

# Impact of dust exposure on mixed bacterial cultures and during eukaryotic cell co-culture infections

Mariam Bado<sup>1</sup> · Syntia Kwende<sup>1</sup> · Shishir Shishodia<sup>1,2</sup> · Jason A. Rosenzweig<sup>1,2</sup>

Received: 25 April 2017 / Revised: 20 July 2017 / Accepted: 22 July 2017 / Published online: 3 August 2017  
© Springer-Verlag GmbH Germany 2017

**Abstract** On a daily basis, humans, and their colonizing microbiome, are exposed to both indoor and outdoor dust, containing both deleterious organic and inorganic contaminants, through dermal contact, inhalation, and ingestion. Recent studies evaluating the dust exposure responses of opportunistic pathogens, such as *Escherichia coli* and *Pseudomonas aeruginosa*, revealed significant increases in biofilm formation following dust exposure. In this study, the effects of dust exposure on mixed bacterial cultures as well as HT-29 co-cultures were evaluated. As it was observed in pure, single bacterial cultures earlier, neither indoor nor outdoor dust exposure (at concentrations of 100 µg/mL) influenced the growth of mixed bacterial liquid cultures. However, when in paired mixed cultures, dust exposure increased sensitivity to oxidative stress and significantly enhanced biofilm formation (outdoor dust). More specifically, mixed cultures (*E. coli*-*Klebsiella pneumoniae*, *K. pneumoniae*-*P. aeruginosa*, and *E. coli*-*P. aeruginosa*) exhibited increased sensitivity to 20 and 50 mM of H<sub>2</sub>O<sub>2</sub> in comparison to their pure, single bacterial culture counterparts and significantly enhanced biofilm production for each mixed culture. Finally, bacterial proliferation during a eukaryotic gut cell (HT29) co-culture was significantly more robust for both *K. pneumoniae* and *P. aeruginosa* when exposed to both house and road dust; however, *E. coli* only experienced significantly enhanced proliferation, in HT29 co-culture, when exposed to road dust.

Taken together, our findings demonstrate that bacteria respond to dust exposure differently when in the presence of multiple bacterial species or when in the presence of human gut epithelial cells, than when grown in isolation.

**Keywords** Biofilm formation · Oxidative stress responses · Bacterial proliferation · Environmental microbiology · Gut microbiota

## Introduction

With advances in technology and various industrial practices, chemicals such as plasticizers, pesticides, and those found in flame retardants are increasingly entering in the ecosystem (Weschler 2009). Their effect on the environment depends on the amount of chemical released, the type and concentration of chemical, and its source. Some chemicals (e.g., mercury, ozone, and arsenic) are deleterious if released into the environment even when there is no immediate or visible impact. In the worst case scenario, noxious chemicals accumulate in the food chain and persist in the environment for many years. Unfortunately, some of these harmful chemicals (both inorganic and organic) such as arsenic, chromium, mercury, lead, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) can be found in various dust particles (Whitehead 2011; Hogervost et al. 2007).

The presence of some of the aforementioned deleterious chemicals in indoor dust is a cause for concern on account of the increasingly long periods of time people spend indoors. Indoor chemical contamination can be derived from various sources including cooking, smoking/burning candles, deteriorating building materials, cleaning products, cosmetics, biocides, textiles, house furnishing, and electronic devices (Bergh et al. 2011). In the USA, adults spend roughly 21 h/day

✉ Jason A. Rosenzweig  
rosenzweigja@tsu.edu

<sup>1</sup> Department of Environmental and Interdisciplinary Sciences, Texas Southern University, 3100 Cleburne Street, Houston, TX 77004, USA

<sup>2</sup> Department of Biology, Texas Southern University, 3100 Cleburne Street, Houston, TX 77004, USA

indoors, while children spend 17–19 h/day, justifying the need to evaluate the potentially deleterious effect of indoor dust on human health. Some studies (Berkowitz et al. 2003; Simcox 1995) have evaluated human risks of indoor dust exposure on infants and pregnant women. Not surprisingly, for infants and toddlers, the primary route of exposure was through hand-to-mouth activities, such as eating fallen food contaminated with dust (Butte and Heinzow 2002).

Beyond indoor dust, outdoor dust (including road dust), influenced by urbanization and industrialization, is known to contain a wide range of toxins and metals (Adriano 2001; Duong and Lee 2011). Metals found in outdoor dust are derived from diverse sources including atmospheric precipitation, various industrial processes, coal combustion, and vehicle emission (Ahmed and Ishiga 2006). More specifically, platinum group elements (PGEs) are rare natural elements which are being increasingly used in health and commercial products. Consequently, their concentrations in the environment have been steadily increasing (Ravindra et al. 2004). Metals typically enter the human body via three principal routes: ingestion, dermal contact, and inhalation (Garrison et al. 2014; Sahlstrom et al. 2015). In humans, metals have been shown to affect the central nervous system, especially in children (Valko et al. 2005; Yongming et al. 2006; Zheng et al. 2010; Du et al. 2013).

Our previous studies have shown that dust exposure influences bacterial growth, oxidative stress resistance, and virulence potential when bacteria were grown in pure culture (Suraju et al. 2015). More specifically, dust exposure on three representative commensals, who double as opportunistic pathogens (*Escherichia coli*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*), resulted in increased biofilm production in all three organisms following exposure to both indoor and outdoor dust; however, only *E. coli* exhibited increased sensitivity to oxidative stress following exposure to dust (Suraju et al. 2015).

Unfortunately, though, little is known about the physiological effect of dust exposure on bacterial co-cultures, which more accurately reflects native microbiological environments where multiple organisms co-exist and often compete with one another. Two primary types of competition can occur when two bacterial strains are co-cultured. In exploitative competition, species compete for limited nutrients while interference competition results in species directly antagonizing one another (Shoaie et al. 2013). In fact, some molecules produced by bacteria can influence the behavior and fitness of neighboring, yet unrelated, species within the same ecosystem. In some cases, they can be beneficial, but in most cases they are antagonistic (Iwase et al. 2010). The purpose of this study was to broaden our earlier preliminary findings (Suraju et al. 2015) by characterizing the impact of dust exposure (containing PGEs as well as other contaminants) on mixed-culture opportunistic bacterial pathogens that associate with the human microbiome as well as bacterial and eukaryotic cell co-cultures.

## Materials and methods

### Dust

House dust (SRM 2585) was purchased from the National Institute of Standards and Technology, and a list of organic components found in the house dust can be found in Table 1. Road dust (BCR 723, Sigma) was sampled in Austria in 1998, and a list of its inorganic can be found in Table 2.

### Bacterial strains, and culture conditions

For all studies, either house dust (Sigma-Aldrich NIST SRM 2585) or road dust (Sigma-Aldrich BCR 723) was used at concentrations of 100 µg/mL in PBS unless otherwise noted. All dust samples were diluted in PBS and plated to ensure sterility before use. *E. coli* K-12 (Carolina Biological, Burlington, NC, USA—155065A), *P. aeruginosa* PA01 (Carolina Biological, Burlington, NC, USA—155250A), and *Klebsiella pneumoniae* (Carolina Biological, Burlington, NC, USA—155095A) were used in all studies. For all experiments, Brain Heart Infusion (BHI) medium (Sigma-Aldrich) was used to grow bacterial strains with agitation (250 rpm) at 37 °C. All absorbance readings were taken using a BioTek™ ELx800™ microplate reader at 595 nm.

### Eukaryotic cell lines

HT-29 cells (ATCC HTB38) were cultured as previously described (Petiot et al. 2000) with some minor modifications. DMEM (Thermo Fisher 11965092) supplemented with 10% FBS (Thermo Fisher 16140071) and 5% pen-strep (Thermo Fisher 15140122) was used. Cells were incubated at 37 °C with 5% CO<sub>2</sub>. Flask medium was changed every 3 days.

### Growth curve analysis

For liquid growth assays, phosphate-buffered saline (PBS) (Amresco)-suspended indoor (house) or outdoor (road) dusts were diluted into BHI broth at a final concentration of 100 µg/mL each. Saturated cultures of *K. pneumoniae*, *E. coli*, and *P. aeruginosa* were diluted to an optical density (595 nm) of 0.2 in a 96-well plate (final volume, 200 µL/well). For control wells, only PBS was added to each well. Growth rates were monitored every 30 min for 8 h. All growth experiments were conducted in triplicate. To enumerate and distinguish individual bacteria strains in mixed cultures, either MacConkey agar (Difco) or eosin methylene blue (EMB) agar (Difco) was used. On EMB agar, *E. coli* colonies were metallic green, *K. pneumoniae* colonies were pinkish-purple, and *P. aeruginosa* colonies appeared colorless.

**Table 1** Organic contaminants of house dust (SRM 2585)

Selected PAHs in SRM 2585	Mass fraction (dry-mass basis) ( $\mu\text{g}/\text{kg}$ )
Naphthalene	266 $\pm$ 8
Phenanthrene	1920 $\pm$ 20
Methylphenanthrene	293 $\pm$ 36
2-Methylphenanthrene	352 $\pm$ 40
Methylphenanthrene	205 $\pm$ 16
Fluoranthene	4380 $\pm$ 100
Pyrene	3290 $\pm$ 30
Benzo[ghi]fluoranthene)	317 $\pm$ 11
Benzo[c]phenanthrene	288 $\pm$ 10
Benz[a]anthracene	1160 $\pm$ 54
Triphenylene	589 $\pm$ 17
Chrysene	2260 $\pm$ 60
Benzo[b]fluoranthene	2700 $\pm$ 90
Benzo[j]fluoranthene	1320 $\pm$ 110
Benzo[e]pyrene	2160 $\pm$ 80
Benzo[ghi]perylene	2280 $\pm$ 40
Selected PCB congeners in SRM 2585	Mass fraction (dry-mass basis) ( $\mu\text{g}/\text{kg}$ )
PCB 18 (2,2',5-trichlorobiphenyl)	12.8 $\pm$ 1.0
PCB 28 (2,4,4'-trichlorobiphenyl)	13.4 $\pm$ 0.5
PCB 31 (2,4',5-trichlorobiphenyl)	14.0 $\pm$ 0.5
PCB 44 (2,2'3,5'-tetrachlorobiphenyl)	18.1 $\pm$ 1.9
PCB 52 (2,2',5,5'-tetrachlorobiphenyl)	21.8 $\pm$ 1.9
PCB 56 (2,3,3',4-tetrachlorobiphenyl)	4.42 $\pm$ 0.28
PCB 70 (2,3',4',5-tetrachlorobiphenyl)	13.1 $\pm$ 1.2
PCB 74 (2,4,4',5-tetrachlorobiphenyl)	5.22 $\pm$ 0.51
PCB 87 (2,2',3,4,5'-pentachlorobiphenyl)	16.6 $\pm$ 0.8
PCB 92 (2,2',3,5,5'-pentachlorobiphenyl)	5.48 $\pm$ 0.72
PCB 95 (2,2',3,5',6-pentachlorobiphenyl)	22.7 $\pm$ 2.6
PCB 99 (2,2',4,4',5-pentachlorobiphenyl)	11.6 $\pm$ 0.4
PCB 101 (2,2',4,5,5'-pentachlorobiphenyl)	29.8 $\pm$ 2.3
PCB 105 (2,3,3',4,4'-pentachlorobiphenyl)	13.2 $\pm$ 1.4
PCB 107 (2,3,3',4,5'-pentachlorobiphenyl)	4.14 $\pm$ 0.47
PCB 110 (2,3,3',4',6-pentachlorobiphenyl)	28.1 $\pm$ 3.7
PCB 118 (2,3',4,4',5-pentachlorobiphenyl))	26.3 $\pm$ 1.7
PCB 138 (2,2',3,4,4',5'-hexachlorobiphenyl)	27.6 $\pm$ 2.1
PCB 146 (2,2',3,4',5,5'-hexachlorobiphenyl)	4.89 $\pm$ 0.38
PCB 149 (2,2',3,4',5',6-hexachlorobiphenyl)	24.4 $\pm$ 1.9
PCB 151 (2,2',3,5,5',6-hexachlorobiphenyl)	6.92 $\pm$ 0.64
PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl)	40.2 $\pm$ 1.8
PCB 158 (2,3,3',4,4',6-hexachlorobiphenyl)	4.50 $\pm$ 0.43
PCB 163 (2,3,3',4',5,6-hexachlorobiphenyl)	7.2 $\pm$ 1.2
PCB 170 (2,2',3,3',4,4',5-heptachlorobiphenyl)	8.8 $\pm$ 1.0
PCB 174 (2,2',3,3',4,5,6'-heptachlorobiphenyl)	8.83 $\pm$ 0.47
PCB 180 (2,2',3,4,4',5,5-heptachlorobiphenyl))	18.4 $\pm$ 3.2
PCB 183 (2,2',3,4,4',5',6-heptachlorobiphenyl)	5.27 $\pm$ 0.39
PCB 187 (2,2',3,4',5,5',6-heptachlorobiphenyl)	11.3 $\pm$ 1.4
PCB 206 (2,2',3,3',4,4',5,5',6-nonachlorobiphenyl)	3.81 $\pm$ 0.13
Selected chlorinated pesticides in SRM 2585	Mass fraction (dry-mass basis) ( $\mu\text{g}/\text{kg}$ )
4,4'-DDE	261 $\pm$ 2(b)

**Table 1** (continued)

Selected PAHs in SRM 2585	Mass fraction (dry-mass basis) ( $\mu\text{g}/\text{kg}$ )
4,4'-DDD	27.3 $\pm$ 0.8
2,4'-DDT	44.5 $\pm$ 3.9
4,4'-DDT	111 $\pm$ 23
Selected chlorinated pesticides in SRM 2585	Mass fraction (dry mass basis) ( $\mu\text{g}/\text{kg}$ )
<i>cis</i> -Chlordane	174 $\pm$ 45
<i>trans</i> -Chlordane	277 $\pm$ 96
<i>cis</i> -Nonachlor	28.0 $\pm$ 0.6
<i>trans</i> -Nonachlor	130 $\pm$ 38
Heptachlor	166 $\pm$ 34
Heptachlor epoxide	11.3 $\pm$ 0.6
Dieldrin	88 $\pm$ 21
<i>gamma</i> -Hexachlorocyclohexane	4.06 $\pm$ 0.55
Mirex	6.89 $\pm$ 0.25
Pentachlorobenzene	20.9 $\pm$ 1.6

### Protein expression

Saturated bacterial cultures, grown at 37 °C with agitation, of *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, were diluted to an optical density (600 nm) of 0.2 in 2 mL of BHI medium (Difco) in either the presence of 100  $\mu\text{g}/\text{mL}$  of house, road, or no dust. Five hundred-microliter samples were removed from each

growing subculture at 0-, 3-, and 6-h time points. Cells were harvested via centrifugation (14,000 $\times g$ ), and bacterial pellets were resuspended in 100  $\mu\text{L}$  RIPA buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na<sub>2</sub>EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1  $\mu\text{g}/\text{mL}$  leupeptin) for 45 min on ice. Samples were then centrifuged at 14,000 rpm for 5 min. After determination of total protein concentration using the Bradford assay (Bio-Rad), 15  $\mu\text{g}$  total protein from each sample was loaded on 10% Tris-polyacrylamide gel and then Coomassie (Bio-Rad) stained.

**Table 2** Inorganics contaminants of road dust (BCR 723)

Compounds	Mass fraction (dry mass basis)	Units
Pd	6.1 $\pm$ 1.9	$\mu\text{g}/\text{kg}$
Pt	81.3 $\pm$ 2.5	$\mu\text{g}/\text{kg}$
Rh	12.6 $\pm$ 1.3	$\mu\text{g}/\text{kg}$
Al	3.75 $\pm$ 0.22	%
Ba	0.46 $\pm$ 0.04	$\text{g}/\text{kg}$
Cd	2.5 $\pm$ 0.4	$\text{mg}/\text{kg}$
Co	29.8 $\pm$ 1.6	$\text{mg}/\text{kg}$
Cr	440 $\pm$ 18	$\text{mg}/\text{kg}$
Fe	3.29 $\pm$ 0.2	%
Hf	2.2 $\pm$ 0.7	$\text{mg}/\text{kg}$
Mn	1.28 $\pm$ 0.04	$\text{g}/\text{kg}$
Mo	40.0 $\pm$ 0.6	$\text{mg}/\text{kg}$
Ni	171 $\pm$ 3	$\text{mg}/\text{kg}$
Pb	866 $\pm$ 16	$\text{mg}/\text{kg}$
Rb	75 $\pm$ 5	$\text{mg}/\text{kg}$
Sb	28.2 $\pm$ 2.3	$\text{mg}/\text{kg}$
Sr	254 $\pm$ 19	$\text{mg}/\text{kg}$
Ti	2.58 $\pm$ 0.13	$\text{g}/\text{kg}$
Th	4.8 $\pm$ 0.5	$\text{mg}/\text{kg}$
V	74.9 $\pm$ 1.9	$\text{mg}/\text{kg}$
Y	12.5 $\pm$ 1.8	$\text{mg}/\text{kg}$
Zn	1.66 $\pm$ 0.10	$\text{g}/\text{kg}$

### Oxidative stress assays

PBS-suspended indoor (house) or outdoor (road) dust was diluted into BHI broth to a final concentration of 100  $\mu\text{g}/\text{mL}$ . Saturated cultures of *K. pneumoniae*, *E. coli*, and *P. aeruginosa* were diluted to an optical density (595 nm) of 0.2 in a 96-well plate (final volume, 200  $\mu\text{L}$ ). For negative control wells, only PBS was added. Following 1 h of growth, H<sub>2</sub>O<sub>2</sub> was added to the subcultures at final concentrations of 0, 20, and 50 mM, and growth was monitored for 7 h. All experiments were conducted in triplicate.

### Biofilm formation assay

For our biofilm formation assay, we employed our previously described methods (Suraju et al. 2015) with some modifications. Briefly, saturated cultures of *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, grown in BHI, were diluted to an optical density (595 nm) of 0.2 in a 96-well plate (final volume, 200  $\mu\text{L}/\text{well}$ ). Microtiter plates were incubated for 24 h with agitation ( $\sim$  100 rpm) at 37 °C, after which optical densities at 595 nm were measured. Wells were washed with water and

incubated with 0.1% (vol/vol) crystal violet (total volume, 250  $\mu\text{L}$ /well) for 1 h at ambient temperature. Unbound crystal violet was removed by washing with water, and wells were dried overnight. Biofilm-bound crystal violet was dissolved in 250  $\mu\text{L}$  of 30% acetic acid. Optical densities of solubilized crystal violet were measured at 570 nm. Biofilm produced was normalized based on relative biomass (optical densities of planktonic cells) to account for any differences in the growth rates of the various bacterial strains used. All experiments were carried out in triplicate or quadruplicate.

#### MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

HT29 cells were seeded in a 96-well plate at a density of 5000 cells/well  $\sim$  24 h preceding the experiment. The following day, treatment of designated wells using 25, 50, and 100  $\mu\text{g}/\text{mL}$  of indoor (house) or outdoor (road) dust occurred for 0, 3, 6, or 12 h. Twenty microliters of 5 mg/mL MTT was added to each well, followed by incubation at 37  $^{\circ}\text{C}$  with 5%  $\text{CO}_2$ . After 4 h, medium was gently removed from each well and replaced with 100  $\mu\text{L}$  of DMSO. Cells were agitated on an orbital shaker for 5 to 10 min, and absorbencies were read at 570 nm with a reference filter of 640 nm.

#### Bacterial co-culture with eukaryotic cells

HT29 cells were seeded into 24-well plates at densities of  $\sim 1 \times 10^5$ /well, 24 h prior to bacterial infection. At this seeding density, monolayers were subconfluent ( $\sim$  60–80% confluency) at the time of the experiment. Bacteria were grown to saturation in BHI broth at 37  $^{\circ}\text{C}$  with agitation ( $\sim$  250 rpm), washed with 1 $\times$  PBS, and diluted to optical densities (600 nm) of 1.0 in DMEM + 10% FBS. Diluted cultures of *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, were further diluted (as necessary) to achieve multiplicities of infection of 1. Following a 30-min attachment period, each well was washed with PBS, and DMEM containing 100  $\mu\text{g}/\text{mL}$  of either indoor or outdoor dust was added to each well. Viable colony plate counts were enumerated for both the 0- and 6-h end points, and fold increases over that time period were calculated. All plates were incubated for 24 h at 37  $^{\circ}\text{C}$ .

#### Statistical analysis

Statistical analysis was conducted using GraphPad Prism software (version 6). All experiments were performed in triplicate, and error bars on the graphs represent standard error of the mean. For comparing sets of data, the Student's *t* test was used, and statistical significance was considered for *P* values  $<0.05$ .

## Results

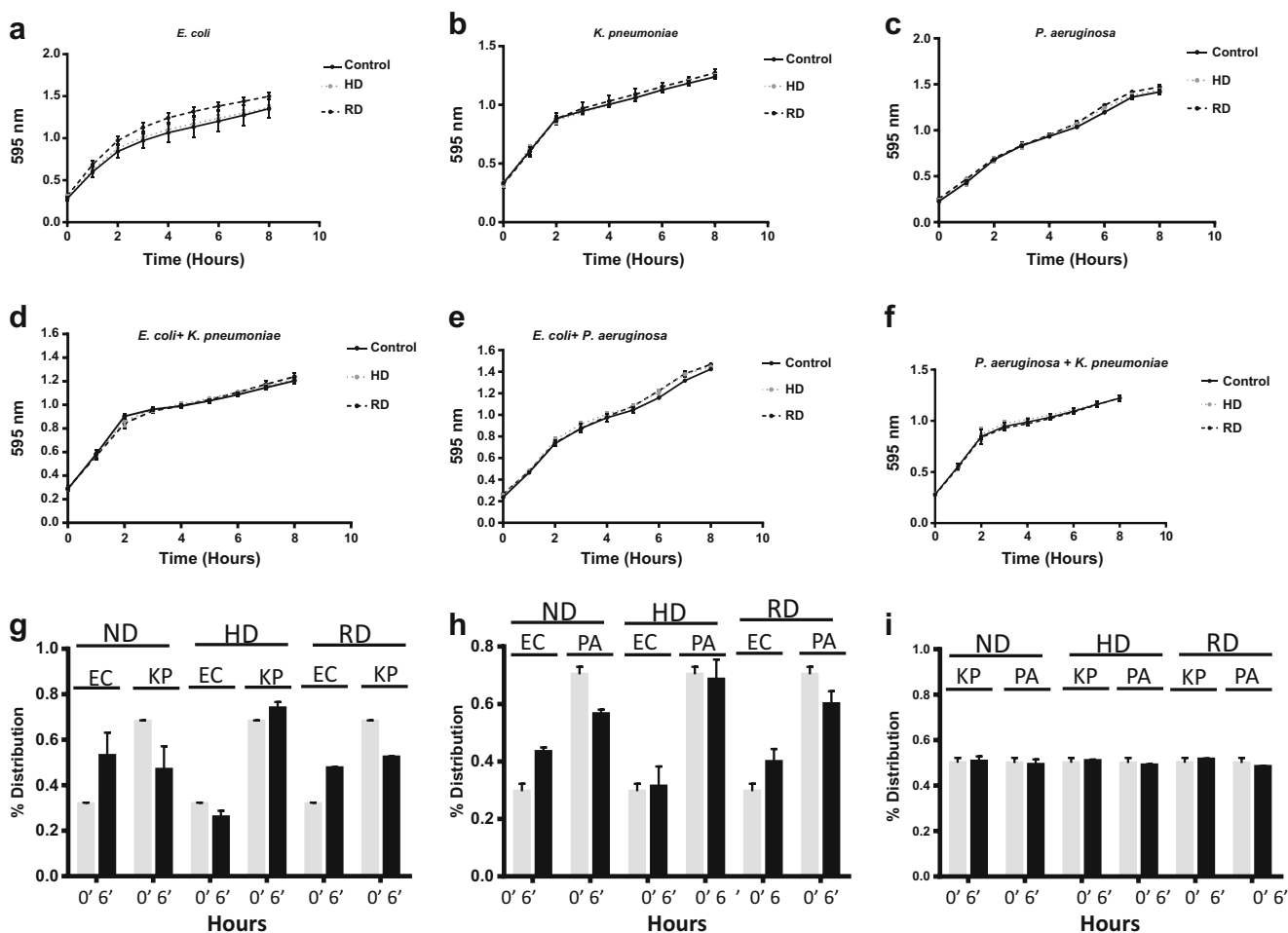
### Dust exposure did not influence bacterial growth in either pure or mixed culture

To determine the impact of dust (indoor and outdoor) exposure on bacterial growth, we first exposed pure bacterial liquid cultures of *E. coli*, *K. pneumoniae*, or *P. aeruginosa* to 100  $\mu\text{g}/\text{mL}$  of dust (indoor and outdoor) while measuring their biomass every 30 min for 8 h (Fig. 1a–c). In agreement with our earlier findings in which the growth of *E. coli*, *E. faecalis*, and *P. aeruginosa* was not influenced by dust exposure for 4 h (Suraju et al. 2015), exposure to 100  $\mu\text{g}/\text{mL}$  of either indoor or outdoor dust similarly did not impact bacterial growth for all three organisms (*E. coli*, *K. pneumoniae*, or *P. aeruginosa*) evaluated in this study during an 8-h period (Fig. 1a–c). Similarly, dust exposure (both indoor and outdoor) of 100  $\mu\text{g}/\text{mL}$  had no impact on mixed-culture growth (of (1) *E. coli*–*P. aeruginosa*, (2) *K. pneumoniae*–*E. coli*, and (3) *K. pneumoniae*–*P. aeruginosa*) when compared to the same mixed cultures grown in the absence of dust (Fig. 1d–f).

To further evaluate the impact of dust exposure on mixed bacterial cultures, we employed selective solid medium (eosin methylene blue) plates to determine whether one specific organism, within the mixed culture, outgrew its counterpart. We observed that although bulk biomass remains unaffected, as observed in liquid cultures (Fig. 1d–f), some representatives within the mixed cultures appear to outgrow their companion strains on solid EMB selective medium. More specifically, when exposed to house dust (but not road dust), *K. pneumoniae* appeared to outgrow *E. coli* in an *E. coli*–*K. pneumoniae* mixed culture (Fig. 1g). Similarly, when exposed to both house and road dust, *P. aeruginosa* appeared to outgrow *E. coli* in an *E. coli*–*P. aeruginosa* mixed culture (Fig. 1h). Interestingly, in the *K. pneumoniae*–*P. aeruginosa* mixed culture, neither organism appeared to outgrow/outcompete its companion (Fig. 1i). Taken together, employing the more refined selective solid medium enabled us to determine that, although mixed cultures experienced no alternations in net growth (as measured by biomass), dust exposure could enhance the ability of select members of the gut microbiota to expand at the expense of neighboring unrelated organisms.

### Dust exposure did not grossly alter bacterial protein production

To determine whether dust exposure could grossly affect protein production in exposed bacteria, we challenged *E. coli*, *K. pneumoniae*, or *P. aeruginosa* with either 100  $\mu\text{g}/\text{mL}$  of house, road, or no dust for 0, 3, or 4 h timepoints. At the aforementioned times, cells were harvested, lysed, loaded on polyacrylamide gels, and subjected to electrophoresis. For



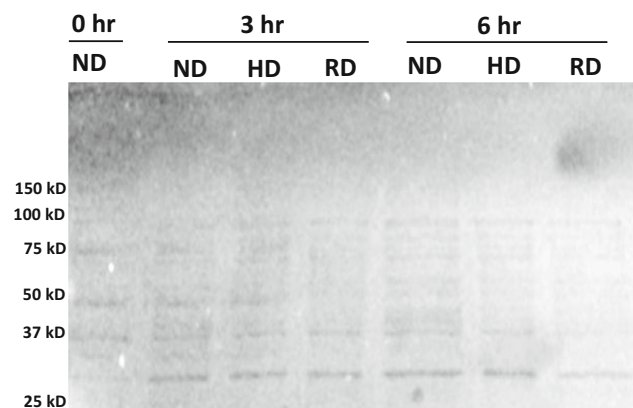
**Fig. 1** Bacterial growth in response to dust exposure. Growth curve analysis of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* in pure culture (a–c) or in mixed cultures (d–f), is shown in response to house and road dust exposure. Distribution of individual strains within a mixed

culture following dust exposure is also shown (g–i). Representative experiment shown is in triplicate, and errors of the mean are also shown. Student's *t* test was used to determine any statistical differences

each of the timepoints evaluated, there was no apparent difference in *E. coli*'s total protein production when exposed to either 100  $\mu\text{g}/\text{mL}$  of house, road, or no dust (Fig. 2). Similarly, we did not observe any dramatic alterations in protein production in either *K. pneumoniae* or *P. aeruginosa* when exposed to 100  $\mu\text{g}/\text{mL}$  of house, road, or no dust for 0, 3, or 4 h timepoints (data not shown). Although, these data did not reveal any gross-level differences in protein expression, a more refined look at specific subsets of proteins and or gene expression (i.e., mRNA transcript levels) could reveal dust responsive bacterial genes and/or gene products.

### Dust exposure increased the sensitivity of mixed bacterial cultures to oxidative stress

Earlier, we demonstrated that exposure to dust (indoor and outdoor) increased the sensitivity of *E. coli*, but not of *E. faecalis*, and *P. aeruginosa*, to  $\text{H}_2\text{O}_2$  challenge (Suraju et al. 2015). In this study, and in agreement with our earlier finding (Suraju



**Fig. 2** Evaluation of gross level protein production in dust exposed *E. coli*. A 5.0-mL *E. coli* subculture was started at an optical density 600 nm of 0.2, and 100- $\mu\text{L}$  aliquots were removed at 0-, 3-, and 6-h time points for bacterial cell harvest. After total protein quantification using the Bradford assay, 15  $\mu\text{g}$  of total protein was loaded in each well of a 10% polyacrylamide gel. The gel was Coomassie stained, and protein bands and their intensities are displayed

et al. 2015), *E. coli* exhibited increased sensitivity to 20 and 50 mM of H<sub>2</sub>O<sub>2</sub> when exposed to both indoor and outdoor dust (Fig. 3 compare panels b, c to e, f, and h, i). In extending our earlier studies (Suraju et al. 2015), we also evaluated the impact of dust exposure on the oxidative stress response of *K. pneumoniae* (Fig. 2). Like *E. coli*, *K. pneumoniae* similarly experienced increased sensitivity to 20 mM H<sub>2</sub>O<sub>2</sub> (compare panel Fig. 3b–h) following road dust exposure but did not experience altered sensitivity to 20 mM H<sub>2</sub>O<sub>2</sub> when exposed to house dust (Fig. 3e). This again underscores the unique components found in each dust type as well as the unique responses of the individual bacteria. In sharp contrast to both *E. coli* and *K. pneumoniae*, *P. aeruginosa* demonstrated significantly better intrinsic resistance to both 20 and 50 mM H<sub>2</sub>O<sub>2</sub> exposure (Fig. 3b, c) in the absence of dust challenge. Further, in the presence of both house (Fig. 3e, f) and road dust (Fig. 3h, i), *P. aeruginosa* was significantly more resistant than either *E. coli* or *K. pneumoniae* to subsequent 20 mM (Fig. 3e, h) and 50 mM (Fig. 3f, i) H<sub>2</sub>O<sub>2</sub> challenge.

Since bacteria live mostly in heterogeneous, mixed-culture native environments, we further investigated the sensitivity of bacterial mixed cultures to H<sub>2</sub>O<sub>2</sub>. More specifically, *E. coli* and *K. pneumoniae*, *E. coli*–*P. aeruginosa*, and *K. pneumoniae*–*P. aeruginosa* mixed cultures, initially conditioned in either house or road dust (100 µg/mL) for 1 h, were subsequently challenged with 0, 20, or 50 mM of H<sub>2</sub>O<sub>2</sub>. *E. coli*–*K. pneumoniae* mixed cultures exhibited greater sensitivity to 20 mM H<sub>2</sub>O<sub>2</sub> when in the presence of 100 µg/mL of house dust (compare Fig. 3b, e) than the pure culture of each individual bacterial cultures (Fig. 3b, e). Interestingly, the *E. coli*–*K. pneumoniae* mixed culture was significantly more resistant to 20 mM H<sub>2</sub>O<sub>2</sub> when conditioned/exposed to road dust than the two individually pure cultures (Fig. 3h) but not house dust (Fig. 3f). However, the aforementioned enhanced resistance was not observed when the *E. coli*–*K. pneumoniae* mixed culture was challenged with 50 mM H<sub>2</sub>O<sub>2</sub> in the presence of road dust (Fig. 3i).

Despite *P. aeruginosa* being significantly the most resistant to both 20 and 50 mM H<sub>2</sub>O<sub>2</sub> challenge in the presence of both house (Fig. 3e, f) and road (Fig. 3h, i) dust, the *E. coli*–*P. aeruginosa* mixed culture was significantly more sensitive to 50 mM of H<sub>2</sub>O<sub>2</sub> in the presence of house dust than either of the two individually pure cultures (Fig. 3f). Similarly, in the presence of road dust, the *E. coli*–*P. aeruginosa* mixed culture was significantly more sensitive to 50 mM of H<sub>2</sub>O<sub>2</sub> than the *P. aeruginosa* pure culture (Fig. 3i).

The *K. pneumoniae*–*P. aeruginosa* mixed culture exhibited significantly greater sensitivity to 20 mM of H<sub>2</sub>O<sub>2</sub> with 100 µg/mL of road dust than *K. pneumoniae* and *P. aeruginosa* pure cultures (Fig. 3h). Similarly, when challenged with 50 mM H<sub>2</sub>O<sub>2</sub>, the *K. pneumoniae*–*P. aeruginosa* mixed culture exhibited significantly greater sensitivity than the *P. aeruginosa* and *K. pneumoniae* pure culture (Fig. 3i). Taken together, these data suggest that in a mixed culture,

*P. aeruginosa* loses its intrinsic resistance to H<sub>2</sub>O<sub>2</sub> challenges when otherwise grown independently, suggesting perhaps antagonistic relationships in mixed bacterial cultures.

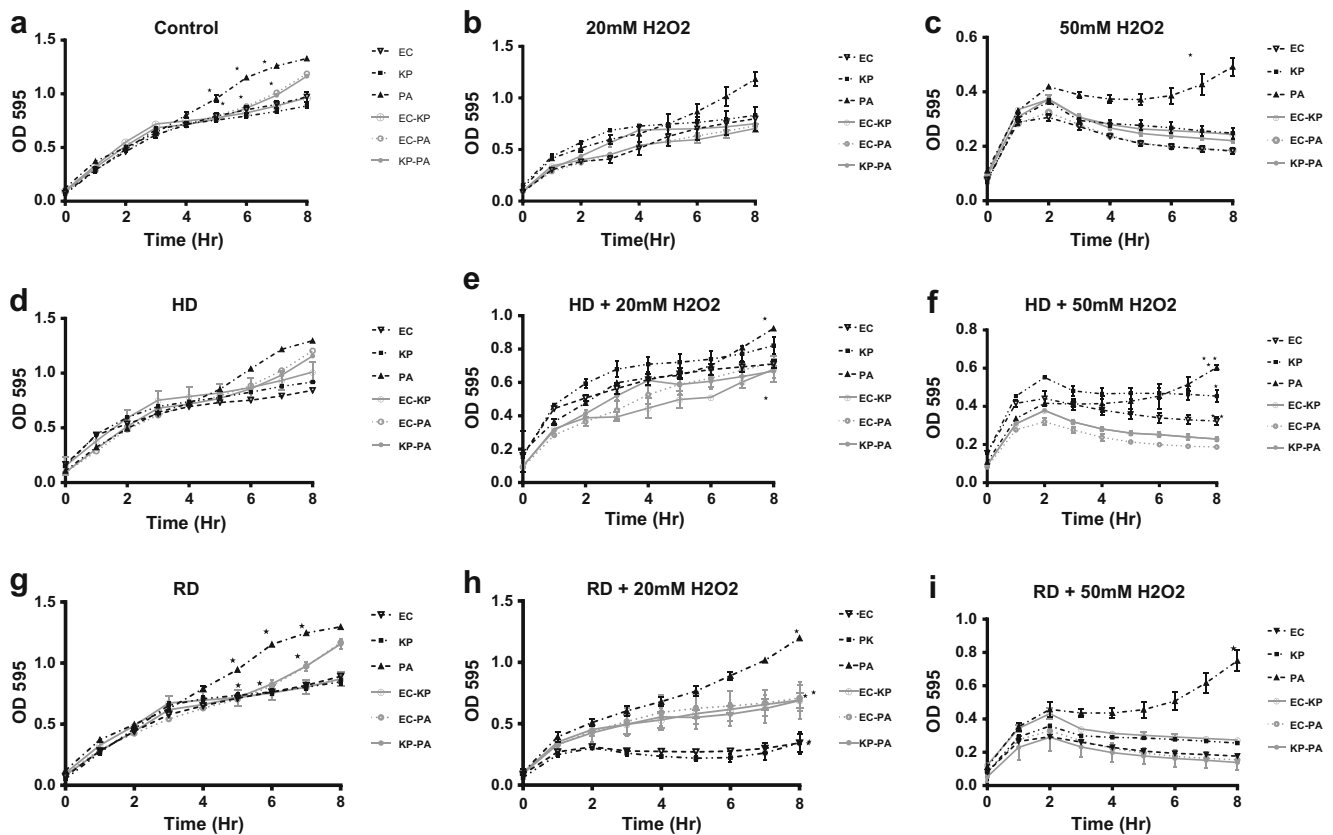
### Enhanced biofilm production in opportunistic bacterial mixed cultures exposed to dust

For our biofilm assays, we chose to employ concentrations of 100 µg/mL of indoor and outdoor dust, as we had done earlier (Suraju et al. 2015), on account of the aforementioned concentration not adversely affecting the growth kinetics of all bacteria evaluated in either mixed or pure culture conditions (Fig. 1). In agreement with our earlier findings (Suraju et al. 2015), exposure to either 100 µg/mL of indoor or outdoor dust resulted in significantly increased biofilm production in both *E. coli* and *P. aeruginosa* (Fig. 4a). Mirroring *E. coli* and *P. aeruginosa*, *K. pneumoniae* similarly exhibited significantly enhanced biofilm production following exposure to either 100 µg/mL of indoor or outdoor dust (Fig. 4a).

Curious about whether the same phenomenon would be observed in mixed bacterial cultures, we exposed *E. coli*–*P. aeruginosa*, *K. pneumoniae*–*P. aeruginosa*, and *E. coli*–*K. pneumoniae* mixed cultures to either 100 µg/mL of indoor, outdoor, or no dust. Interestingly, despite each individual pure culture exhibiting significantly increased biofilm formation following exposure to 100 µg/mL of indoor dust (Fig. 4a), only the *K. pneumoniae*–*P. aeruginosa* mixed culture demonstrated significantly enhanced biofilm formation among all mixed cultures tested. More specifically, no change in biofilm formation was observed for either the *E. coli*–*P. aeruginosa* or *E. coli*–*K. pneumoniae* mixed cultures (Fig. 4b). This somewhat unexpected finding suggests that in mixed culture, *E. coli*–*P. aeruginosa* and *E. coli*–*K. pneumoniae* could be antagonizing to one another in response to house dust exposure resulting in compromised biofilm production. In sharp contrast, all three of the aforementioned mixed cultures tested experienced significantly enhanced biofilm production following exposure to 100 µg/mL of outdoor dust (Fig. 4c), further underscoring the unique physiological consequences triggered by the disparate contents found within each type of dust (Suraju et al. 2015).

### HT29 gut epithelial cells are not influenced by 100 µg/mL of either indoor or outdoor dust

“To determine whether any gross-level changes observed in HT29 gut epithelial- bacterial co-culture infections were a result of bacterial responses to the tested dust alone and not due to any negative influence on HT29 viability, an MTT assay was performed on HT29 cells (alone) following dust exposure to gauge dust-induced host cytotoxicity.”



**Fig. 3** Oxidative stress resistance of single and mixed bacterial cultures. Single and mixed cultures of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were exposed to 0, 20, and 50 mM H<sub>2</sub>O<sub>2</sub> without dust (a–c, respectively), with 100 µg/mL of house dust (d–f, respectively), or 100 µg/mL of road

dust (g–i, respectively). Representative experiments shown are in triplicate, and errors of the mean are also shown. Student's *t* test was used to determine any statistical differences. *p* < 0.05 was considered significant

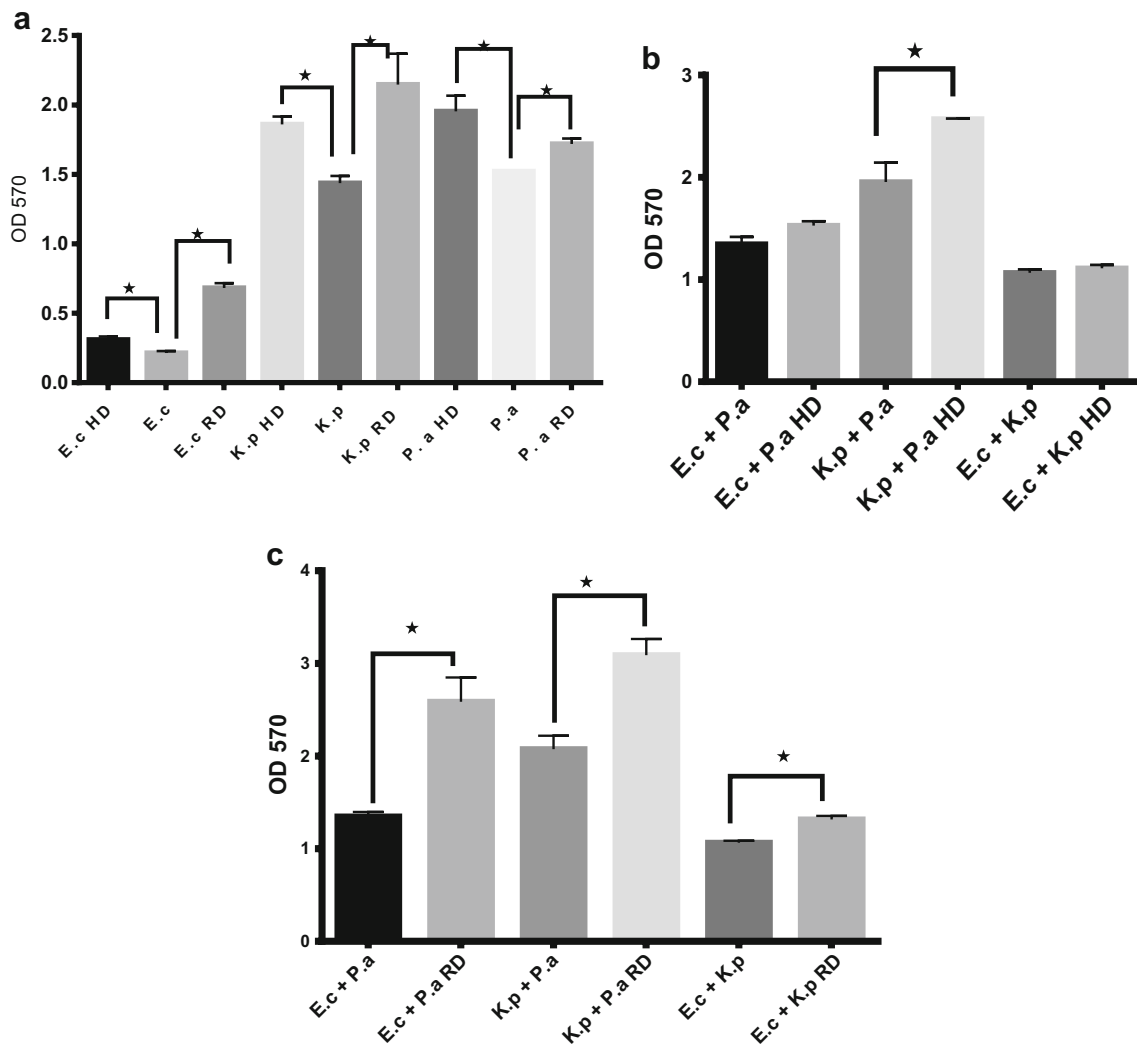
More specifically, prior to conducting bacterial co-culture experiments with eukaryotic cells, we sought to determine whether our working concentrations of 100 µg/mL (of either dust type) had any deleterious effects on HT29 human colonic gut epithelial cells. To determine this, we exposed cultured HT29 cells to 25, 50, and 100 µg/mL of either house or road dust for 0, 3, 6, and 8 h. Fortunately, none of the three concentrations tested for either house (Fig. 5a) or road (Fig. 5b) dust significantly influenced HT29 cell viability at any of the times evaluated (Fig. 5). This finding enabled us to next evaluate the impact of dust exposure on bacterial HT29 co-cultures, knowing that if differences were observed they would not be due to adverse effects on either bacterial or HT29 viability.

### HT29 bacterial co-cultures as a model for gut exposure to dust

When *E. coli* was co-cultured with HT29 cells, interestingly, exposure to 100 µg/mL of house dust did not significantly influence bacterial proliferation during the 6-h infection period; however, exposure to road dust (at the same concentration) did significantly enhance *E. coli*'s proliferation during the same infection period (Fig. 6a). This finding again underscores the

importance of dust components in promoting bacterial responses (since the components found in road and house dust differ). Unlike *E. coli*, which only seemed to respond to road dust exposure in co-culture (Fig. 6a), *K. pneumoniae* and *P. aeruginosa* responded to both dust types (Fig. 6b, c). More specifically, when *K. pneumoniae* (in an HT29 co-culture) was exposed to both house and road dust, 1000-fold increases were observed in bacterial proliferation counts for each, both significantly higher rates of proliferation than the ~ 100-fold proliferation observed for *K. pneumoniae* when no dust was present in the co-culture (Fig. 6b). Similarly, when *P. aeruginosa* (in an HT29 co-culture) was exposed to both house and road dust, > 1500-fold increases were observed in bacterial proliferation counts for each, both significantly higher rates of proliferation than the ~ 600-fold proliferation observed for *P. aeruginosa* when no dust was present in the co-culture (Fig. 6c). Taken together, these data indicate that within the gut microenvironment (HT29 cells), not all resident flora representatives respond similarly to dust exposure (mimicking dust ingestion in humans). Further, the source of the dust (indoor vs. outdoor) could also influence bacterial responses and proliferation, potentially promoting some opportunistic pathogen-induced disease states.





**Fig. 4** Biofilm formation of single and mixed bacterial cultures. Single cultures of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were exposed to 100 µg/mL of house dust, road dust, or no dust (a). Mixed cultures of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were exposed to 100 µg/mL of house dust or no dust (b). Mixed cultures of *E. coli*, *K. pneumoniae*, and

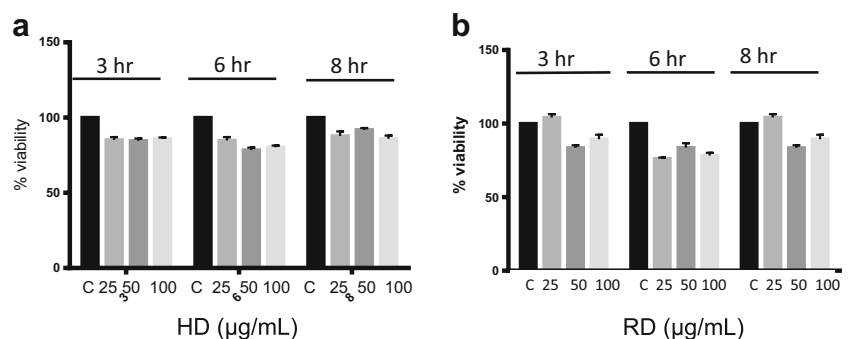
*P. aeruginosa* were exposed to 100 µg/mL of road dust or no dust (c). Representative experiments shown are in triplicate, and errors of the mean are also shown. Student’s *t* test was used to determine any statistical differences. *p* < 0.05 was considered significant

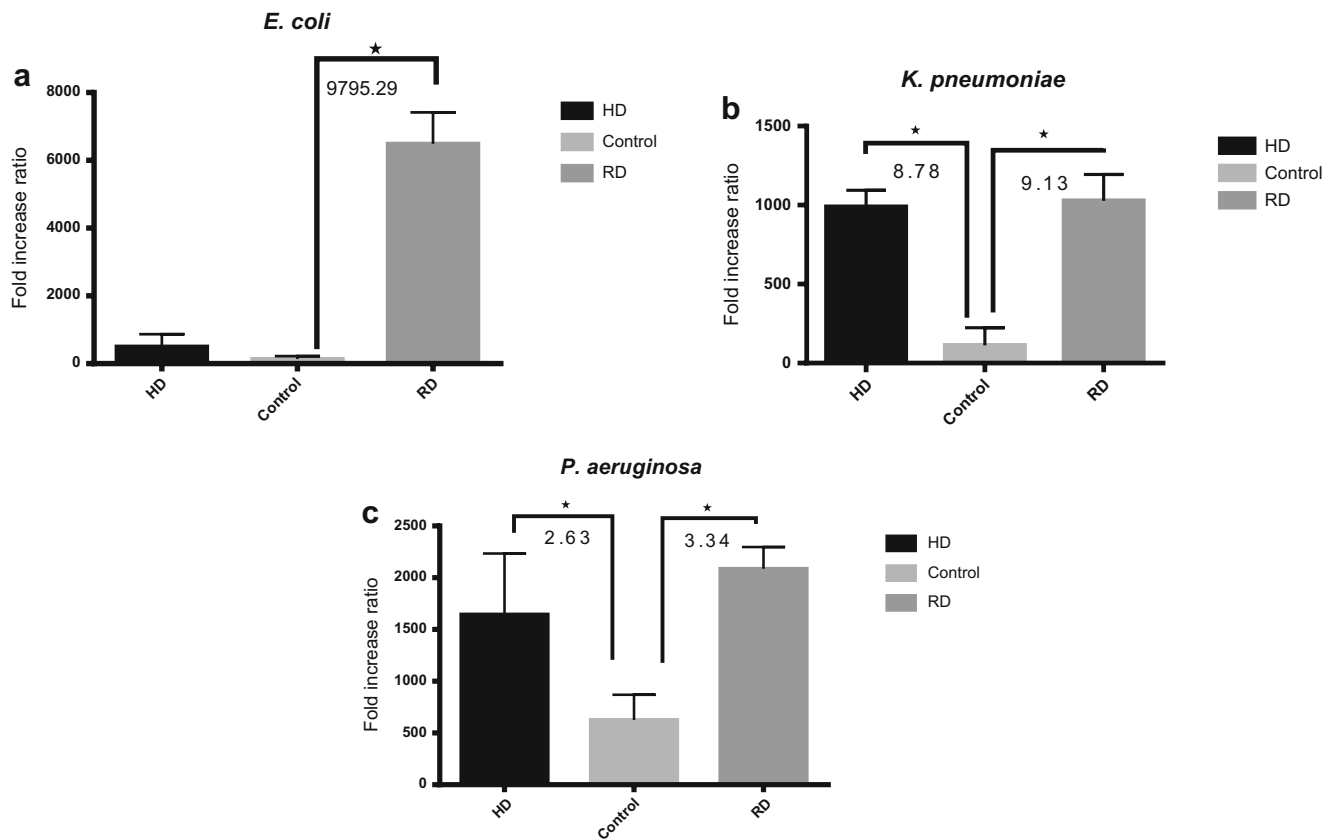
**Discussion**

Various contaminants (many of them noxious) present in dust can enter the human body through inhalation, direct ingestion

(e.g., consumption of vegetables grown in contaminated fields), and/or dermal contact (Bjerg et al. 2015; Garrison et al. 2014; Karotki et al. 2014; Sahlstrom et al. 2015; Qu et al. 2012). Several studies have attempted to evaluate

**Fig. 5** Effect of dust on human gut epithelial HT29 cells. MTT assays were performed to determine cytotoxicity of 25, 50, and 100 µg/mL of either house dust (a) or road dust (b) on human HT29 cells. Representative experiment shown is in triplicate, and errors of the mean are also shown. Student’s *t* test was used to determine any statistical differences





**Fig. 6** Dust-exposed bacterial cells co-cultured with HT29 cells modeling the gut microenvironment. HT29–*E. coli* (a), HT29–*K. pneumoniae* (b), and HT29–*P. aeruginosa* (c) co-cultures exposed to 100  $\mu\text{g}/\text{mL}$  of house, road, or no dust were evaluated for bacterial proliferation

following a 6-h infection period. Representative experiments shown are in triplicate, and errors of the mean are also shown. Student's *t* test was used to determine any statistical differences.  $p < 0.05$  was considered significant. Values shown represent fold increases over time

population health risks due to heavy metal exposure such as arsenic, cadmium, lead, and mercury, through various exposure pathways, especially through the soil and food chain (MacIntosh et al. 1996; Hough et al. 2004; Bastrup et al. 2008; Albering et al. 1999; Man et al. 2010; Mari et al. 2009). While it has been easier to evaluate the effect of inhaled dust contaminants, it has been much more challenging to determine similar effects in the human gut, mainly on account of ingested dust contaminants rapidly being metabolized and excreted (Colombo et al. 2008b). With regards to the human gut, the intestinal microbiota plays a critical role in human health by enhancing host metabolism, producing some nutritional factors, providing protection against transient flora (many of which are pathogenic), and improving host immune function (Guinane and Cotter 2013). In fact, perturbations in the gut microbiota can result in serious consequences for human health, either beneficial or harmful (Ley et al. 2006a). For example, disruptions of the gut microbiota can lead to intestinal disease states including obesity (Ley et al. 2006b; Zhang et al. 2009), malnutrition (Kau et al. 2011), inflammatory bowel disease (IBD), encompassing ulcerative colitis (UC), and Crohn's disease (Frank et al. 2011). Further, gut

microbiota disruptions can also lead to systematic diseases such as diabetes (Qin et al. 2012). Furthermore, epidemiological studies linked air pollution exposure to gastrointestinal diseases, including inflammatory bowel disease (IBD) (Kaplan et al. 2010; Ananthkrishnan et al. 2011), appendicitis (Kaplan et al. 2013), irritable bowel syndrome (Kaplan et al. 2012), and enteric infections in infants (Chen et al. 2008). Additionally, pollutant particulate matter can adulterate food and water supply in significant amounts (Beamish et al. 2011; De Brouwer et al. 2012). According to Lomer et al. (2004),  $10^{12}$ – $10^{14}$  particles are ingested per day by an individual on a typical Western diet, with an estimated mucosal uptake of  $\sim 1\%$  ( $10^9$ – $10^{12}$  per day).

Our recent studies demonstrated that dust exposure can affect bacterial growth, oxidative stress resistance, and the virulence potential of *E. coli*, *E. faecalis*, and *P. aeruginosa*, through enhanced biofilm production, when in pure culture (Suraju et al. 2015). In this manuscript, we employed three representative opportunistic pathogens, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (which are also part of the human gut microbiota), to determine their behaviors in mixed cultures (with each other) and co-culture with human gut epithelial HT29 cells. In so doing, this approach

better enables us to model how gut microbiota (in their native environment) could react when exposed to dust contaminants. In using 100- $\mu\text{g}/\text{mL}$  concentrations of both house and road dust previously determined to not adversely affect the growth rates of pure bacterial cultures (Suraju et al. 2015), we similarly observed that mixed culture growth rates are also not adversely affected in liquid culture. However, when we employed selective medium (EMB) to enumerate individual bacterial colonies within the mixed cultures, we observed that although bulk biomass remains unaffected (as observed in liquid cultures), some representatives within the mixed cultures appear to outgrow their paired companion strains. More specifically, when exposed to house (but not road) dust, *K. pneumoniae* appeared to outgrow *E. coli* in an *E. coli*–*K. pneumoniae* mixed culture. Similarly, when exposed to both house and road dust, *P. aeruginosa* appeared to outgrow *E. coli* in an *E. coli*–*P. aeruginosa* mixed culture.

We observed that mixed *E. coli*–*K. pneumoniae*, *K. pneumoniae*–*P. aeruginosa*, and *E. coli*–*P. aeruginosa* cultures exhibited increased sensitivity to 20 and 50 mM of  $\text{H}_2\text{O}_2$  in comparison to the pure bacteria culture. Studies have shown that the Cpx and  $\sigma\text{E}$  stress responses exist in many Gram-negative bacterial pathogens (Raivio et al. 1999; Raivio and Silhavy 2001; Ades 2004). It is possible that, only when grown independently, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, all Gram-negative bacteria possessing  $\sigma\text{E}$ , Cpx, and Bae envelope stress response factors, benefit from protection against a variety of stressors, including oxidative stress (Raivio 2005); however, when grown together in mixed culture, this stress response benefit becomes over-ridden by some unknown mechanism(s).

With regards to biofilm production, the specific type of dust exposure had disparate influences on the mixed culture biofilm production relative to their pure culture counterparts. For example, while exposure to both house and road dust significantly enhanced biofilm production in all three individual cultures, only road dust exposure was able to emulate that response for all three mixed cultures evaluated (i.e., *E. coli*–*P. aeruginosa*, *K. pneumoniae*–*P. aeruginosa*, and *E. coli*–*K. pneumoniae*). When exposed to house dust, only the *K. pneumoniae*–*P. aeruginosa* mixed culture produced significantly higher biofilm than the untreated mixed-culture controls. It is entirely possible that this disparity could be due, in part, to the unique contaminants found within each dust type. Another possible contributing factor could be due to bacterial competition/antagonism. In fact, some molecules produced by bacteria can influence the behavior and fitness of other species within the same ecosystem. In some cases, they can be beneficial, but in most cases they are antagonistic (Iwase et al. 2010).

In co-culture with gut HT29 cells following road dust challenge, we observed *E. coli*, *K. pneumoniae*, and *P. aeruginosa* all exhibiting significantly enhanced proliferation over a 6-h infection period than the untreated controls. Curiously, of the three organisms evaluated, only *E. coli* did not respond to

house dust challenge with significantly enhanced proliferation, likely owing to the different components within each dust type (Suraju et al. 2015). Perhaps the enhanced proliferation is due, in part, to the upregulation of adhesive pili, in response to dust exposure. For example, a *P. aeruginosa* and *Roseobacter denitrificans* co-culture study showed the activation of various defense mechanisms (Conway et al. 2012), while another study revealed enhanced fermentation of mixed microbial communities (Anburajan et al. 2017). With regards to *K. pneumoniae*, it uses two adhesive pilus structures, the mannose-sensitive type 1 pili (T1P), composed of a major fimbrial FimA subunit and a minor tip adhesin FimH, and the mannose-resistant type 3 pili (T3P or MR/K), composed of the major pilus subunit MrkA and the minor tip adhesin MrkD (Gerlach et al. 1988; Gerlach et al. 1989). Perhaps contaminants in dust (indoor and outdoor) can trigger an upregulation of these different pilus components promoting enhanced cell adhesion and subsequent proliferation in the HT29 co-cultures. Although our gross level-protein expression analysis did not reveal any dramatic differences in protein levels following dust exposure at various time points, it is still entirely possible that differences in some key stress-response factor levels could be at play, accounting for at least some of the physiological responses we have observed. In the future, it would be useful to combine and monitor some of the changes in gene expression of our study's suggested factors and proteins (e.g., pili,  $\sigma\text{E}$ , Cpx, and Bae factors) with these types of physiological and growth experiments. Ultimately, future studies will need to be conducted evaluating meta-transcriptomics coupled with mixed bacterial cultures co-cultured with HT29 cells to more accurately reflect changes to the diversity found in the human microbiome in response to dust contaminants.

**Acknowledgements** We would like to thank Dr. McClean from the University of Houston for sharing all bacterial strains with us. We would also like to thank Mohammed Suraju, Shari Galvin, Hyun-Min Hwang, and Daniel Vrinceanu for stimulating discussions and thoughtful criticism of our work. Finally, we would like to thank Nkem Azu for her assistance with the MTT assay performed. This work was supported by the National Science Foundation RISE (HRD-1345173; awarded to JR and SS).

**Compliance with ethical standards** This article does not contain any studies with human participants or animals performed by any of the authors.

**Funding** This study was funded by the National Science Foundation (HRD-1345173).

**Conflict of interest** The authors declare that she has no conflict of interest.

## References

- Ades SE (2004) Control of the alternative sigma factor  $\sigma^F$  in *Escherichia coli*. *Curr Opin Microbiol* 7:157–162
- Adriano DC (2001) Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals. Springer, New York
- Ahmed F, Ishiga H (2006) Trace metal concentration in street dusts of Dhaka city, Bangladesh. *Atmos Environ* 40:3835–3844
- Albering HJ, van Leusen SM, Moonen EJC, Hoogewerff JA, Kleinjans JCS (1999) Human health risk assessment: a case study involving heavy metal soil contamination after the flooding of the river Meuse during the winter of 1993–1994. *Environ Health Perspect* 107:37–43
- Ananthkrishnan AN, McGinley EL, Binion DG, Saeian K (2011) Ambient air pollution correlates with hospitalizations for inflammatory bowel disease: an ecologic analysis. *Inflamm Bowel Dis* 17(5):1138–1145
- Anne Petiot A, Ogier-Denis E, Blommaert EFC, Alfred J, Meijer AJ, Codogno P (2000) Distinct classes of phosphatidylinositol 3'-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. *J Biol Chem* 275(2):992–998
- Anburajan P, Park JH, Sivagurumathan P, Pugazhendhi A, Kumar G, Choi CS, Kim SH (2017) Mixed-culture H2 fermentation performance and the relation between microbial community composition and hydraulic retention times for a fixed bed reactor fed with galactose/glucose mixtures. *J Biosci Bioeng* S1389-1723(16):30578–30573
- Baastrup R, Sørensen M, Balstrøm T, Frederiksen K, Larsen CL (2008) Arsenic in drinking-water and risk for cancer in Denmark. *Environ Health Perspect* 116:231
- Beamish LA, Osomio-Vargas AR, Wine E (2011) Air pollution: an environmental factor contributing to intestinal disease. *J Crohns Colitis* 5(4):279–286
- Bergh C, Torgrip R, Emenius G, Östman C (2011) Organophosphate and phthalate esters in air and settled dust—a multi-location indoor study. *Indoor Air* 21(1):67–76
- Berkowitz GS, Obel J, Deych E, Lapinski R, Godbold J, Liu Z, Landrigan PJ, Wolff MS (2003) Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort. *Environ Health Perspect* 111(1):79–84
- Bjerg A, Ronmark EP, Hagstad S, Eriksson J, Andersson M, Wennergren G, Toren K, Ekerljung L (2015) Gas, dust, and fumes exposure is associated with mite sensitization and with asthma in mite-sensitized adults. *Allergy* 70(5):604–607
- Butte W, Heinzow B (2002) Pollutants in house dust as indicators of indoor contamination. *Rev Environ Contam Toxicol Rev Environ Contam Toxicol* 175:1–46
- Chen H, Goldberg MS, Villeneuve PJ (2008) A systematic review of the relation between long-term exposure to ambient air pollution and chronic diseases. *Rev Environ Health* 23(4):243–249
- Colombo C, Monhemius AJ, Plant JA (2008) Platinum, palladium and rhodium release from vehicle exhaust catalysts and road dust exposed to simulated lung fluids. *Ecotoxicol Environ Saf* 71(3):722–730
- Conway CA, Esiobu N, Lopez JV (2012) Co-cultures of *Pseudomonas aeruginosa* and *Roseobacter denitrificans* reveal shifts in gene expression levels compared to solo cultures. *ScientificWorldJournal* 2012:120108
- De Brouwere K, Buekers J, Cornelis C, Schlekot CE, Oller AR (2012) Assessment of indirect human exposure to environmental sources of nickel: oral exposure and risk characterization for systemic effects. *Sci Total Environ* 419:25–36
- Du Y, Gao B, Zhou H, Ju X, Hao H, Yin S (2013) Health risk assessment of heavy metals in road dusts in urban parks of Beijing, China. *Procedia Environ Sci* 18:299–309
- Duong TT, Lee B (2011) Determining contamination level of heavy metals in road dust from busy traffic areas with different characteristics. *J Environ Manag* 92(3):554–562
- Frank DN, Robertson CE, Hamm CM, Kpadeh Z, Zhang T, Chen H, Zhu W, Sartor RB, Boedeker EC, Harpaz N, et al. (2011) Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. *Inflamm. Bowel Dis* 17:179–184
- Garrison VH, Majewski MS, Konde L, Wolf RE, Otto RD, Tsuneoka Y (2014) Inhalable desert dust, urban emissions, and potentially biotoxic metals in urban Saharan–Sahelian air. *Sci Total Environ* 500–501:383–394
- Gerlach GF, Allen BL, Clegg S (1988) Molecular characterization of the type 3 (MR/K) fimbriae of *Klebsiella pneumoniae*. *J Bacteriol* 170:3547–3553
- Gerlach GF, Clegg S, Allen BL (1989) Identification and characterization of the genes encoding the type 3 and type 1 fimbrial adhesins of *Klebsiella pneumoniae*. *J Bacteriol* 171:1262–1270
- Guinane CM, Cotter PD (2013) Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. *Ther Adv Gastroenterol* 6(4):295–308
- Hogervorst J, Plusquin M, Vangronsveld J, Nawrot T, Cuypers A, Van Hecke E, Roels HA, Carleer R, Staessen J (2007) House dust as possible route of environmental exposure to cadmium and lead in the adult general population. *Environ Res* 103(1):30–37
- Hough RL, Breward N, Young SD, Crout NMJ, Tye AM (2004) Assessing potential risk of heavy metal exposure from consumption of home-produced vegetables by urban populations. *Environ Health Perspect* 112(2):215–221
- Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, Agata T, Mizunoe Y (2010) *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature* 465(7296):346–349
- Kaplan GG, Hubbard J, Korzenik J, Sands BE, Panaccione R, Ghosh S, Wheeler AJ, Villeneuve PJ (2010) The inflammatory bowel diseases and ambient air pollution: a novel association. *Am J Gastroenterol* 105(11):2412–2419
- Kaplan GG, Tanyingoh D, Dixon E, Johnson M, Wheeler AJ, Myers RP, Bertazzon S, Saini V, Madsen K, Ghosh S (2013) Ambient ozone concentrations and the risk of perforated and nonperforated appendicitis: a multicity case-crossover study. *Environ Health Perspect* 121(8):939–943
- Kaplan GG, Szyszkwicz M, Fichna J, Rowe BH, Porada E, Vincent R, Madsen K, Ghosh S, Storr M (2012) Non-specific abdominal pain and air pollution: a novel association. *PLoS One* 7(10):e47669
- Karotki DG, Beko G, Clausen G, Madsen AM, Andersen ZJ, Massling A, Ketzel M, Ellermann T, Lund R, Sigsgaard T, Moller P, Loft S (2014) Cardiovascular and lung function in relation to outdoor and indoor exposure to fine and ultrafine particulate matter in middle-aged subjects. *Environ Int* 73:372–381
- Kau A, Ahern P, Griffin N, Goodman A, Gordon J (2011) Human nutrition, the gut microbiome and the immune system. *Nature* 474:327–336
- Ley R, Peterson D, Gordon J (2006a) Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124:837–848
- Ley R, Turnbaugh P, Klein S, Gordon J (2006b) Microbial ecology: human gut microbes associated with obesity. *Nature* 444:1022–1023
- Lomer MC, Hutchinson C, Volkert S, Greenfield SM, Catterall A, Thompson RP, Powell JJ (2004) Dietary sources of inorganic micro-particles and their intake in healthy subjects and patients with Crohn's disease. *Br J Nutr* 92(6):947–955
- MacIntosh DL, Spengler JD, Ozkaynak H, Tsai L, Ryan PB (1996) Dietary exposures to selected metals and pesticides. *Environ Health Perspect* 2:202–209

- Man YB, Sun XL, Zhao YG, Lopez BN, Chung SS (2010) Health risk assessment of abandoned agricultural soils based on heavy metal contents in Hong Kong, the world's most populated city. *Environ Int* 36:570–576
- Mari M, Nadal M, Schuhmacher M, Domingo JL (2009) Exposure to heavy metals and PCDD/Fs by the population living in the vicinity of a hazardous waste landfill in Catalonia, Spain: health risk assessment. *Environ Int* 35:1034–1039
- Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490:55–60
- Qu CS, Ma ZW, Yang J, Yang L, Jun Bi J, Huang L (2012) Human exposure pathways of heavy metals in a lead zinc mining area, Jiangsu Province, China. *PLoS One* 7(11):e46793
- Raivio TL (2005) Envelope stress responses and gram-negative bacterial pathogenesis. *Mol Microbiol* 56(5):1119–1128
- Raivio TL, Silhavy TJ. (2001) Periplasmic stress and ECF sigma factors. *Annu Rev Microbiol.* 55:591–624
- Raivio TL, Popkin DL, Silhavy TJ. (1999) The Cpx envelope stress response is controlled by amplification and feedback inhibition. *J Bacteriol.* 181(17):5263–72.
- Ravindra K, Bencs L, Van Grieken R (2004) Platinum group elements in the environment and their health risk. *Sci Total Environ* 318(1–3):1–43
- Sahlstrom LM, Sellstrom U, de Wit CA, Lignell S, Darnerud PO (2015) Estimated intakes of brominated flame retardants via diet and dust compared to internal concentrations in a Swedish mother–toddler cohort. *Int J Hyg Environ Health* 218(4):422–432
- Shoae S, Karlsson F, Mardinoglu A, Nookaew I, Bordel S, Nielsen J (2013) Understanding the interactions between bacteria in the human gut through metabolic modeling. *Sci Rep* 3:2532
- Simcox NJ (1995) Pesticides in household dust and soil: exposure pathways for children of agricultural families. *Environ Health Perspect* 103(12):1126–1134
- Suraju MO, Lalinde-Barnes S, Sanamvenkata S, Esmaeili M, Shishodia S, Rosenzweig JA (2015) The effects of indoor and outdoor dust exposure on the growth, sensitivity to oxidative-stress, and biofilm production of three opportunistic bacterial pathogens. *Sci Total Environ* 538:949–958
- Valko M, Morris H, Cronin MTD (2005) Metals, toxicity and oxidative stress. *Curr Med Chem* 12(10):1161–1208
- Weschler CJ (2009) Changes in indoor pollutants since the 1950s. *Atmos Environ* 43(1):153–169
- Whitehead T (2011) Estimating exposures to indoor contaminants using residential dust. *J Expo Sci Environ Epidemiol* 21:549–564
- Yongming H, Peixuan D, Junji C, Posmentier ES (2006) Multivariate analysis of heavy metal contamination in urban dusts of Xi'an, Central China. *Sci Total Environ* 355(1–3):176–186
- Zhang H, DiBaise J, Zuccolo A, Kudrna D, Braidotti M, Yu Y (2009) Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci U S A* 106:2365–2370
- Zheng N, Liu J, Wang Q, Liang Z (2010) Health risk assessment of heavy metal exposure to street dust in the zinc smelting district, northeast of China. *Sci Total Environ* 408(4):726–733