich, Shanghai, China). Agar was purchased from Fisher Scientific. Phosphate buffer saline (PBS; pH 7.4) and 2′, 7′-dichlorofluorescein diacetate (DCFH-DA) were obtained from Invitrogen (Invitrogen, Shanghai, China). Kanamycin (Km), chloromycetin (Chl), and thiourea (CH_4N_2S, TU) were supplied from TCI (TCI, Tokyo, Japan). RNAisoPlus kit (Cat. No. D9108A) and reverse transcription kit (Cat. No. 2680A) were purchased from TaKaRa (TaKaRa, Dalian, China).

2.2. Combustion experiments and particle sampling

Four types of particle samples were collected from the combustion of four different petrol and diesel fuels, including 97 octane petrol, 93 octane petrol, light diesel oil, and marine heavy diesel oil, in a laboratory-scale burning chamber, which was described in detail in our previous research (Wu et al., 2017). The raw samples were then size-reduced in a ball mill, sieved to 200 nm, and sterilized by X-ray irradiation. Then, these particles samples were suspended in deionized water containing 0.01% Tween 80 as the dispersant. The stock solutions of each sample were prepared at a concentration of 10,000 mg/L and diluted to proper concentrations for exposure. The ranges of particles concentrations were selected based on the $PM_{2.5}$ levels that occur in urban air environments in the field traffic emission systems (Xu et al., 2014). The particles samples were ultrasonicated for at least 30 min before each experiment to minimize aggregation. The analysis of morphology characterization, elemental compositions, and chemical components like polycyclic aromatic hydrocarbons (PAHs) and heavy metals were described in detail in our previous publication (Wu et al., 2017).

2.3. Bacterial strains, plasmid and culture conditions

The donor *E. coli* S17-1 harbors the transferable pCM184-Cm plasmid that is controlled by RP4 DNA segment residing in the host's chromosome (Zhang et al., 2017). Plasmid pCM184-Cm carries the ampicillin (Amp), tetracycline (Tet), and Chl resistance genes, origin of transfer (*oriT*) and replication origin (*oriC*), but not the operons that codes for the DNA processing machinery and produces mating pair formation functions (Tra1 operon and Tra2 operon) (F. Chen et al., 2015). The RP4 DNA segment carries functional Tra1 operon and Tra2 operon, which regulate the transfer of mobilizable plasmid pCM184-Cm (7625 bp) between the donor and recipient bacteria (Samuels et al., 2000). *E. coli* S17-1 was cultured in Luria–Bertani (LB; pH 7.4) medium prepared with 10 g/L of tryptone, 5 g/L of yeast extract and 10 g/L of NaCl, containing 20 mg/L of Chl. The recipient *E. coli* K12 MG1655 harbors an un-transferable pUA139 plasmid carrying the Km resistance genes, and it was grown in LB culture medium containing 100 mg/L of Km. The donor and the recipient strains were both incubated in 37 °C for approximately 16–18 h, with shaking at 200 rpm prior to the conjugation experiments. The concentrations of bacteria were determined using the LB agar plate counting method, which used LB agar plates containing 20 mg/L of Chl for *E. coli* S17-1 and 100 mg/L of Km for *E. coli* K12. LB agar plates containing 20 mg/L of Chl and 100 mg/L of Km were adopted to select, verify and count the transconjugants after the conjugation process.

2.4. Cytotoxicity of petrol and diesel exhaust particles

The growth inhibition effects of four types of petrol and diesel exhaust particles on the donor (*E. coli* S17-1) and recipient (*E. coli* K12) were evaluated. The overnight-grown cultures of *E. coli* S17-1 and *E. coli* K12 strains were exposed to different concentrations of particles samples along with controls (without particles exposure, incubation in LB medium only) for 4 h. The concentrations of bacteria after particles treatment and controls were determined using the LB agar plate counting method as described above. Growth inhibition rates were determined against the controls in triplicates.

2.5. Evaluation of the conjugative transfer efficiencies of ARGs upon exposure to petrol and diesel exhaust particles

In this study, we used the optimized conjugation model to evaluate the horizontal transfer of ARGs between two *E. coli* strains (Zhang et al., 2017). Briefly, the suspension of the donor (*E. coli* S17-1) and recipient (*E. coli* K12) was treated with serial concentrations of exhaust particles collected from combustion of four types of petrol and diesel as described above, along with controls (without particles treatment). Following a 4 h exposure at 37 °C, the transconjugants were determined by using LB plates containing both 20 mg/L of Chl and 100 mg/L of Km at 37 °C after 24 h incubation. The conjugative transfer efficiency was determined by dividing the number of transconjugants by the total number of recipients. The individual recipient concentrations treated with various concentrations of particles were determined by using LB agar plates containing 100 mg/L of Km, as described above.

2.6. Determination of intracellular ROS levels

The intracellular ROS production in the donor and recipient cells was measured by staining with DCFH-DA dye (Zhang et al., 2017). The bacterial mixed cultures, at a density of 10^6–10^7 CFU/mL, were incubated with 10 μM of DCFH-DA at 37 °C for 0.5 h, and gently shaken every 3–5 min. Following washing in PBS thrice and removing extracellular DCFH-DA, the bacterial cultures were exposed to four types of particles at various concentrations for 2 h at 37 °C. Then, each sample was transferred into a 96-well plate (200 μL per well), and the fluorescence intensity (FI; 488 nm/525 nm) was measured by a microplate reader (SynergyH1, Synergy HTMulti-Mode, Biotech, Winooski, VT, USA). The FI values of the treatment groups were divided by the values of the untreated control to indicate enhanced ROS formation as the "relatively elevated level". All of the samples and controls were performed in triplicate.

In addition, a separate ROS scavenging experiment was conducted using TU, a scavenger of ROS, where TU was supplemented to each bacterial culture at 100 mM of final concentrations together with the particles samples. The conjugative transfer rates in the presence of the ROS scavenger were compared with the experiments without it to confirm the role of ROS in the conjugation process and ARGs transfers.

2.7. Transmission electron microscope (TEM) analysis

The impacts of particles on cell membrane surface structures and morphology were evaluated by observing the interactions of particles and the cells via TEM (Qiu et al., 2012). The conjugative bacterial mixtures (10^8–10^9 CFU/mL) treated with petrol and diesel exhaust particles for 4 h, along with control mating mixtures without exposure to particles, were placed onto 400-mesh formvar-coated copper grids and left to dry for 10 min. Then, the samples were observed by a TEM (H-600 Electron Microscope, Hitachi, Japan) operating at 75 kV. Images were recorded by a CCD camera MegaView III (Olympus Soft Imaging Solutions GmbH, Johann-Krane-Weg 39, 48149 Münster, Germany) using the iTEM software from the same company.

2.8. The mRNA expression analysis by quantitative reverse transcription PCR (qRT-PCR)

Bacterial cells treated with diverse concentrations of petrol and diesel exhaust particles were gathered by centrifugation at 10,000 rpm for 15 min, and the total RNA was extracted from individual samples utilizing an RNAisoPlus kit. Then, cDNA was generated from the RNA template by an RT-PCR assay with a reverse transcription kit according to the manufacturer's instructions. The qPCR analysis was performed in a LightCycler480 instrument (Roche, Basel, Switzerland) to quantify the expressions of the 15 genes involved in SOS response (*lexA*, *recA*, and *umuD*), oxidative stress (*soxS*, *soxR*, and *oxyR*), and cell outer membrane proteins (*ompA* and *ompC*), as well as conjugative transfer related genes involved in the global regulator genes of horizontal transfer (*korA*, *korB*, and *trbA*), mating pair formation (Mpf) system genes (*trbBp* and *traF*) and DNA transfer and replication (Dtr) system genes (*trfAp* and *traJ*). The 16S rRNA was also tested as an amplification internal control. The PCR primers are presented in Table S1, and the detailed information about the qRT-PCR mixtures and protocols were reported in Zhang et al. (2017).

2.9. Statistical analysis

One-way analysis of variance (ANOVA) and independent sample *t*-tests were run on SPSS 23.0 (IBM-SPSS, Chicago, IL, USA) to determine statistical significance. A result was considered significant if $P < 0.05$, highly significant if $P < 0.01$, and extremely significant if $P < 0.001$, according to the previous studies (Qiu et al., 2012).

3. Results and discussion

3.1. Cytotoxicity of petrol and diesel exhaust particles

Fig. S1 shows the dose-dependent growth inhibition curves of the donors and recipients upon exposure to various concentrations of particles (from 5 to 160 mg/L). The results showed that the comparative cytotoxicity levels of the particles generated from the petrol and diesel sources varied depending on the *E. coli* strain. For example, particles from light diesel oil exhibited the lowest level of cytotoxicity to *E. coli* S17-1, but second highest cytotoxicity level to *E. coli* K12. Particles emitted from the combustion of 97 octane petrol seemed to exert the highest inhibition effect on the growth of both recipient *E. coli* K12 and donor *E. coli* S17-1 strain (Fig. S1). This may be due to the higher contents of important heavy metals and polycyclic aromatic hydrocarbons (PAHs) in particles emitted from 97 octane petrol, compared with other three different petrol and diesel fuels (Wu et al., 2017). Petrol and diesel exhaust particles contain a myriad of inorganic and organic compounds, among which only a very small fraction have been identified (Yadav et al., 2010; Wu et al., 2017). Our previous analysis results indicated that the particles samples used in the present study mostly have dimensions < 100 nm (Fig. 4f), and were mainly composed of black carbon, basic elements (e.g., C, O, S, K, and Si), metallic elements (e.g., Fe, Mn, Cr, V, and Ni), and organic compounds, such as PAHs (e.g., naphthalene, acenaphthylene, acenaphthene, fluorene, and phenanthrene) (Wu et al., 2017).

3.2. Effects of petrol and diesel exhaust particles exposure on conjugative transfer frequency

To determine whether the fine particles could facilitate the horizontal ARGs transfer, we evaluated the impact of the four types of petrol and diesel exhaust particles on the conjugative transfer of ARGs from *E. coli* S17-1 to *E. coli* K12. The results showed that all the petrol and diesel exhaust particles induced concentration-dependent (5–160 mg/L) increases in conjugative transfer rates by 2.2–5.3, 1.4–2.0, 2.0–5.1 and 1.2–2.4 folds compared with the controls, which were emitted from the combustions of 97 octane petrol, 93 octane petrol, light diesel oil, and marine heavy diesel oil, respectively (Fig. 1).

For all types of petrol and diesel exhaust particles evaluated, the conjugative transfer efficiencies declined at the exposure concentration of 320 mg/L (Fig. 1). These results can be explained by the high concentrations (320 mg/L) of particles exposure, which resulted in the inactivation of the donor and recipient (Fig. S1). This finding was consistent with the previous findings that inhibitory levels of disinfectants, antibiotics, and antibacterial biocides caused the low conjugative transfer frequency by inactivation of the bacteria in the mating system (Zhang et al., 2017; Jutkina et al., 2017). Furthermore, high concentrations of particles interfered with cell-to-cell contact by adsorbing onto the whole surface of both donor and recipient cells, resulting in the decreased conjugative transfer efficiencies. The TEM results also indicated that the *E. coli* cells were covered with particles at concentrations of 320 mg/L (data not shown).

It is also notable that particles emitted from 97 octane petrol and light diesel oil combustions led to higher transfer frequencies than those emitted from 93 octane petrol and marine heavy diesel oil combustions ($P = 0.0003$, independent sample *t*-test) (Fig. 1). This may be due to the different chemical and toxic effects of these four types of petrol and diesel exhaust particles (Wu et al., 2017). It is recognized that the particles emission from automobiles and stationary combustion sources are highly sensitive to engine type, operating conditions, injector design, fuel types and road conditions (Goel and Guttikunda, 2015). Although the laboratory-based measurements may not represent all the real-world emissions (Choudhary and Gokhale, 2016), most research studied certain specific laboratory-conditions to simulate the real-world vehicle emissions and stationary combustions (Chang et al., 2004;

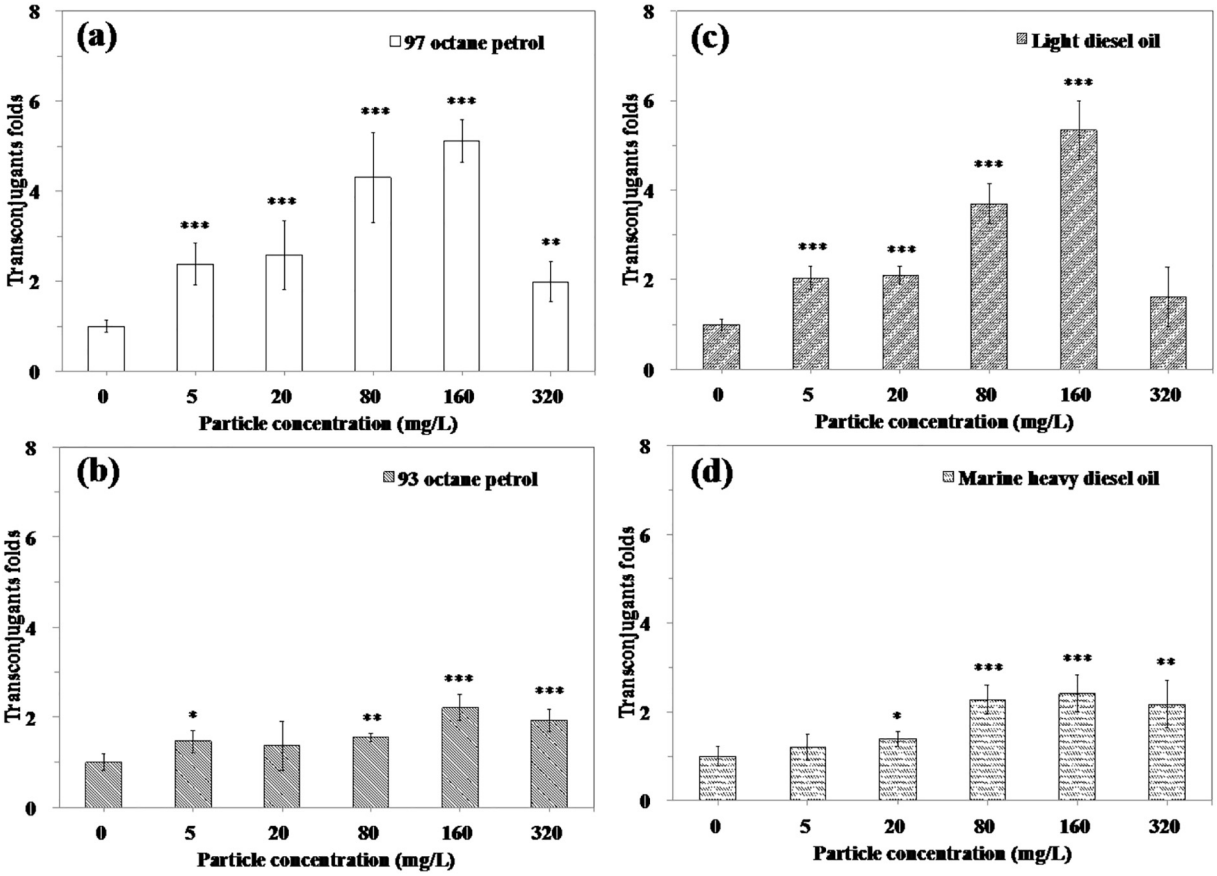

Fig. 1. Impacts of particles emitted from combustions of 97 octane petrol (a), 93 octane petrol (b), light diesel oil (c), and marine heavy diesel oil (d) on the conjugative transfer from *E. coli* S17-1 to *E. coli* K12. All particles samples had significant impacts on the conjugative ARGs transfer (ANOVA, $P < 0.05$); significant differences between individual particle-treated groups and the control group without particles treatment were tested with independent sample *t*-test and shown with * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

Borrás et al., 2009). In this study, four typical petrol and diesel fuels were selected to represent those widely used in China (Wu et al., 2017). Even though the combustion conditions were different from the real automobiles and stationary combustion, the morphology and major chemical components of diesel and petrol exhaust particles in the present study were similar to the $PM_{2.5}$ samples collected from urban air (Goel and Guttikunda, 2015; Fu et al., 2012). Thus, the present results imply that $PM_{2.5}$ in urban air potentially can enhance the spread of ARGs between *E. coli*.

3.3. Mechanisms involved in enhanced conjugative transfer by petrol and diesel exhaust particles

3.3.1. Intracellular ROS formation induced by petrol and diesel exhaust particles

Intracellular ROS, causing oxidative stress, are highly reactive molecules, such as superoxide ($O_2\cdot$), nonspecific hydroxyl radicals ($\cdot OH$) and hydrogen peroxide (H_2O_2), which can damage numerous biomolecules and therefore disrupt a series of cellular processes (Imlay, 2003). In this study, concentration-dependent increases in intracellular ROS levels were found in the donors and recipients upon exposure to four types of particles emitted from petrol and diesel combustions (Fig. 2a). Importantly, statistically significant positive correlations between fold changes of transconjugant and moderate ROS formation levels (1–2.5 folds) were observed for the treatment of 5–160 mg/L of particles emitted from the combustions of 97 octane petrol ($R^2 = 0.96$), 93 octane petrol ($R^2 = 0.96$), light diesel oil ($R^2 = 0.95$), and marine heavy diesel oil ($R^2 = 0.82$) (Fig. 2b). Obviously, the slopes of the correlation curves varied among the different types of particles samples (Wu et al., 2017). The results indicated that exceeding levels of ROS production induced by the particles at the highest concentration (320 mg/L) likely resulted in serious oxidative damage and cell death, which consequently decreased the conjugative transfer frequency, similar to the observations previously reported (Zhang et al., 2017; Simon et al., 2000). Several research studies have provided strong evidence that transition metals and redox cycling organic components in fine particles could elicit ROS production, which is closely associated with lipid peroxidation, oxidative stress, as well as alterations in cell structures (Gerlofs-Nijland et al., 2013; Durga et al., 2014). The multifarious metals and PAHs detected in particles derived from petrol and diesel combustion were assumed to play important roles in increasing ROS (Wu et al., 2017; Valavanidis et al., 2008).

Furthermore, the ROS scavenging experiment with TU showed a reduced number of transconjugants with the supplementation of TU in comparison to those without the TU addition (Fig. S2), suggesting that moderate ROS levels induced by particles exposure were likely responsible for the promotion of conjugative ARGs transfer.

3.3.2. Induction of oxidative stress and SOS response by petrol and diesel exhaust particles

Quantitative real-time PCR was performed to detect the mRNA expression levels of critical genes related to oxidative stress (*soxS*, *soxR*, and *oxyR*) and SOS response (*lexA*, *recA*, and *umuD*) pathways, both of which have been reported to play important roles in the horizontal transfer of ARGs and chromosome mutation (Andersson and Hughes, 2014; Beaber et al., 2004; Qiu et al., 2012; Zhang et al., 2017). As shown in Figs. 3 and S3, the mRNA expression levels of genes (*soxS*, *soxR*, *oxyR*, *recA*, *lexA*, and *umuD*) involved in oxidative stress and SOS response pathways were significantly increased when exposed to 5–160 mg/L of particles originating from petrol and diesel combustion

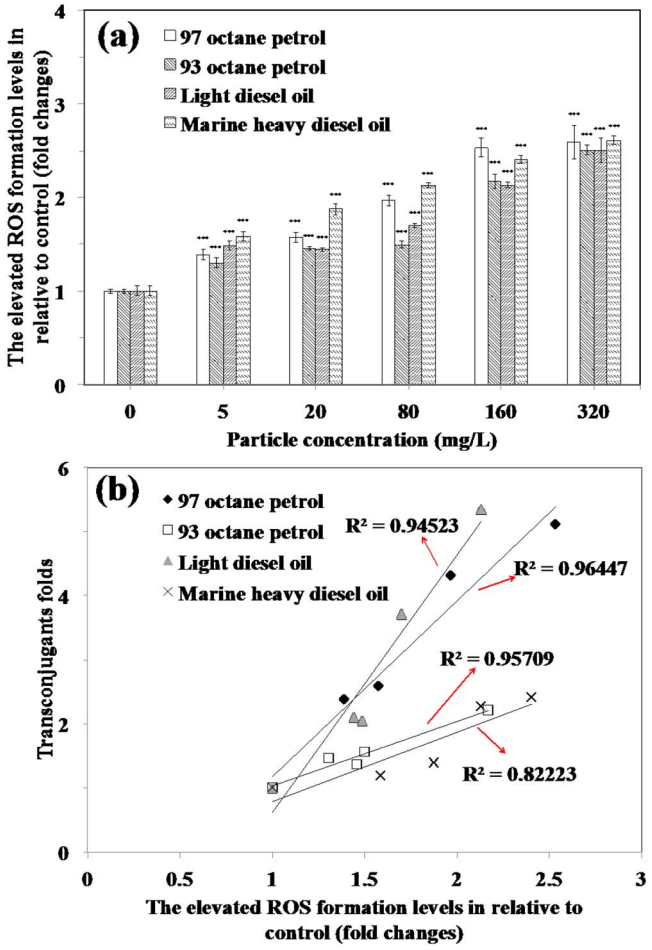

Fig. 2. Petrol and diesel exhaust particles increase intracellular ROS formation and correlate with conjugative gene transfer. (a) The elevated ROS formation levels compared to control (fold changes) during conjugative transfer induced by particles emitted from combustions of 97 octane petrol, 93 octane petrol, light diesel oil, and marine heavy diesel oil. (b) Correlations between fold changes in transconjugant in relative to control and the elevated ROS formation levels (fold changes) during conjugative transfer for the treatments described in (a). All particles had significant impacts on the ROS formation in the donor and recipient bacteria (ANOVA, $P < 0.05$); significant differences in ROS formation between individual particle-exposed groups and the non-exposed control group were tested with an independent sample *t*-test and are shown with * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

in comparison to the control.

SoxRS and OxyR are the major transcriptional regulators that mediate the physiological signal monitoring and antioxidant defense system in *E. coli* (Pomposiello and Demple, 2001; Kabir and Shimizu, 2006). The activation of SoxR enhances the transcription of gene *soxS*, which produces the SoxS protein to further increase the resistance to oxidants and antibiotics (Kabir and Shimizu, 2006). The present results indicated that mRNA expression levels of *soxS*, *soxR* and *oxyR* genes were significantly increased when exposed to 5–160 mg/L of particles (Figs. 3 and S3), which may have contributed to the enhanced conjugative transfer (Fig. 1).

Previous evidences suggested that the initiated SOS response could stimulate horizontal gene transfer due to the influence on the expression of conjugation-related genes and conjugational (i.e., interchromosomal) recombination (Andersson and Hughes, 2014; Beaber et al., 2004; Zhang et al., 2017; Cambray et al., 2011). The present results also showed that particles from various petrol and diesel sources could produce intracellular ROS (Fig. 2) and consequently induce oxidative stress and SOS response (Fig. 3). *RecA* and *lexA* genes are up-regulated when the SOS response is activated (McCool et al., 2004). Similarly, the mRNA expression levels of genes (*recA*, *lexA*, and *umuD*) involved in SOS response were up-regulated with exposure to 5–160 mg/L of the particles compared with the controls (Figs. 3 and S3), which consequently mediated the enhancement of conjugative transfer.

3.3.3. Impact of petrel and diesel exhaust particles on cellular membrane permeability

Increased membrane permeability of bacteria is a crucial factor for promoting the horizontal transfer of ARGs (Alekshun and Levy, 2007; Qiu et al., 2012). To determine if particles could promote the horizontal transfer of plasmid encoded antibiotic resistance via their impact on membrane structure and permeability, the interaction of particles and the donor and recipient cells was observed by TEM. The membrane structure of *E. coli* cells without exposure to particles was intact and smooth (Fig. 4a). The TEM photomicrographs (Fig. 4b–f) showed that the soots with various diameters (< 100 nm) were absorbed onto the *E. coli* cell surface, and the surrounding areas had spherical primary particles and fractal-like chain structures, which were the dominant composition in all particles samples in the present study (Wu et al., 2017). Compared with normal bacterial cells, the bacterial outer structure was damaged and formed pores after particle samples treatment (Fig. 4b–e), which might facilitate the resistance plasmids transfer among the bacterial cells. Previous reports proposed that petrol and diesel exhaust particles enriched with chemical components, such as organic chemicals and metals, because of their fine and ultrafine compositions, were predominantly associated with oxidative cellular damage and membrane lipid peroxidation (Valavanidis et al., 2008; Knuckles and Dreher, 2007).

The cell membrane permeability is tightly associated with two outer membrane proteins (OMPs), namely, OmpA and OmpC (Zhang et al., 2017; Wang et al., 2015). The mRNA expression levels of *ompA* and *ompC* genes were significantly affected by particles originating from 97 octane petrol, 93 octane petrol, light diesel oil and marine heavy diesel combustions, which were up to 1.8–6.3, 1.3–2.0, 1.8–2.9 and 1.2–1.8 folds compared to the controls, respectively (Figs. 3 and S4). The facilitating roles of particles emitted from 97 octane petrol and light diesel oil combustion, as indicated by their impact on mRNA expression levels of OMP genes (*ompA* and *ompC*), were distinctively higher than the particles emitted from 93 octane petrol and marine heavy diesel oil combustions (Figs. 3 and S4). The subsequent enhanced OmpC and OmpA could augment membrane permeability via pore forming and the membrane transport of plasmids carrying ARGs (Wang et al., 2015). Interestingly, the particles source-dependent and dose-dependent trends were observed in the changes in the *ompA* and *ompC* gene expression levels among the various particles (Figs. 3 and S4). Below a certain threshold, particles led to elevated expression of *ompA* and *ompC* genes, along with the increase of PM concentrations, and above which the effect would diminish (Fig. S4), likely due to the adverse influence of the excessive PM-induced ROS and oxidative stress on the OMPs of the donors and recipients.

3.3.4. The expression of conjugative transfer-related genes

As the donor, *E. coli* S17-1 harbors the transferable pCM184-Cm plasmid that is manipulated by the broad-host-range RP4 located on the host's chromosome and containing a series of conjugative transfer-related genes. Changes in the expressions of DNA-transfer-and-replication (Dtr) genes (*trfAp* and *traJ*) were associated with the mating-pair formation (Mpf) genes (*trbBp* and *traF*) with their corresponding involvement in intra- or inter-genera conjugative transfer (Qiu et al., 2012; Zhang et al., 2017; Wang et al., 2015).

Dtr genes include three operons and the origin of transfer (*oriT*), an intergenic region between the leader and the relaxase operons, and the Dtr functions participate in the initiation of the complicated transfer-replication process (Burton and Dubnau, 2010; Giusti et al., 2012). The particles sources and dose-dependent changes in mRNA expression of

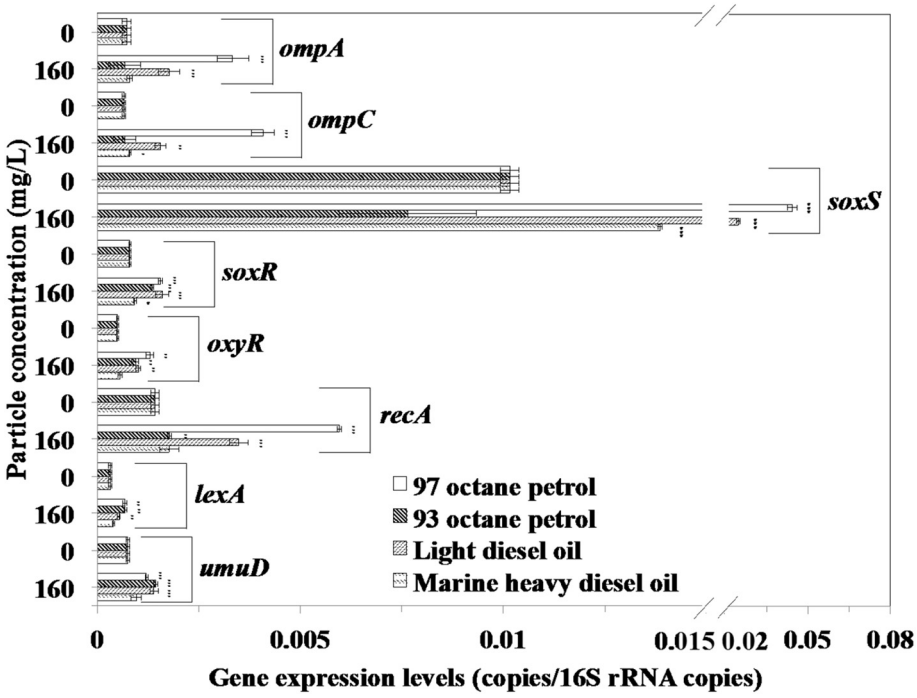

Fig. 3. Impacts of particles emitted from combustions of 97 octane petrol, 93 octane petrol, light diesel oil, and marine heavy diesel oil on the mRNA expression levels of outer membrane protein genes (*ompA* and *ompC*), oxidative stress-related genes (*soxS*, *soxR*, and *oxyR*), and SOS response-related genes (*lexA*, *recA*, and *umuD*) during conjugative transfer from *E. coli* S17-1 to *E. coli* K12. Significant differences in the gene expression levels between individual particle-exposed groups and the non-exposed control group were tested with independent-sample *t*-tests and are shown with * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

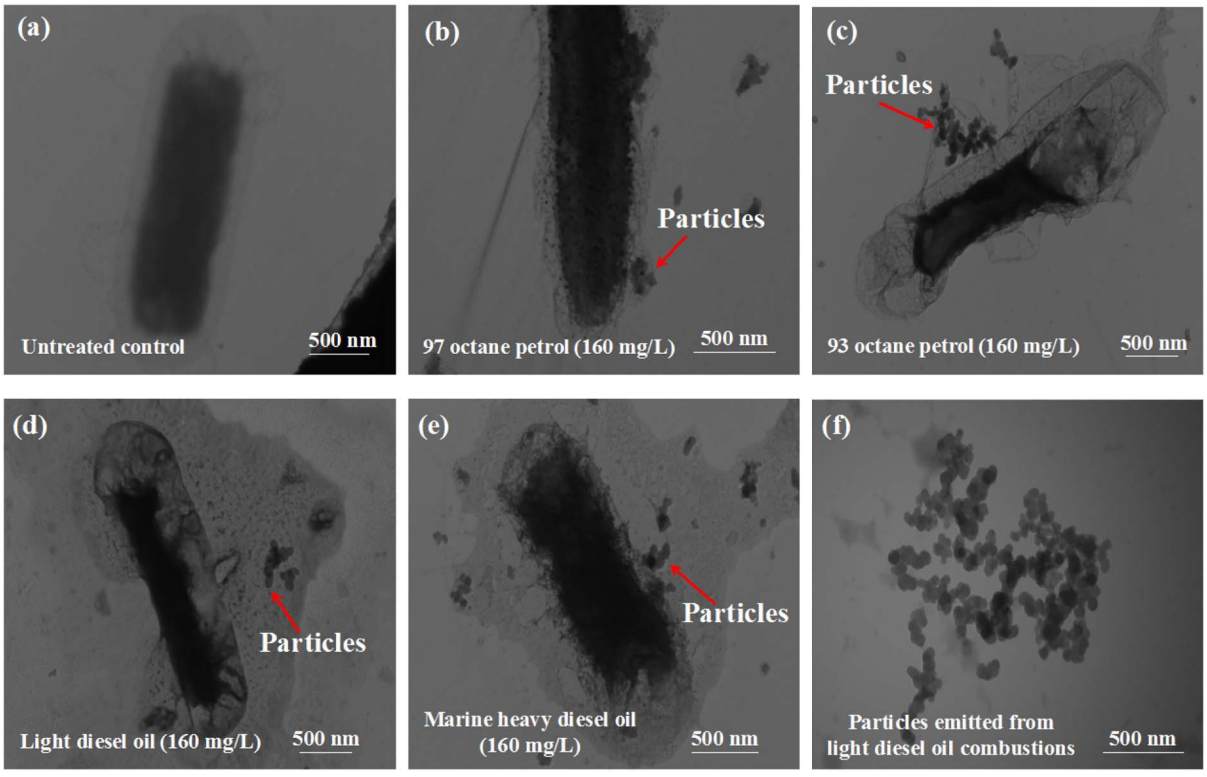

Fig. 4. TEM images of *E. coli* in the control group (no particles exposure) (a) and after exposure to particles emitted from the combustions of 97 octane petrol (b), 93 octane petrol (c), light diesel oil (d), and marine heavy diesel oil (e), and the particles emitted from light diesel oil combustions (f). The arrows show the presences of particles.

Dtr genes (*trfAp* and *traJ*) are shown in Figs. 5 and S5. For example, the mRNA expression levels of *trfAp* and *traJ* genes were significantly increased due to exposure to particles emitted from 97 octane petrol and light diesel oil combustion, by up to 2.7 and 2.5 folds compared to the controls, respectively (Figs. 5 and S5). Gene *trfA*, which is essential for the replication gene operon, was observed to be up-regulated at an increasing level as the particles concentration increased (Figs. 5 and S5). TraJ, the product of the *traJ* gene, binds to the *oriT* and is involved in forming the relaxosome that triggers the strand-specific nick in circular plasmids (Ziegelin et al., 1989). Particles generally exhibited stronger effects on the up-regulation of the expression of *trfA* gene than those of the *traJ* gene, both of which belong to the Dtr system.

The Mpf system is conducive to the emergence of membrane-related proteins and channels, which are conjugative channels for single-stranded DNA transfer (Burton and Dubnau, 2010; Grahn et al., 2000). The mRNA expression levels of Mpf gene *trbBp* were significantly

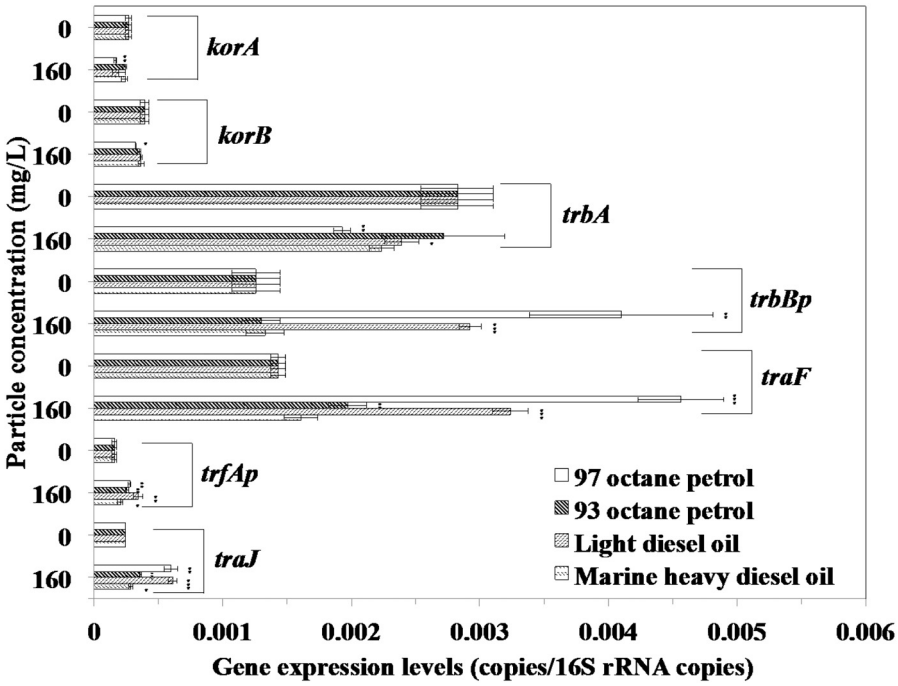

Fig. 5. Impacts of particles emitted from combustions of 97 octane petrol, 93 octane petrol, light diesel oil, and marine heavy diesel oil on the mRNA expression levels of the global regulatory genes (*korA*, *korB*, and *trbA*) and conjugative genes (*trbBp*, *traF*, *trfAp* and *traJ*) during conjugative transfer from *E. coli* S17-1 to *E. coli* K12. Significant differences in the gene expression levels between individual particle-exposed groups and the non-exposed control group were tested with independent-sample *t*-tests and are shown with * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

affected by particles originating from 97 octane petrol and light diesel oil combustion ($P = 0.0026$; $P = 0.0002$, independent sample *t*-test), by up to 3.3 and 2.3 folds compared to the controls, respectively (Figs. 5 and S5). Similarly, compared to the control samples, up to a 3.2-fold increase in the expression levels of *traF* gene was observed after exposure to particles generated from petrol and diesel combustion, especially 97 octane petrol and light diesel oil (Figs. 5 and S5b). TraF is one of the pilus assembly proteins, and its expression levels are positively associated with horizontal gene transfer among bacterial populations (Grahn et al., 2000).

As shown in Figs. 5 and S6, the presence of particles also affected the expression of three well-known global regulatory genes, namely, *kor*A, *kor*B, and *trb*A, which play vital roles in the conjugal transfer, replication, and maintenance of the mobilizable plasmid (Qiu et al., 2012; Zhang et al., 2017; Wang et al., 2015). Specifically, particles led to the down-regulation of genes *korA*, *korB*, and *trbA* by 16.4%, 14.6%, and 16.3%, respectively, compared to the controls. Previous research proved that the repressed expression of *korA* and *korB* genes can activate the *trfAp* gene, and the simultaneous down-regulation of both *korB* and *trbA* genes can stimulate the expression of the *trbBp* gene (Qiu et al., 2012). Up-regulation of both *trfAp* and *trbBp* genes were shown to facilitate the conjugative ARGs transfer and transconjugant formation (Qiu et al., 2012).

4. Environmental implications

There are growing concerns of fine particles pollution and antimicrobial resistance, which poses risks to public health worldwide (Gandolfi et al., 2011; Pal et al., 2016; McEachran et al., 2015). Our laboratory scale study demonstrated that petrol and diesel exhaust particles significantly promoted the horizontal transfer of ARGs between two *E. coli* stains. Although the particles samples used in present study were emitted from the laboratory-scale burning chamber, the morphology and principal chemical components of these samples were similar to that emitted from the field engine work or collected from urban air (Wu et al., 2017; Fu et al., 2012; Riddle et al., 2007). Therefore, we predicted that the fine particles in urban atmospheric environment are likely to promote the frequency of horizontal transfer of ARGs among bacteria.

The underlying mechanisms for the increased conjugative transfer of ARGs between *E. coli* caused by petrol and diesel exhaust particles were systematically explored. The intracellular ROS, oxidative stress, and SOS response in conjugative mating were induced to varying degrees following exposure to particles, which was suspected to be triggered by the diverse chemical constituents of particles, such as metals and PAHs (Wu et al., 2017; Verma et al., 2015; Gerlofs-Nijland et al., 2013). The nano-scale particles would interact with bacterial cells to affect cell membranes and pores, which consequently affected the cell membrane permeability, as indicated by the up-regulation of two permeability-related OMPs genes (*ompA* and *ompC*). Furthermore, the induction of SOS response presumably impacted the interchromosomal recombination and the expression of conjugation-related genes, consequently promoting horizontal gene transfer (Andersson and Hughes, 2014).

Our results have important implications for the possibility that atmospheric PM pollutants may exacerbate ARGs transfer among pathogenic bacteria. This could further cause adverse effects on clinical drug resistance and public health, as well as aggravating the antimicrobial resistance diffusion in waterbodies and soils via $PM_{2.5}$ particles from atmospheric deposition. Furthermore, this study provides a novel evaluation method to further our understanding of the ecological effect of particles on antimicrobial resistance dissemination, and implies the urgency of strengthening efficacious policy for controlling airborne PM pollution.

Notes

The authors declare no competing financial interest.

Acknowledgements

The content is solely the responsibility of the authors. This work was supported by grants from the National Natural Science Foundation of China (No. 91643106, 21477024, 21527814, and 21507012), United States National Science Foundation (NSF, CBET-1437257, CBET-1810769, IIS-1546428), National Institute of Environmental Health Sciences (NIEHS, PROTECT 3P42ES017109 and CRECE 1P50ES026049) and Environmental Protection Agency (EPA, CRECE

83615501).

Appendix A. Supplementary data

The Supporting Information is available free of charge on the ACS Publications website at https://doi.org/10.1016/j.envint.2018.02.038. Table S1 and Figs. S1–S6.

References

Aguilera, I., Pedersen, M., Garcia-Esteban, R., Ballester, F., Basterrechea, M., Esplugues, A., Fernandez-Somoano, A., Lertxundi, A., Tardon, A., Sunyer, J., 2013. Early-life exposure to outdoor air pollution and respiratory health, ear infections, and eczema in infants from the INMA study. Environ. Health Perspect. 121 (3), 387–392.

Alekshun, M.N., Levy, S.B., 2007. Molecular mechanisms of antibacterial multidrug resistance. Cell 128 (6), 1037–1050.

Andersson, D.I., Hughes, D., 2014. Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol. 12 (7), 465–478.

Beaber, J.W., Hochhut, B., Waldor, M.K., 2004. SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature 427 (6969), 72–74.

Bengtsson-Palme, J., Larsson, D.G.J., 2015. Antibiotic resistance genes in the environment: prioritizing risks. Nat. Rev. Microbiol. 13 (6).

Borrás, E., Tortajada-Genaro, L.A., Vázquez, M., Zielinska, B., 2009. Polycyclic aromatic hydrocarbon exhaust emissions from different reformulated diesel fuels and engine operating conditions. Atmos. Environ. 43 (37), 5944.

Burton, B., Dubnau, D., 2010. Membrane-associated DNA transport machines. Cold Spring Harb. Perspect. Biol. 2 (7), a000406.

Cambray, G., Sanchez-Alberola, N., Campoy, S., Guerin, E., Da Re, S., Gonzalez-Zorn, B., Ploy, M.C., Barbe, J., Mazel, D., Erill, I., 2011. Prevalence of SOS-mediated control of integron integrase expression as an adaptive trait of chromosomal and mobile integrons. Mob. DNA 2 (1), 6.

Chang, M.C., Chow, J.C., Watson, J.G., Hopke, P.K., Yi, S.M., England, G.C., 2004. Measurement of ultrafine particle size distributions from coal-, oil-, and gas-fired stationary combustion sources. J. Air Waste Manage. Assoc. 54 (12), 1494–1505.

Chen, F., Cao, Y.J., Wei, S., Li, Y.Z., Li, X.Y., Wang, Q., Wang, G.J., 2015a. Regulation of arsenite oxidation by the phosphate two-component system PhoBR in *Halomonas* sp. HAL1. Front. Microbiol. 6, 923.

Chen, Q., Andersson, A., Mecklenburg, M., Xie, B., 2015b. A biosensing strategy for the rapid detection and classification of antibiotic resistance. Biosens. Bioelectron. 73 (412–413), 251–255.

Chen, Q., An, X., Li, H., Su, J., Ma, Y., Zhu, Y.G., 2016. Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. Environ. Int. 92-93, 1–10.

Chmielarczyk, A., Wojkowska-Mach, J., Romaniszyn, D., Adamski, P., Helwich, E., Lauterbach, R., Pobiega, M., Borszewska-Kornacka, M., Gulczynska, E., Kordek, A., Heczko, P.B., 2014. Mode of delivery and other risk factors for *Escherichia coli* infections in very low birth weight infants. BMC Pediatr. 14.

Choudhary, A., Gokhale, S., 2016. Urban real-world driving traffic emissions during interruption and congestion. Transport. Res. D-Tr. E. 43, 59–70.

Durga, M., Nathiya, S., Rajasekar, A., Devasena, T., 2014. Effects of ultrafine petrol exhaust particles on cytotoxicity, oxidative stress generation, DNA damage and inflammation in human A549 lung cells and murine RAW 264.7 macrophages. Environ. Toxicol. Pharmacol. 38 (2), 518–530.

Fu, H., Zhang, M., Li, W., Chen, J., Wang, L., Quan, X., Wang, W., 2012. Morphology, composition and mixing state of individual carbonaceous aerosol in urban Shanghai. Atmos. Chem. Phys. 12 (2), 693–707.

Gandolfi, I., Franzetti, A., Bertolini, V., Gaspari, E., Bestetti, G., 2011. Antibiotic resistance in bacteria associated with coarse atmospheric particulate matter in an urban area. J. Appl. Microbiol. 110 (6), 1612–1620.

Gerlofs-Nijland, M.E., Totlandsdal, A.I., Tzamkiozis, T., Leseman, D.L.A.C., Samaras, Z., Lag, M., Schwarze, P., Ntziachristos, L., Cassee, F.R., 2013. Cell toxicity and oxidative potential of engine exhaust particles: impact of using particulate filter or biodiesel fuel blend. Environ. Sci. Technol. 47 (11), 5931–5938.

Giusti, M.D., Pistorio, M., Lozano, M.J., Tejerizo, G.A.T., Salas, M.E., Martini, M.C., Lopez, J.L., Draghi, W.O., Del Papa, M.F., Perez-Mendoza, D., Sanjuan, J., Lagares, A., 2012. Genetic and functional characterization of a yet-unclassified rhizobial Dtr (DNA-transfer-and-replication) region from a ubiquitous plasmid conjugal system present in *Sinorhizobium meliloti*, in *Sinorhizobium medicae*, and in other nonrhizobial Gram-negative bacteria. Plasmid 67 (3), 199–210.

Goel, R., Guttikunda, S.K., 2015. Evolution of on-road vehicle exhaust emissions in Delhi. Atmos. Environ. 105, 78–90.

Grahn, A.M., Haase, J., Bamford, D.H., Lanka, E., 2000. Components of the RP4 conjugative transfer apparatus form an envelope structure bridging inner and outer membranes of donor cells: implications for related macromolecule transport systems. J. Bacteriol. 182 (6), 1564–1574.

He, C., Salonen, H., Ling, X., Crilley, L., Jayasundara, N., Cheung, H.C., Hargreaves, M., Huygens, F., Knibbs, L.D., Ayoko, G.A., Morawska, L., 2014. The impact of flood and post-flood cleaning on airborne microbiological and particle contamination in residential houses. Environ. Int. 69, 9–17.

Hesterberg, T.W., Long, C.M., Lapin, C.A., Hamade, A.K., Valberg, P.A., 2010. Diesel exhaust particulate (DEP) and nanoparticle exposures: what do DEP human clinical studies tell us about potential human health hazards of nanoparticles? Inhal. Toxicol. 22 (8), 679–694.

Imlay, J.A., 2003. Pathways of oxidative damage. Annu. Rev. Microbiol. 57, 395–418.

Jin, L., Luo, X.S., Fu, P.Q., Li, X.D., 2017. Airborne particulate matter pollution in urban China: a chemical mixture perspective from sources to impacts. Natl. Sci. Rev. 4, 593–610.

Jutkina, J., Marathe, N.P., Flach, C.-F., Larsson, D.G.J., 2017. Antibiotics and common antibacterial biocides stimulate horizontal transfer of resistance at low concentrations. Sci. Total Environ. 616–617 (11 − 12), 172–178.

Kabir, M.M., Shimizu, K., 2006. Investigation into the effect of soxR and soxS genes deletion on the central metabolism of *Escherichia coli* based on gene expressions and enzyme activities. Biochem. Eng. J. 30 (1), 39–47.

Katouli, M., 2010. Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections. Iran J. Microbiol. 2 (2), 59–72.

Knuckles, T.L., Dreher, K.L., 2007. Fine oil combustion particle bioavailable constituents induce molecular profiles of oxidative stress, altered function, and cellular injury in cardiomyocytes. J. Toxicol. Environ. Health. A. 70 (21), 1824–1837.

Lv, L., Jiang, T., Zhang, S.H., Yu, X., 2014. Exposure to mutagenic disinfection byproducts leads to increase of antibiotic resistance in *Pseudomonas aeruginosa*. Environ. Sci. Technol. 48 (14), 8188–8195.

MacIntyre, E.A., Gehring, U., Molter, A., Fuertes, E., Klumper, C., Kramer, U., Quass, U., Hoffmann, B., Gascon, M., Brunekreef, B., Koppelman, G.H., Beelen, R., Hoek, G., Birk, M., de Jongste, J.C., Smit, H.A., Cyrys, J., Gruzieva, O., Korek, M., Bergstrom, A., Agius, R.M., de Vocht, F., Simpson, A., Porta, D., Forastiere, F., Badaloni, C., Cesaroni, G., Esplugues, A., Fernandez-Somoano, A., Lerxundi, A., Sunyer, J., Cirach, M., Nieuwenhuijsen, M.J., Pershagen, G., Heinrich, J., 2014. Air pollution and respiratory infections during early childhood: an analysis of 10 European birth cohorts within the ESCAPE project. Environ. Health Perspect. 122 (1), 107–113.

McCool, J.D., Long, E., Petrosino, J.F., Sandler, H.A., Rosenberg, S.M., Sandler, S.J., 2004. Measurement of SOS expression in individual *Escherichia coli* K-12 cells using fluorescence microscopy. Mol. Microbiol. 53 (5), 1343–1357.

McEachran, A.D., Blackwell, B.R., Hanson, J.D., Wooten, K.J., Mayer, G.D., Cox, S.B., Smith, P.N., 2015. Antibiotics, bacteria, and antibiotic resistance genes: aerial transport from cattle feed yards via particulate matter. Environ. Health Perspect. 123 (4), 337–343.

O'Neill, J., 2014. Antimicrobial Resistance: Tackling a Crisis for the Future Health and Wealth of Nations. http://amr-review.org/sites/default/files/AMR (Review Paper - Tackling a crisis for the health and wealth of nations_1.pdf).

Pal, C., Bengtsson-Palme, J., Kristiansson, E., Larsson, D.G., 2016. The structure and diversity of human, animal and environmental resistomes. Microbiome. 4 (1), 54.

Pomposiello, P.J., Demple, B., 2001. Redox-operated genetic switches: the SoxR and OxyR transcription factors. Trends Biotechnol. 19 (3), 109–114.

Qiu, Z.G., Yu, Y.M., Chen, Z.L., Jin, M., Yang, D., Zhao, Z.G., Wang, J.F., Shen, Z.Q., Wang, X.W., Qian, D., Huang, A.H., Zhang, B.C., Li, J.W., 2012. Nanoalumina promotes the horizontal transfer of multiresistance genes mediated by plasmids across genera. P. Natl. Acad. Sci. USA. 109 (13), 4944–4949.

Riddle, S.G., Jakober, C.A., Robert, M.A., Cahill, T.M., Charles, M.J., Kleeman, M.J., 2007. Large PAHs detected in fine particulate matter emitted from light-duty gasoline vehicles. Atmos. Environ. 41 (38), 8658–8668.

Samuels, A.L., Lanka, E., Davies, J.E., 2000. Conjugative junctions in RP4-mediated mating of *Escherichia coli*. J. Bacteriol. 182 (10), 2709–2715.

Simon, H.U., Haj-Yehia, A., Levi-Schaffer, F., 2000. Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis Int. J. Programmed Cell Death. 5 (5), 415–418.

Valavanidis, A., Fiotakis, K., Vlachogianni, T., 2008. Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. J. Environ. Sci. Health C. 26 (4), 339–362.

Verma, V., Fang, T., Xu, L., Peltier, R.E., Russell, A.G., Ng, N.L., Weber, R.J., 2015. Organic aerosols associated with the generation of reactive oxygen species (ROS) by water-soluble $PM_{2.5}$. Environ. Sci. Technol. 49 (7), 4646–4656.

Wang, Q., Mao, D.Q., Luo, Y., 2015. Ionic liquid facilitates the conjugative transfer of antibiotic resistance genes mediated by plasmid RP4. Environ. Sci. Technol. 49 (14), 8731–8740.

World Health Organization (WHO), 2014. Antimicrobial Resistance: Global Report on Surveillance.

Wu, D., Zhang, F., Lou, W.H., Li, D., Chen, J.M., 2017. Chemical characterization and toxicity assessment of fine particulate matters emitted from the combustion of petrol and diesel fuels. Sci. Total Environ. 605–606, 172–179.

Xu, P., Chen, Y.F., Ye, X.J., 2013. Haze, air pollution, and health in China. Lancet 382 (9910), 2067.

Xu, P., Wang, W., Ji, J.W., Yao, S.Y., 2014. Analysis of the contribution of the road traffic industry to the $PM_{2.5}$ emission for different land-use types. Comput. Intel. Neurosci. 2014, 821973.

Yadav, V.K., Prasad, S., Patel, D.K., Khan, A.H., Tripathi, M., Shukla, Y., 2010. Identification of polycyclic aromatic hydrocarbons in unleaded petrol and diesel exhaust emission. Environ. Monit. Assess. 168 (1–4), 173–178.

Zhang, Y.Z., Yao, Z.L., Shen, X.B., Liu, H., He, K.B., 2015. Chemical characterization of $PM_{2.5}$ emitted from on-road heavy-duty diesel trucks in China. Atmos. Environ. 122, 885–891.

Zhang, Y., Gu, A.Z., He, M., Li, D., Chen, J.M., 2017. Subinhibitory concentrations of disinfectants promote the horizontal transfer of multidrug resistance genes within and across genera. Environ. Sci. Technol. 51 (1), 570–580.

Zhu, Y.G., Johnson, T.A., Su, J.Q., Qiao, M., Guo, G.X., Stedtfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. P. Natl. Acad. Sci. USA. 110 (9), 3435–3440.

Ziegelin, G., Furste, J.P., Lanka, E., 1989. TraJ protein of plasmid RP4 binds to a 19-base pair invert sequence repetition within the transfer origin. J. Biol. Chem. 264 (20), 11989–11994.