



The impact of prenatal exposure to air pollution on childhood wheezing and asthma: A systematic review



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ABSTRACT

Background and objectives: There has been no clear consensus about whether prenatal exposure to air pollution contributes to the development of wheezing and asthma in children. We conducted a systematic review to analyze the association between exposure to different pollutants during pregnancy and the development of childhood wheezing and asthma.

Methods: We systematically reviewed epidemiological studies published through June 6, 2017 available in the MEDLINE and Web of Science databases. We included studies that examined the association between prenatal exposure to any air pollutants except tobacco smoke and the incidence or prevalence of “wheezing” or “asthma” from birth to 14 years of age. We extracted key characteristics of each included study using a template of predefined data items. We used the Critical Appraisal Skills Programme checklists to assess the validity of each included study. We conducted overall and subgroup meta-analyses for each summary exposure-outcome association. Pooled odds ratios (OR) with 95% confidence intervals (CI) were estimated by using a random effects model.

Results: Eighteen studies met our eligibility criteria. There was notable variability in exposure assessment methods. The overall random effects risk estimates (95% CI) of different pollutants were 1.04 (0.94–1.15) aromatic hydrocarbons (PAH), 1.04 (1.01–1.07) NO₂, 1.4 (0.97–2.03) PM_{2.5} for childhood wheeze and 1.07 (1.01–1.14) NO₂, 1 (0.97–1.03) PM_{2.5}, 1.02 (0.98–1.07) SO₂, 1.08 (1.05–1.12) PM₁₀ for childhood asthma. Minimal heterogeneity was seen for PAH and SO₂, while some heterogeneity was observed for PM₁₀, PM_{2.5} and NO₂.

Conclusions: The overall and subgroup risk estimates from the meta-analyses showed statistically significant associations between prenatal exposures to NO₂, SO₂, and PM₁₀ and the risk of wheezing and asthma development in childhood. There is insufficient evidence to show an effect of prenatal exposure to BC, CO, and O₃ on childhood wheezing and asthma. Further studies are needed to examine the individual compounds' effects.

1. Introduction

It is believed that a complex interaction of environmental exposures and genetic susceptibility contributes to the development of wheezing and asthma (Spanier, 2015). Asthma, the most frequent non-infectious disease in children (Zar and Ferkol, 2014), affects 235 million people globally with an increasing prevalence in low to middle income countries. Air pollution is a complex and ubiquitous mixture of pollutants including particulate matter, chemical substances and biological materials (Mannucci et al., 2015). There is growing awareness of the adverse impacts of air pollution on health following both prenatal and postnatal exposure (Gruzieva et al., 2017; Gehring et al., 2015).

There is substantial evidence showing that postnatal air pollution

can exacerbate pre-existing wheezing and asthma (Orellano et al., 2017). Postnatal exposure to the main components of air pollution (nitrogen dioxide (NO₂), fine particulate matter (diameter < 2.5 μm, PM_{2.5}; diameter < 10 μm PM₁₀), black carbon (BC), sulphur dioxide (SO₂), etc.) is correlated with an increased incidence of wheezing and asthma (Khreis et al., 2017; Fan et al., 2016; Veras et al., 2017). Among observational and systematic studies examining prenatal exposure to substances, maternal tobacco, folic acid, paracetamol, and biphenyl A use were all correlated with an increased incidence of childhood asthma (Rosas-Salazar and Hartert, 2017; Brown et al., 2014; Xie et al., 2016). Until now, there has been no clear evidence regarding the relationship between prenatal air pollution exposure and childhood wheezing and asthma. This is likely attributable, at last in part, to the difficulty in

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conducting adequate epidemiological studies required to address this question.

Earlier studies and reviews primarily focused on the impact of postnatal and early life air pollution on the development of asthma and related symptoms during childhood. These studies generally showed significant effects (Lee et al., 2017; Prieto-Parra et al., 2017; Ranciere et al., 2017). There are also many original studies evaluating the impact of prenatal exposure to tobacco smoke (Underner et al., 2015) and indoor air pollution (mold, house dust mites, mildew, cooking fumes, etc.) on the development of childhood asthma and wheezing (Underner et al., 2015; Vanker et al., 2015; Bruce et al., 2000). These studies have shown that tobacco exposure during pregnancy is positively correlated with childhood asthma and wheezing.

The prenatal period could represent a critical exposure window for fetal lung development (Lacasana et al., 2005). The impact of air pollution exposure on maternal health can directly impact neonatal growth and the developmental of the fetal immune system (Vieira, 2015; Wong et al., 2015).

Studies have shown that air pollution during pregnancy can lead to adverse neonatal and pregnancy outcomes (Proietti et al., 2013; Filippini et al., 2015), as well as poor pediatric health and even the development of conditions including lung disease, respiratory problems, leukemia, retinoblastoma, and cognitive disorders., etc. the impact of air pollution exposure on mother's health could directly affect neonates' growth and immune system development (Vieira, 2015; Wong et al., 2015).

2. Objectives

In this systematic review, we provide a contemporary synthesis of observational epidemiological studies examining the association between prenatal air pollution exposure and the subsequent development of asthma and wheezing in children from birth to 14 years of age. We hypothesize that prenatal exposure to major air pollutants (PM_{2.5}, NO₂, SO₂, PM₁₀, PAH) increases the risk of subsequent asthma and wheezing.

To our knowledge, this is the first systematic review specifically focused on prenatal air pollution exposure and its impact on childhood asthma and wheezing. We selected wheezing as one of the key study points because it is one of the most important components in making the diagnosis of asthma and is directly correlated with pediatric respiratory health, and the inclusion of wheezing as an outcome might help with under-diagnosis due to lack of access to healthcare in the US studies.

This paper synthesized case control and cohort studies with no start time limitations, specifically focused on prenatal air pollution exposures as a potential cause for childhood wheezing and the developmental of asthma. The aims of this systematic review included: 1) to increase study power in order to effectively detect associations between prenatal air pollution exposure and the development of childhood wheezing and asthma with increased precision and 2) to explore the impact of different pollutants and potential drivers of heterogeneity.

3. Methods

We conducted this systematic review in accordance with established guidelines published by the University of York's Centre for Reviews and Dissemination (Akers et al., 2009). We completed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (Stroh et al., 2012), attached in the [Supplementary materials](#).

3.1. Search methods

Searches were performed on June 6, 2017. We searched the following databases: Pubmed and Web of Science. Relevant studies were identified using the searching strategies listed below. To enhance a high recall ratio, we did not use terms such as prenatal, perinatal, or

pregnancy to minimize limitation of retrieved results.

#1: (air pollution) or (carbon monoxide) or (sulphur dioxide) or (nitrogen dioxide) or (ozone) or (particulate matter) or (air pollutants) or (PM₁₀) or (PM_{2.5}) or (traffic pollution) or (outdoor pollution) or (PAH) or (polycyclic aromatic hydrocarbons)

#2: (asthma) or (wheeze) or (respiratory tract allergy) or (respiratory sounds) or (bronchial hyperactivity)

#3: children OR childhood

#1 AND #2 AND #3

We applied no limits on the initial publication date. We subsequently hand searched the reference lists of all included studies and of previous reviews on this topic (Veras et al., 2017; Korten et al., 2017; Goldizen et al., 2016; Webb et al., 2016), and one extra study (Olsson et al., 2014) was found. Studies were exported into a NoteExpress 3.2 library and duplicates were automatically removed.

3.2. Inclusion criteria

We selected studies that met all of the following criteria:

- (1) Published epidemiological/observational studies including case-control, cohort and cross-sectional studies. We elected to include these types of studies since they can all offer evidence regarding risk factors for disease onset if appropriately designed;
- (2) Explicitly specified the term “asthma” or “wheeze” in children as an outcome for investigation;
- (3) Examined prenatal exposure of the mother to any designated air pollutant including carbon monoxide (CO), black carbon (BC), nitrogen oxides (NO_x), nitric oxide (NO), nitrogen dioxide (NO₂), hydrocarbons, diameter of Particulate Matter within 2.5 μm (PM_{2.5}), diameter of Particulate Matter within 10 μm (PM₁₀), or polycyclic aromatic hydrocarbons (PAH); and
- (4) Examined and reported associations between preceding maternal exposure to air pollution and subsequent risk of childhood asthma or wheezing reported as incidence or lifetime prevalence from birth until 18 years of age.

We excluded studies that:

- (1) Were reviews, commentaries, governmental reports, letters, or animal and experimental studies, conference papers;
- (2) Only examined postnatal air pollution or indoor air pollution;
- (3) Only examined air pollution metrics including indoor air pollution, proximity to point sources and tobacco smoke;
- (4) Only examined the association between the exposure and other allergic or respiratory diseases or symptoms excluding asthma and wheezing;
- (5) Only examined adulthood asthma.

3.3. Studies selection

Titles and abstracts of all records were screened. A random 20% of studies were independently screened. All potentially relevant studies were retrieved and full manuscripts were reviewed against the inclusion criteria by HH with a random 50% of studies independently reviewed.

3.4. Data extraction

Data was extracted by using a predefined data template (Khreis et al., 2017). Data was primarily extracted from the main bodies of the included studies, and when necessary information was missing from the main manuscripts, data was extracted from the [Supplementary materials](#) and associated publications. Data extraction was undertaken manually (Deng et al., 2016; Sbihi et al., 2016; Liu et al., 2016; Aguilera et al., 2013; Rosa et al., 2011; Esplugues et al., 2011; Clark et al., 2010; Jedrychowski et al., 2010, 2005, 2009, 2014; Miller et al., 2004;

Jerzynska et al., 2017; Hsu et al., 2015; Chiu et al., 2014; Jung et al., 2011; Patel et al., 2011).

3.5. Quality assessment

Using the checklists and procedure provided in the Critical Appraisal Skills Programme (CASP), we evaluated each study's validity across six key parameters: (1) potential for selection bias; (2) outcome measurements or classification bias; (3) exposure measurements, recall or classification bias; (4) identification of and adjustment for important confounders; (5) length and completion of follow-up; and (6) any special characteristics. The CASP checklists are provided in the form of twelve questions for cohort studies. These checklists are designed to help the assessor evaluate the validity of each study and are answered by a "yes," "no," or "can't tell." The cohort study checklist was used for cross-sectional studies. All included papers were independently evaluated.

3.6. Analysis

For each given outcome in every study, we sought to obtain an odds ratio (OR) or relative risk (RR) with a 95% confidence interval (CI) for any prenatal exposure to the following pollutants: PAH, NO₂, SO₂, PM₁₀, PM_{2.5}, CO, O₃, and BC. Meta-analyses were performed to calculate weighted effect measures and 95% confidence intervals across studies by using random effects models using the DerSimonian and Laird methods. Heterogeneity was expected across the various methods used to estimate pollutant exposure and across the age distribution of children. Heterogeneity was assessed using I² and subgroup analyses were conducted to investigate potential explanations. The presentation of the meta-analyses adhered to the Meta-analysis of Observational Studies in Epidemiology guidelines. All analyses were performed using Stata version 10.

4. Results

Our search yielded 8618 unique articles and from this, 22 records were identified for full-text review (Fig. 1). Eighteen studies published between 2004 and June 6, 2017 met our inclusion criteria. Table 1 provides a summary of each study. Ages of participants ranged from 0

to 10 years old. Air pollutants exposure levels were measured during the whole (or the second) period of pregnancy. Sample sizes ranged from 152 to 63,542 participants. Follow-up periods ranged from 1 to 10 years. Seven studies were conducted in Europe, 9 in North America, and 2 in China. Fourteen studies were cohort studies, two were nested case control studies in a birth cohort, and 2 studies were cross-sectional in design.

4.1. Outcome definitions

All included studies met our inclusion criteria. The majority (15) of studies exclusively relied on responses to questionnaires using parental or self-reporting of doctor-diagnosed asthma and wheezing. Three studies relied on confirmed asthma/wheezing diagnoses from pediatricians/allergists, physician records of diagnoses/admissions, and records of billing/discharge, respectively.

4.2. Exposure assessment methods and pollutant studies

There were various models and methods used to assess pollutant exposure levels during pregnancy according to the different kinds of pollutants. Land-use regression (LUR) models, which utilize targeted pollutant concentrations measured at a limited number of sites (usually 20–100 sites) and land use characteristics derived from a geographic information system (GIS) to predict pollutant concentrations at unmeasured locations, were predominantly (9 studies) used to calculate traffic-related air pollutant exposure, such as NO₂, SO₂, PM₁₀, and NO. Seven studies involved the wearing of personal monitoring samplers to measure air pollution exposure levels. Of these studies, five evaluated PAH exposure and two evaluated PM_{2.5}. Two studies used cord blood and urine sample test results to determine PAH exposure levels. Three studies used the Inverse Distance Weighted (IDW) method, which is another methodology that estimates air pollution concentrations at the destination area based on data obtained at monitoring stations.

In studies using measured or modeled pollutant concentrations in main analyses to represent prenatal exposures, PAH and NO₂/X were the pollutants most studied, with 7 studies each, followed by PM_{2.5} (6 studies), SO₂ and PM₁₀ (4 studies each), BC (3 studies), and NO, CO and O₃ (2 studies each).

Prenatal air pollution exposures were predominantly assigned based

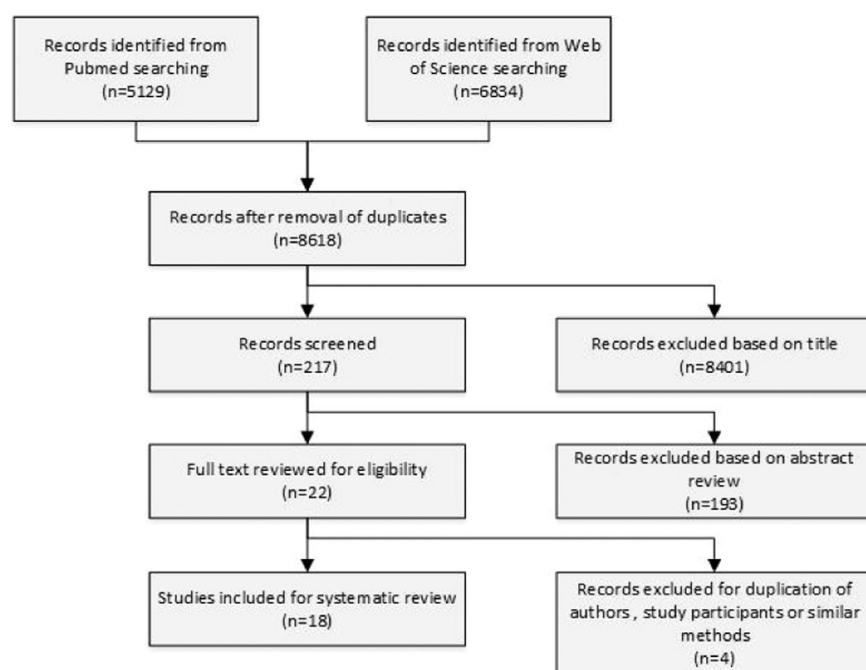


Fig. 1. Flow chart of study screening process.

Table 1
Main characteristics of studies included in the systematic review.

City, country	Reference (year, author)	Study Design	Maternal Age	Age Group	Participants	Exposure Assessment Method	Pollutants	Pregnancy Phase	Related Outcome	Incidence of Cases	Outcome Definition	Adjustment Variables	CASP Notes
Lodz, Polish	Jerzynska et al. (2017)	cohort	Not available	1 y.o–2 y.o	455	urine samples	PHA	second/third clinic visit during pregnancy	wheezing	36(12.1%)	pediatrician /allergist	number of infections and paternal smoking	no clear distinction of prenatal versus postnatal exposure to PAH, no assessment of the level of bias in the sample
Changsha, China	Deng et al. (2016)	cohort	Not available	3 y.o–6 y.o	2598	IDW	NO ₂ SO ₂ PM ₁₀	Three trimesters	asthma	175(6.8%)	Parents report doctor diagnosed	child's sex, age, birth season, breastfeeding, parental atopy, maternal productive age, parental SES by house size, ETS, new furniture, house re-decoration, visible mold/damp stains, window condensation in winter, mosquito repellent use, other air pollutants in the same time window, effects of the air pollutant in other time windows	Excluding low birth weight/premature birth children may bias results towards the null, no adjustment for socioeconomic status, potential for selection bias by excluding kindergartners with low response rates and others with missing data
British Columbia, Canada	Hind et al. (2016)	nested case-control based on cohort	Not available	0 y.o–5 y.o/ 6 y.o–10 y.o	58306/ 63543	IDW/LUR	NO ₂ PM _{2.5} NO CO O ₃ BC SO ₂ PM ₁₀	duration of pregnancy	asthma	6948(11.9%)/ 1711(2.7%)	doctor diagnosis, admission	breastfeeding status at the time of discharge, parity, maternal education (in area-level quartiles), household income (in area-level quintiles), gestational length and birthweight	Administrative data are not collected for research purposes and lack individual-level information (e.g. socioeconomic status measures), and therefore may be subject to residual confounding, no adjustment for heredity
Shanghai, China	Liu et al. (2016)	Cross-sectional	Not available	4 y.o–6 y.o	3344	dispersion model	NO ₂ SO ₂ PM ₁₀	gestational period	asthma	274(8.4%)	doctor diagnosis, mother report questionnaire	age, sex, family history of atopy, ownership of residence, breastfeeding, home dampness, distance of residence from the nearest main traffic road, usage of heating during	Potential for recall and reporting bias due to the retrospective questionnaire study, higher likelihood that exposures include other sources of emissions

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Table 1 (continued)

City, country	Reference (year, author)	Study Design	Maternal Age	Age Group	Partici- pants	Exposure Assessment Method	Polluta-nts	Pregnancy Phase	Related Outcome	Incidence of Cases	Outcome Definition	Adjustment Variables	CASP Notes
Brigham , Boston , Ameri- ca	Hsu et al. (2015)	cohort	≥ 18	1–6 y.o	736	LUR & spatio- temporal model	PM _{2.5}	over the pregnancy	asthma	110(15%)	Maternal reported, clinician-diagnosed	winter, renovating the residence or buying new large furniture during early lifetime and household ETS	Potential for recall and reporting bias due to the questionnaire report, focused on a higher-risk sample may lead to selected bias
Boston, Massachusetts, America	Chiu et al. (2014)	cohort	≥ 18	0.3–2 y.o	708	LUR	PM _{2.5} BC	duration of pregnancy	> 2 episodes constituting repeated wheezing	85(12%)	mother report	gender, race/ ethnicity, maternal education, birth season, maternal atopy, and cockroach antigen	focused on community violence and ambient air pollution, higher likelihood that exposures include other sources of emissions, lacking report of adjustment of confounders
Krakow, Poland	Jedrychowski et al. (2014)	cohort	18–35	1 y.o–4 y.o	257	personal monitor	PHA	second trimester	wheezing	40%	Parental report	gender of child, season of birth, parity, maternal education, maternal atopy, prenatal or postnatal exposure to PAH, ETS	relatively short follow-up period and a limited size of the subsample
Stockholm, Swedish	Olsson et al. (2014)	cohort	Not available	1–6 y.o	78770	dispersion model	NOX	gestational period	asthma	9.70%	receiving prescribed asthma medication during the 6th year of life	family history, gestational age, born preterm, and other factors not available	exposures include other sources of emissions, exposure estimated methods are of low accuracy
Asturias/Gipuzkoa/ Sabadell/ Valencia, Spanis- h	Aguilera et al. (2013)	cohort	≥ 16	1 y.o–1.5 y.o	2199	LUR	NO ₂	duration of pregnancy	wheezing	804(37.5%)	interviewer-led questionnaires	sex, age, duration of breastfeeding, child care attendance, siblings at birth, paternal allergy, parental asthma, maternal age at delivery, maternal education, pre- pregnancy body mass index, maternal smoking during pregnancy, paternal postnatal	Potential for recall and reporting bias due to the retrospective questionnaire study

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Table 1 (continued)

City, country	Reference (year, author)	Study Design	Maternal Age	Age Group	Partici- pants	Exposure Assessment Method	Polluta-nts	Pregnancy Phase	Related Outcome	Incidence of Cases	Outcome Definition	Adjustment Variables	CASP Notes
Manhattan/South Bronx-New York, America	2006 Rosa et al. (2011)	cohort	Not available	1 y.o–6 y.o	290	sampler	PHA	prenatal period	asthma, current wheezing	98(33.8)/ 98(33.8)	doctor diagnosis, mother report	smoking continued at 32 weeks of gestation (log- transformed) maternal asthma, maternal education, race/ ethnicity and sex.	significant ETS- derived PAH contributions, to the total PAH concentrations that were measured cannot be discounted Potential for recall and reporting bias due to the retrospective questionnaire study, lack reporting of adjustment of confounders, no adjustment for heredity
Columbia Center, Canada	Jung et al. (2011)	cohort	Not available	5 y.o/6 y.o	152	sampler	PAH(Σ PAH nonvolatile, pyrene)	prenatal period	current asthma	not available	validated questionnaires	not available	Due to the limitation concerning the number of subjects, results should be interpreted with caution, no adjustment for heredity
Valencia, Spain	Esplugues et al. (2011)	cohort	Not available	1 y.o	706	LUR	NO ₂	entire pregnancy and each trimester	wheezing	25.80%	parental report	sex, smoking at week 12 of pregnancy, number of persons who live together, attendance at a day-care center, caution, no zone of residence, season of birth	Study of Dominicans and African Americans, subjects included in analysis had lower asthma proportions than fully enrolled cohort, no adjustment for heredity
New York, America	Patel et al. (2011)	cohort	Not available	1 y.o–5 y.o	2276	LUR	air pollution combined effect	prenatal address	Asthma, wheezing	373(16.3%)/ 580(25.5%)	parental report	presence of smoker in the home, annual income, residential cockroach allergen concentration, residential mouse allergen concentration, age, and age by GIS variable interaction	Study of Dominicans and African Americans, subjects included in analysis had lower asthma proportions than fully enrolled cohort, no adjustment for heredity, potential for recall bias in defining the outcome
British Columbia, Canada	Clark et al. (2010)	nested case- control based on cohort	31 $\pm$ 5	3 y.o–4 y.o	37401	LUR/IDW	NO ₂ , PM _{2.5} NO CO O ₃ BC SO ₂ P.M. ₁₀	duration of pregnancy	asthma	3482(9.3%)	billing/discharge records	parity, breastfeeding, income quintile (neighborhood level), maternal education status (neighborhood level), birth weight, and	Young age for accurate diagnosis, exclusion of low birth weight/ premature birth children may bias results towards the null, socioeconomic status variables (continued on next page)

Table 1 (continued)

City, country	Reference (year, author)	Study Design	Maternal Age	Age Group	Partici- pants	Exposure Assessment Method	Polluta-nts	Pregnancy Phase	Related Outcome	Incidence of Cases	Outcome Definition	Adjustment Variables	CASP Notes
New York, America	Jedrychowski et al. (2010)	cohort	18–35	1–2 y.o/ 3–4 y.o	339	Maternal /cord blood test/ Monitoring Sampler	PHA PM _{2.5}	post- partum/ second trimester	wheezing	139(41%)	follow-up interview	gender of child, maternal education, parity, maternal atopy, prenatal exposure to ETS and fine particles and mold dampness in household	assigned at the Census dissemination level, no adjustment for heredity no clear distinction between the effect of prenatal PAH and PM _{2.5} exposure from that of postnatal exposure
Krakow, Poland	Jedrychowski et al. (2009)	cohort	18–35	1–2 y.o	468	Monitoring Sampler	PM _{2.5}	prenatal period	wheezing	126(26.9%)	Parental report	gender of child, maternal education, parity, maternal atopy, prenatal ETS, prenatal exposure to fine particles and moldy/damp household	No clear distinction between the effect of prenatal PM _{2.5} exposure from that of postnatal exposure, uncertain whether delayed or immediate effects of prenatal PAHs exposure
Krakow, Poland	Jedrychowski et al. (2005)	cohort	18–35	1 y.o.	331	personal monitor	PHA	second trimester	Wheezing in the chest, Wheezing without cold	61(18.3%)/ 7(2.1%)	questionnaire occurrence of a specific symptom over at least 1 day	Adjusted for gender of child, child's birth weight, season of birth, ETS in postnatal period, mother's allergy, mother's education level, molds at home	No clear distinction between the effect of prenatal from postnatal PAHs exposure, uncertain whether delayed or immediate effects of prenatal PAHs exposure
Washington, America	Miller et al. (2004)	cohort	24.5 ± 5	1 y.o–2 y.o	264	monitoring sampler	PHA	trimester of pregnancy	asthma, wheezing	—	questionnaire	history of asthma in the mother, birth weight, or PAH measurement during the heating season	uncertain whether findings represent delayed effects of prenatal PAH exposure on children or more immediate effects

on the participants' residential addresses and monitoring samplers' data, with a few exceptions where routine measurements from fixed-site stations near children's nurseries and urine/blood samples were used to quantify exposures.

4.3. Quality assessment

Results from the CASP assessment are attached in the [Supplementary \(S1\) material](#). Overall, we found that most of the selected studies are of a sufficiently high quality to accurately evaluate the relationship between prenatal air pollution exposure and the development of wheezing and asthma. Some of the limitations identified relate to inaccurate exposure measurements, evaluation of asthma and wheezing by questionnaires, and failure to adjust for important confounders and imprecise results.

4.4. Wheezing & asthma risks in association with prenatal air pollution exposures

Due to the obvious variance in different exposure measurements, the absence of data on exposure levels, adjustments for various confounders and the limited number of suitable quantitative studies for each kind of pollutant, we were unable to apply the meta-analysis to every pollutant except a few pollutants (PAH, NO₂, SO₂, PM₁₀). The following are the qualitative and quantitative reviews of studies of each exposure.

4.4.1. Risks in association with prenatal PAH exposure

There were seven studies evaluating the correlation between prenatal PAH exposure levels and childhood 1–6 years of age) wheezing (6 studies) and asthma (1 study). A pooled analysis of the six relevant studies revealed that prenatal exposure to PAH was associated with a non-significant increase in risk of onset of wheezing in children (OR = 1.04, 95%CI = 0.94–1.15, I² = 7.8%). Due to a markedly different method of PAH measurement in one study (Jerzynska et al., 2017; Jedrychowski, 2010), we conducted a subgroup analysis by urine or cord blood testing and estimation of PAH exposure by personal monitor, which showed contradictory effect (Table 2) with lower heterogeneity.

Jung et al. (2011) dichotomized PAH into Σ8PAH_{nonvolatile} and pyrene and found a positive association between high pyrene levels and current asthma (OR = 3.01, 95%CI: 1.2–7.5). This study also revealed an inverse association between high Σ8PAH_{nonvolatile} exposure and current asthma (OR = 0.31, 95%CI: 0.11–0.91) and wheezing (OR = 0.24, 95%CI: 0.09–0.67) among non-atopic children. Miller et al. (2004) found that prenatal exposure to PAH alone has no significant effect on wheezing or probable asthma diagnosis but had a positive effect on wheezing (1 year olds, OR = 1.29, 95% CI: 1.03–1.61) and probable asthma diagnosis (2 year olds OR = 1.64, 95% CI: 1.10–2.45) in conjunction with ambient tobacco (ETS) exposure (median PAH: 3.65 ng/m³).

4.4.2. Risks in association with prenatal NO₂/X exposure

There were seven studies focused on the correlation between prenatal NO₂ exposure and childhood 0–10 years of age) wheezing or

asthma occurrence. Two studies were included for pooled analysis of prenatal NO₂ exposure and showed significantly increased risk of childhood wheezing (OR = 1.04, 95% CI = 1.01–1.07, I² = 0.0%). Pooled analysis of four studies showed that prenatal NO₂ exposure was significantly associated with the occurrence of childhood asthma (OR = 1.07, 95% CI = 1.01–1.14, I² = 85%) (see Fig. 2). Heterogeneity may be caused by different NO₂ estimation methods (IDW and LUR) and age differences. Subgroup analysis of estimation methods exhibited positive significant correlation between IDW estimation of NO₂ and asthma, while negative non-significant results with LUR estimation and asthma (Table 3). After excluding one study with results from 6 to 10 year old children, pooled analysis enhanced the effect of prenatal NO₂ exposure on asthma occurrence among children aged 0–5 years old (OR = 1.12, 95% CI = (1.04–1.19), I² = 84%).

A conference paper (Olsson, David, 2014) from a registry-based cohort in Sweden found no association between NO_x levels during pregnancy or infancy and asthma; the trend was found to be negative (ORs = 0.97 and 0.98, respectively). A significantly positive association (Clark, NA, 2010) between asthma incidence 3–4 year olds) and in utero exposure to NO (IDW estimated OR = 1.07, 95% CI: 1.03–1.72, LUR estimated OR = 1.05, 95% CI: 1.02–1.09) was found in another BC birth cohort.

4.4.3. Risks in association with prenatal PM_{2.5} exposure

There were six studies focused on the association between prenatal PM_{2.5} exposure and childhood wheezing or asthma. Two and three studies were included in the pooled analysis of prenatal PM_{2.5} exposure to evaluate wheezing and asthma, respectively; the results were all non-significant (OR = 1.4, 95% CI = 0.97–2.03, I² = 37.6%; OR = 1, 95% CI = 0.97–1.03, I² = 70.7%). Jedrychowski et al. (2009) found in a Polish cohort study that the effect of PM_{2.5} on childhood wheezing was very weak.

4.4.4. Risks in association with prenatal SO₂ exposure

There were four studies evaluating the association between prenatal SO₂ exposure and childhood asthma occurrence. Pooled analysis showed a positive non-significant effect of prenatal SO₂ exposure on childhood asthma (OR = 1.02, 95%CI = 0.98–1.07, I² = 62.8%) (Fig. 3). After excluding the one study evaluating children aged 6–10 years, pooled analysis was significant with no heterogeneity (OR = 1.03, 95% CI = (1.02, 1.05), I² = 0.0%), which suggested that prenatal exposure to SO₂ was associated with an increased risk of asthma occurrence among children aged 0–5 years.

4.4.5. Risks in association with prenatal PM₁₀ exposure

There were four studies focused on the association between prenatal PM₁₀ exposure and childhood asthma occurrence. Pooled analysis showed a significantly positive effect of prenatal PM₁₀ exposure on childhood asthma (OR = 1.08, 95% CI = 1.05–1.12, I² = 30.4%). After excluding the one study evaluating children aged 6–10 years, pooled analysis was non-significant with higher heterogeneity (OR = 1.05, 95% CI = 0.99–1.12, I² = 40.3%) (Fig. 4). This may suggest that prenatal exposure to PM₁₀ was associated with increased risk of asthma occurrence among children aged 0–10 years.

Table 2
Prenatal air pollution exposure and childhood wheezing.

Prenatal Exposure	No. of Studies	Pooled OR/RR	95%CI	I ² %	Subgroup Analysis (pooled OR/RR, 95%CI, I ² %)	Sensitively analysis by excluded one study with children aged 6–10 years old
PAH	6	1.04	0.94–1.15	7.80%	measurement by urine/blood test: 0.84 (0.55, 1.28), 0.0% measurement by personal monitor: 1.05 (0.97, 1.13), 17.0%	none
NO ₂	3	1.04	1.01–1.07	0%	none	none
PM _{2.5}	2	1.4	0.97–2.03	37.60%	none	none

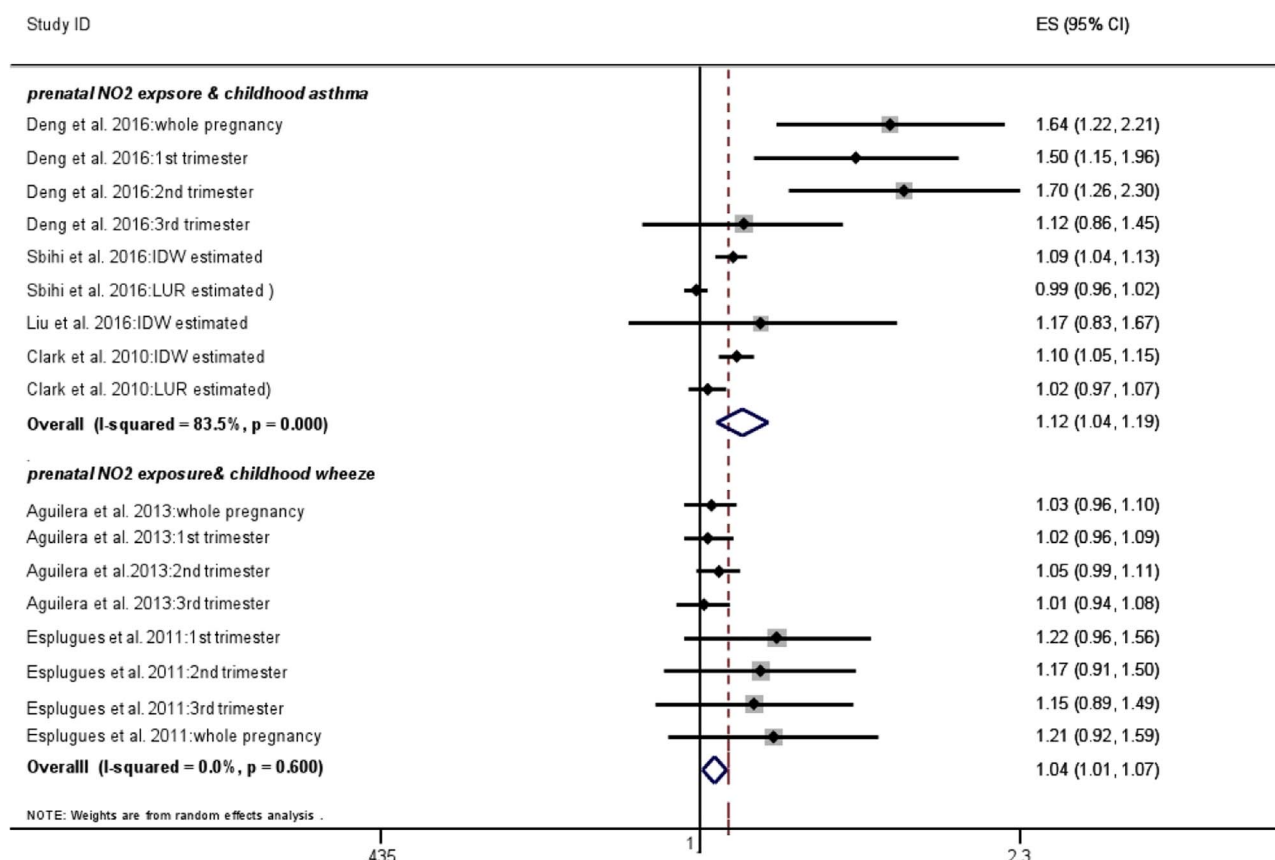


Fig. 2. Prenatal exposure to NO₂ and development of wheezing and asthma among children aged 0–6 years.

Table 3
Prenatal air pollution exposure and childhood asthma.

Prenatal Exposure	No. of Studies	Pooled OR/ RR	95%CI	I ² .%	Subgroup Analysis (pooled OR/RR, 95%CI, I ² .%)	sensitively analysis by excluded one study with children aged 6–10 years old
NO ₂	4	1.07	1.01–1.14	85%	IDW: 1.16 (1.05–1.28), 83.2% LUR: 0.99 (0.96–1.02), 41%	1.12 (1.04–1.19), 84% none
PM _{2.5}	3	1	0.97–1.03	70.70%	none	none
SO ₂	4	1.02	0.98–1.07	62.80%	none	1.03 (1.02, 1.05), 0.0%
PM ₁₀	4	1.08	1.05–1.12	30.40%	none	1.05 (0.99–1.12), 40.3%
CO	2	1.015	0.94–1.09	86.60%	none	1.06 (1.04–1.09), 0.0%
O ₃	2	1.05	0.91–1.21	92.00%	none	none

4.4.6. Risks in association with other pollutants during pregnancy

There were three studies evaluating the association of prenatal exposure to other pollutants (including black carbon (BC), CO and O₃) and the development of childhood asthma. The effect of prenatal exposure to BC was non-significant in 0 to 2 year old and 3–4 year old children (ORs=1.01, 95% CI: 0.99–1.04; ORs=0.99, 95% CI: 0.95–1.05). Results of a pooled analysis of prenatal exposure to CO was non-significant with large heterogeneity (OR=1.02, 95% CI=0.94–1.09, I²=86.6%); the effect was significant (OR=1.06, 95% CI=(1.04–1.09), I²=0.0%) after excluding the one study examining children aged 6–10 years old with no heterogeneity. Pooled analysis of O₃ showed no significant effect of prenatal exposure on childhood asthma occurrence.

5. Discussion

5.1. Overview, strengths and limitations

In this systematic review, we synthesized 18 studies published between 2004 and June of 2017, investigating the association between

prenatal exposure to various air pollutants and subsequent development of childhood wheezing and asthma. We conducted an overall meta-analysis and estimated statistically significant random-effects risk estimates following PAH, NO₂, PM_{2.5}, SO₂ and PM₁₀ exposures. Across the overall meta-analysis and subgroup analyses, the least heterogeneity was seen for PAH and SO₂. Intermediate heterogeneity was seen for PM₁₀ and NO₂ exposure and the greatest heterogeneity was seen for PM_{2.5}, CO and O₃ exposure. Overall, we noted significant variability in different exposure assessment methods and confounder adjustments.

To our knowledge, this is the first and largest review and analysis of current evidence examining the impact of prenatal air pollution exposure on the development of childhood wheezing and asthma. Our systematic review provides evidence for positive associations between prenatal air pollution exposure and childhood wheezing and asthma. Our results are concordant with most previous similar individual studies. The key strengths of our synthesis are its outcome evaluation and its focus on a limited age range 0–5 years of age). In addition, our study specifically focused on the impact of prenatal exposures on subsequent wheezing or asthma in children. This provides a timely contribution to a rapidly evolving field which can dictate the focus and design of future

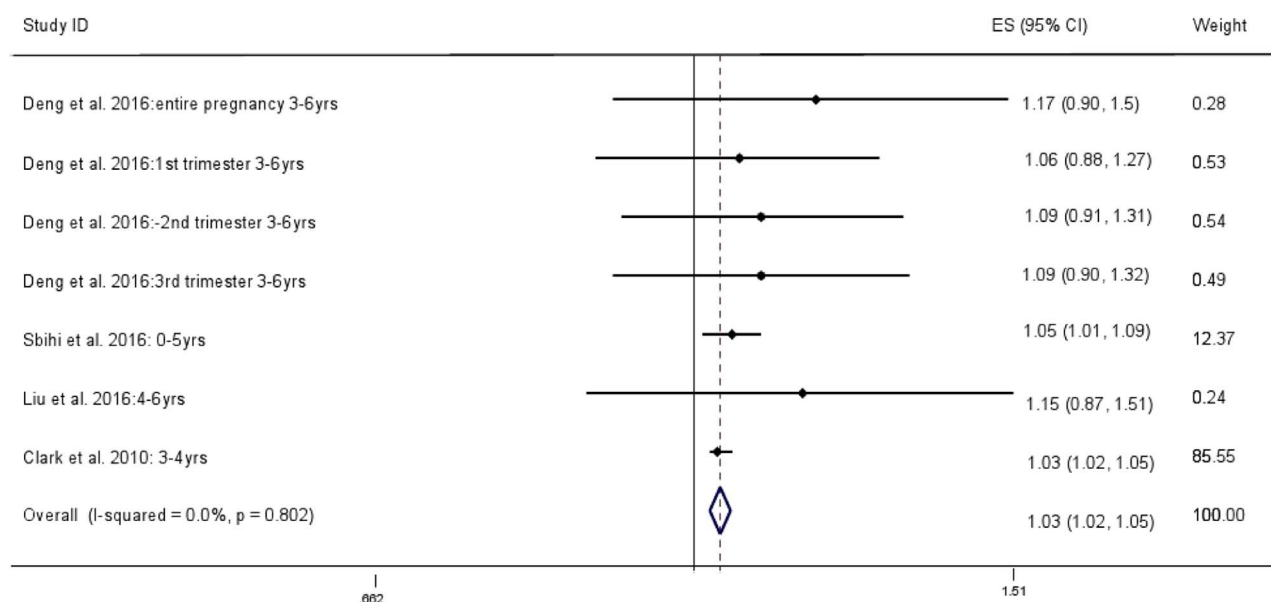


Fig. 3. Prenatal exposure to SO₂ and development of asthma among children aged 0–6 years.

research endeavors.

This study does have some limitations. Due to the limited number of original studies evaluating the association between prenatal air pollution exposure and childhood asthma, the evidence concluded from our synthesis should be confirmed by further studies. Pollutant assessment methods in our studies varied between different pollutants. For example, IDW was utilized to estimate NO₂ exposure; this method is known to have lower accuracy in estimating personal exposure levels than the LUR method. Personal monitors were used for determining PM_{2.5} exposure estimates, which likely resulted in robust evidence in that synthesis. Restriction of analysis to studies published in English is another potential limitation, though in practice this excluded only three non-English language studies. We mainly focus on outcomes of children

at ages 0–5, asthma diagnosis at these ages are most of uncertainty, the high rate of wheezing also may be due to respiratory infection, which might result in false positive correlation between air pollution and wheeze and further studies should be concerned.

5.2. Studied pollutants and meta-analysis interpretation

The focus on evaluating PAH effects was related to its wide distribution in daily life and the availability of its measurement. The majority (6 out of 7) of original studies were focused only on prenatal PAH exposure and childhood wheezing. The subgroup analysis driven two opposite but non-significant effects; subgroup analysis did not change the effects. One study chemically analyzed the contents of PAH

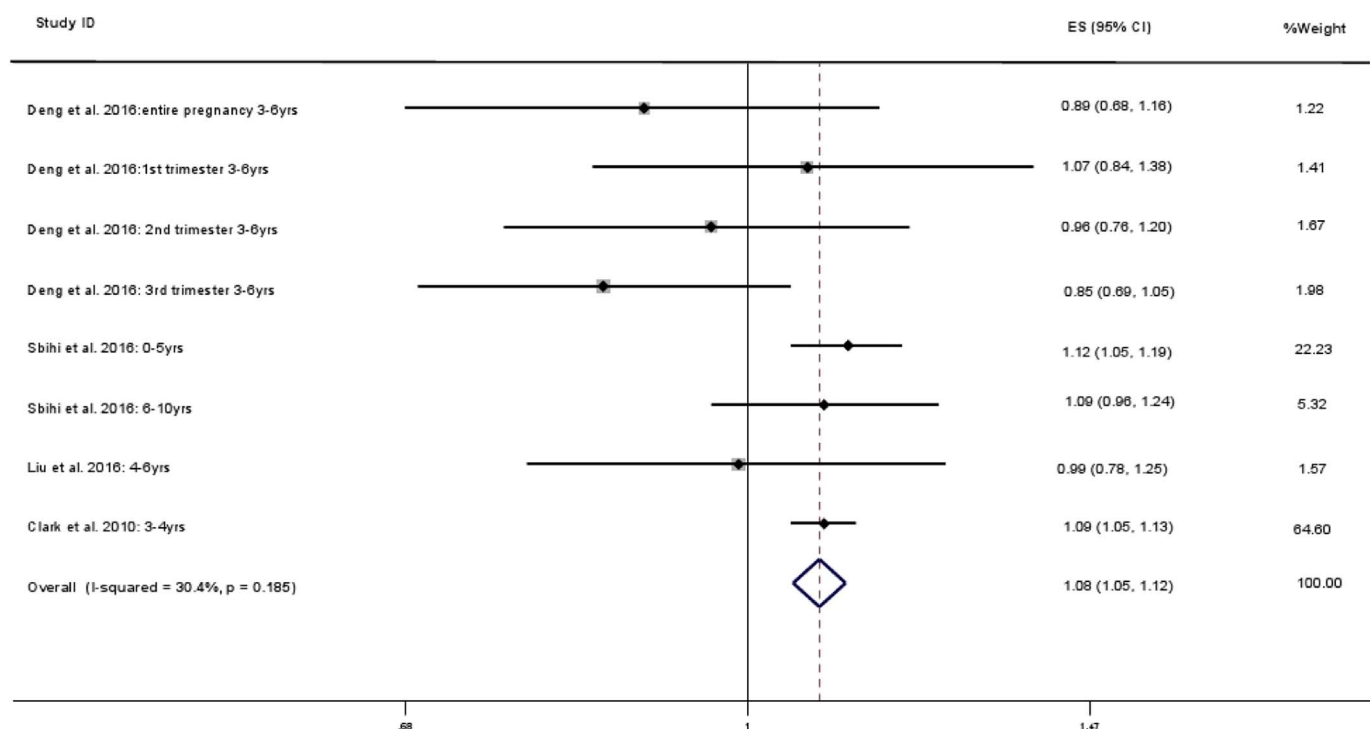


Fig. 4. Prenatal exposure to PM₁₀ and development of asthma among children aged 0–10 years.

and specifically extracted pyrene and PAH_{nonvolatile} concentrations. This study found a high positive correlation between prenatal exposure to pyrene and asthma occurrence, and an inverse effect of PAH_{nonvolatile} on asthma occurrence. Given the previously identified harmful effects of PAH such as DNA methylation, we recommend further research to specifically extract and study the effects of prenatal exposure to pyrene related contents in PAH on asthma in children. NO₂ and PM_{2.5} are surrogate markers of traffic-related air pollution, which has been shown to have a significant effect on childhood asthma with postnatal exposure. Our synthesis showed prenatal exposure to NO₂ to have a positive effect on childhood asthma similar to the effects from postnatal exposure (Veras et al., 2017; Khreis et al., 2017). Prenatal exposure to PM_{2.5} had a weak impact on childhood asthma and wheezing compared with postnatal exposure, which may due to the limited original studies (Hwang et al., 2017; Jung et al., 2017). Our evidence showed prenatal exposure to SO₂ to be positively associated with asthma in children aged 0–5 years old. The effect became weaker for older children; further original studies are needed to support that conclusion. Prenatal exposure to PM₁₀ was overall positively associated with childhood asthma, similar to the results found in other reviews and articles (Veras et al., 2017; Khreis et al., 2017; Mazenq et al., 2017). Due to the more limited number of original studies examining prenatal exposure to BC, CO and O₃, we only calculated the merged effect of exposure to these substances. The results differed from existing studies (Nishimura et al., 2016; Hua et al., 2014).

Recent individual studies had focused on the combined effects of air pollution instead of single pollutant effects on asthma among both outpatients and inpatients. Bharadwaj et al. (2016) found that those exposed to the Great Smog (Britain, 1952) in utero had a 7.91% point increase (95% CI, −2.39 to 18.20) in the likelihood of developing childhood asthma compared with those conceived well before or after the incident and those residing outside the affected area at the time of the smog. This was the first study to link prenatal exposure to later development of asthma using a natural experiment (Bharadwaj et al., 2016). Subsequent studies predominantly assessed prenatal tobacco exposure and the development of respiratory disease, while studies examining the impact of postnatal air pollution including PM_{2.5}, SO₂, PM₁₀, O₃, were mainly positively correlated (Khreis et al., 2017; Fan et al., 2016; Veras et al., 2017; Prieto-Parra et al., 2017; Underner et al., 2015; Olsson et al., 2014; Khreis et al., 2017) with impaired childhood lung function or respiratory disease occurrence among both outpatients and inpatients as well as in emergency settings. There is a need for more original studies examining prenatal air pollution exposure, the results of which can be compared with postnatal exposures.

5.3. Study quality and heterogeneity

Our overall meta-analysis showed positive and statistically significant associations of part of pollutants examined and childhood wheeze and asthma. In fact, as previously mentioned, the high heterogeneity levels found in the NO₂ and PM_{2.5} analyses, in accordance with other studies, may suggest that these pollutants are surrogates for another pollutant or a mixture responsible for the observed effects. A number of other possible factors could explain the heterogeneity identified between the studies. First of all, the studies included have a large variation in number of participants, ranging from 152 to 78,700 pairs of mother and children. Given this, test power may partially explain the heterogeneity. Secondly, the different levels of exposure and constituents of air pollutants in the different geographic areas may explain differences between studies. IDW and LUR models resulted in different test results. Third, the age of children evaluated may account for differences; only one study identified included children aged 6–10 years. After excluding this study, heterogeneity was dramatically decreased. Finally, the definition of “asthma” varied amongst the studies; some cases were parent-reported while others were diagnosed by a physician. In addition, only one study divided asthma into atopic and

non-atopic asthma. We believe that the evidence suggests that prenatal air pollution is correlated with non-atopic asthma.

6. Conclusions and recommendations

Based on this study, we believe there is some evidence to support an association between prenatal exposure to NO₂, SO₂, and PM₁₀ and the development of childhood asthma. Conversely, there is insufficient evidence to support the effect of prenatal exposure to PM_{2.5}, BC, CO, O₃ on wheezing and asthma in childhood. Given that there was found to be some degree of consistency in the findings and conclusions of the individual studies, the results of the prior meta-analysis examining postnatal exposure, and the existing literature evaluating similar pollutants, the hypothesis that prenatal exposure to air pollution contributes to development of childhood wheezing and asthma is reinforced. The question of whether the increase in wheezing and asthma incidence and/or lifetime prevalence we identified in younger children is also seen among older school-aged children cannot be answered based on the current evidence.

Further studies should include greater standardization of study methods including exposure assessment harmonization, outcome typing, and inclusion of all important confounders (e.g. environmental tobacco exposure and heredity factors). Cohort studies should include evaluation of participants from gestation or even from the preconception planning period to childhood through adulthood, since exposure during the gestational period may cause an inevitable or irreversible effect on children's development. Considering that this is the first complete systematic review examining the impact of prenatal air pollution exposure on childhood wheezing and asthma, future synthesis is needed to include more individual analyses with high quality of air pollution estimation methods.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.envres.2017.08.038>.

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Supplementary Material

Sewage treatment effluents in Delhi: A key contributor of β -lactam resistant bacteria and genes to the environment

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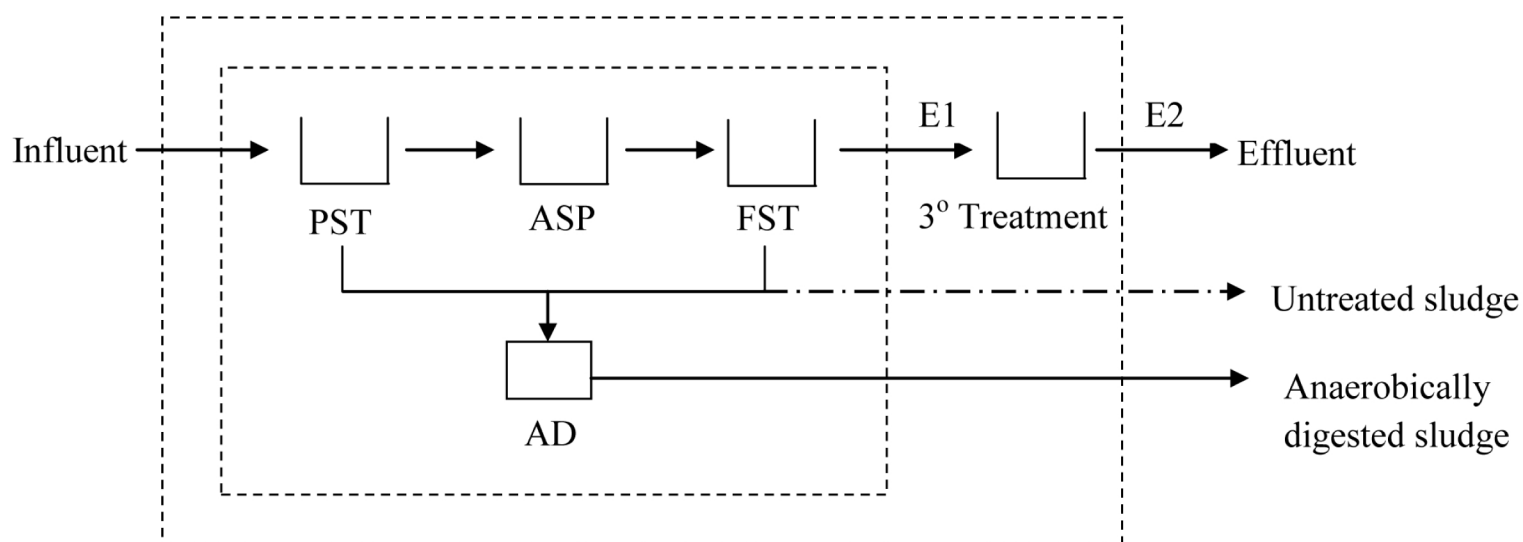


Fig. S1 Configurations of STPs having different stages of operations (PST- Primary settling tank, ASP- Activated sludge process (secondary treatment system), FST – Final settling tank, 3o Treatment (tertiary treatment system), AD- Anaerobic digester, E1- Effluent from FST, E2- Effluent from 3o treatment system)

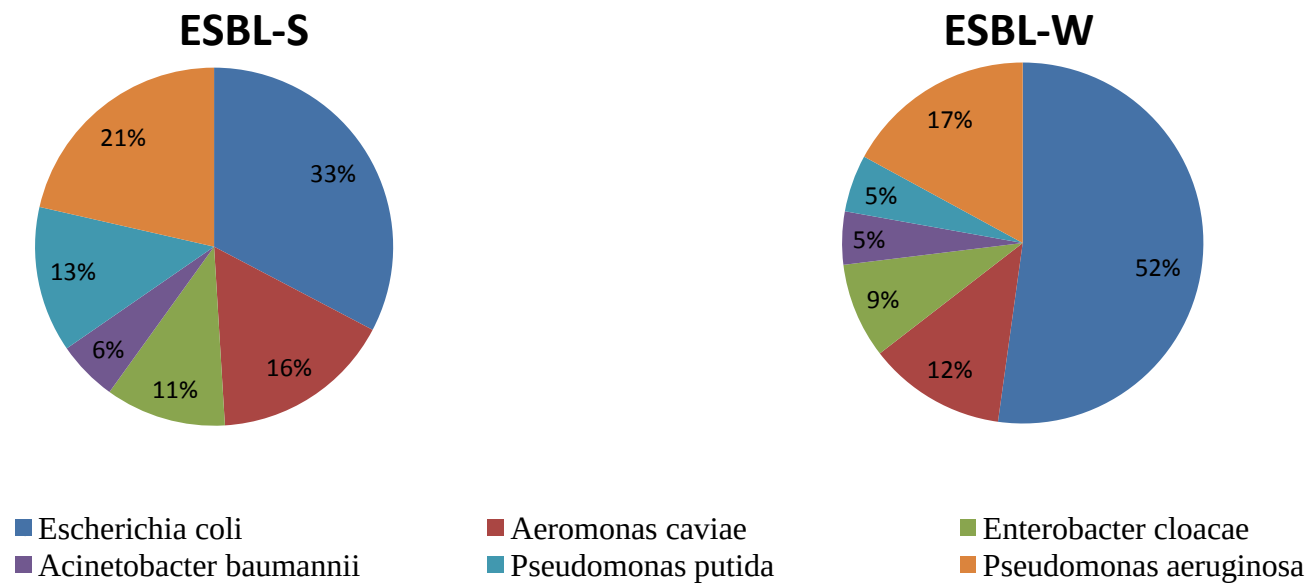


Fig. S2 Proportional abundance of ESBL-resistant bacteria in the samples collected from STP in summer and winter season.

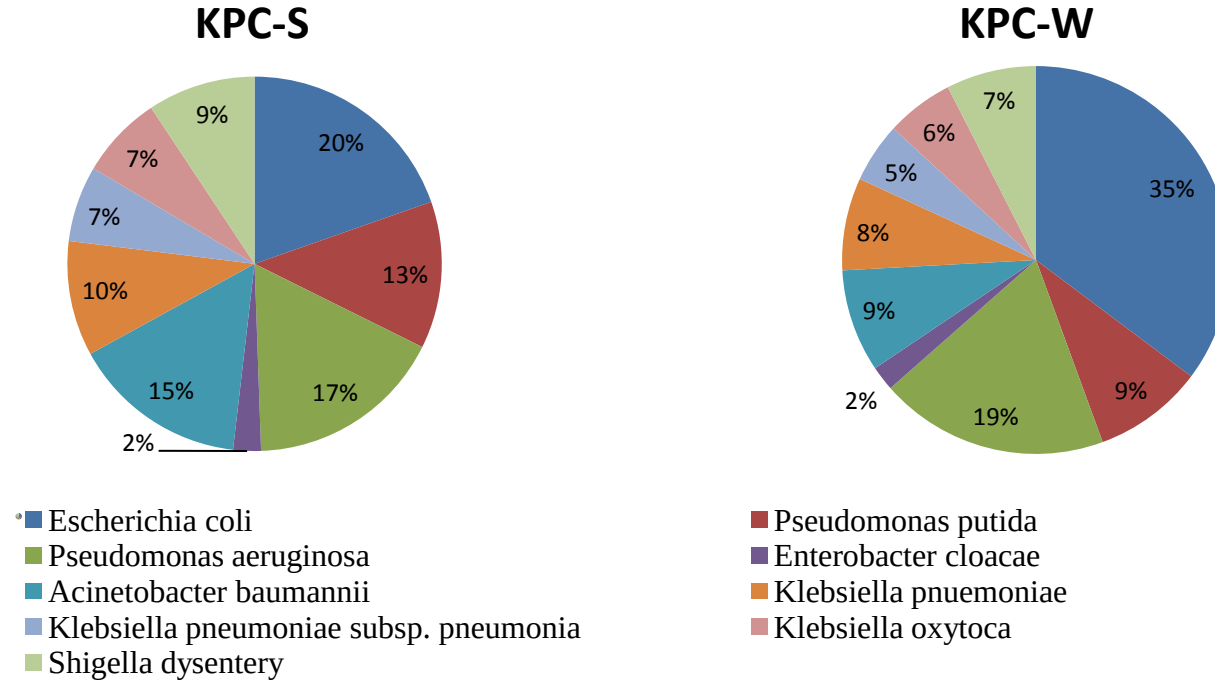


Fig. S3 Proportional abundance of KPC-resistant bacteria in the samples collected from STP in summer and winter season.

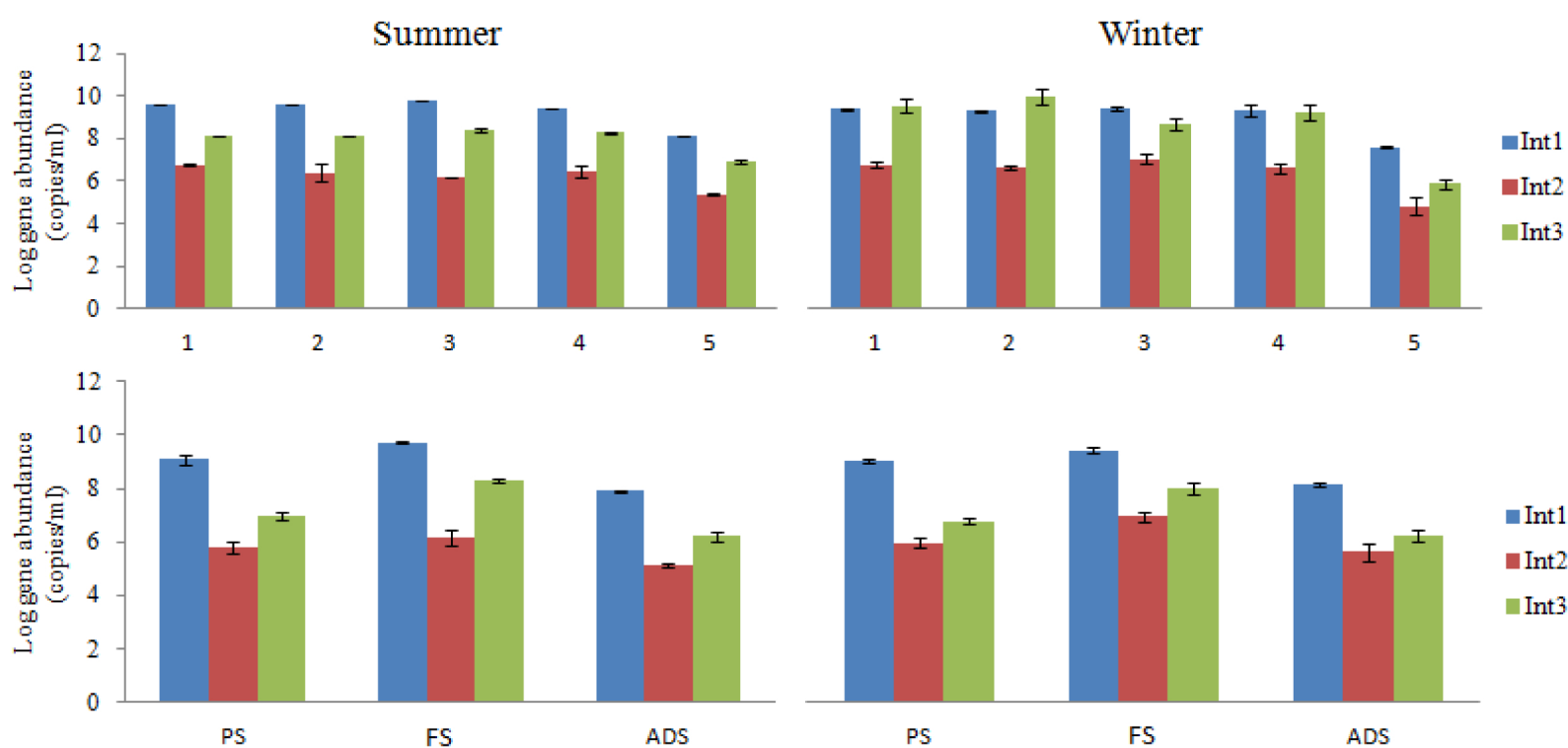


Fig. S4 Abundances of Int1, Int2 and Int3 in water samples obtained from different unit operations of STP and sludge samples collected from PST (PS), FST (FS) and anaerobic digester (ADS) in both summer and winter season. (1-row influent, 2-effluent from PST, 3-effluent from aeration basin, 4-effluent from FST, 5-effluent from tertiary treatment (chlorination)).

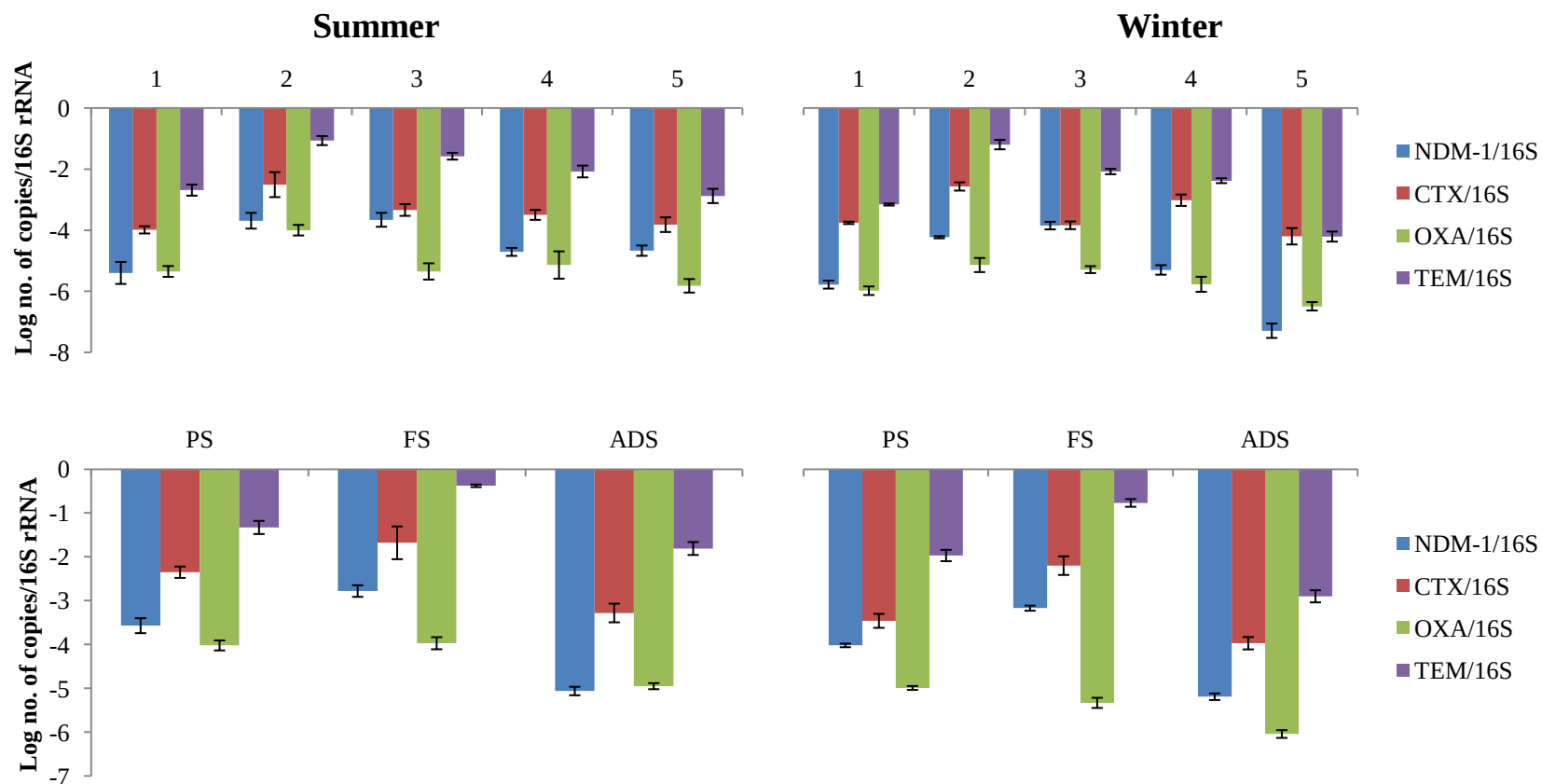


Fig. S5 Relative abundances of NDM-1, CTX, OXA and TEM genes in water and sludge samples collected from different unit operations of STP in both summer and winter seasons. (1-row influent, 2-effluent from PST, 3-effluent from aeration basin, 4-effluent from FST, 5-effluent from tertiary treatment (chlorination), PS- sludge collected from primary settling tank, FS-sludge collected from final settling tank, ADS-sludge collected from anaerobic digester)

Table S1: List of STPs

S.No.	Name of STP	Location	Capacity (MGD)	Treatment Schemes				
				PST	Aeration Basin	FST	Tertiary Treatment	Anaerobic Digester
1	STP 1	South Delhi	45	√	SA	√		
2	STP 2	South Delhi	30	√	D	√	C	√
3	STP 3	South West Delhi	3		SA	√		
4	STP 4	South West Delhi	5		SA	√		
5	STP 5	North West Delhi	40	√	SA	√		
6	STP 6	North West Delhi	40		D	√		√
7	STP 7	North West Delhi	20	√	SA	√		√
8	STP 8	North West Delhi	10	√	SA	√		√
9	STP 9	East Delhi	25	√	SA	√		
10	STP 10	East Delhi	45	√	D	√		√
11	STP 11	West Delhi	20	√	SA+D	√		√
12	STP 12	West Delhi	12	√	D	√	C	√

SA: Surface Aeration C: Chlorination D: Diffusers (Submerged Aeration)

Table S2. Primers used for PCR/qPCR to target the genes in the study.

Target	Primer	Conditions used	Reference
16s-rRNA	Bact338F Bact1046R	Initial enzyme activation at 98°C for 3 mins, 39 cycles of denaturation at 98°C for 5 s, annealing at 60°C for 10 s.	(Huber et al., 2007; Yu et al., 2005)
bla_{TEM}	blaTEM-F blaTEM-R	Initial enzyme activation at 95°C for 2 mins, 39 cycles of denaturation at 95°C for 5 s, annealing at 50°C for 10 s and extension at 72°C for 10 s.	(De Gheldre et al., 2003; Huber et al., 2007)
bla_{OXA}	blaOXA1B14-F blaOXA1-R	Initial enzyme activation at 95°C for 2 mins, 39 cycles of denaturation at 95°C for 5 s, annealing at 55°C for 10 s and extension at 72°C for 10 s.	(Briñas et al., 2003; Moland et al., 2006)
bla_{CTX}	CTX-M-F CTX-M-R	Initial enzyme activation at 95°C for 90 s, 39 cycles of denaturation at 95°C for 5 s, annealing at 58°C for 10 s and extension at 72°C for 10 s.	(Boyd et al., 2004)
bla_{NDM-1}	NDM-1-F NDM-1-R	Initial enzyme activation at 95°C for 5 mins, 49 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 min.	(Naas et al., 2011)
Integron 1	Int1-F Int1-R	Initial enzyme activation at 95 °C for 1 min 30 seconds, 39 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 10 s and extension at 72°C for 10s.	(Barraud et al., 2010)
Integron 2	Int2-F Int2-R	Initial enzyme activation at 95 °C for 1 min 30 seconds, 39 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 10 s and extension at 72°C for 10s.	(Barraud et al., 2010)
Integron 3	Int3-F Int3-R	Initial enzyme activation at 95 °C for 1 min 30 seconds, 39 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 10 s and extension at 72°C for 10s.	(Barraud et al., 2010)

Reference

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Table S3: Water quality (physical) parameters for the influent and effluent samples obtained from various STPs.

Name of the STP	Temperature (°C)		pH		TDS (mg/l)		Conductivity (µS)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
STP1	29.4	29.4	7.2	8.9	835	883	1300	1380
STP2	28.2	30.6	7.1	8.6	828	803	1289	1250
STP3	28.8	28.3	6.7	6.8	450	388	984	776
STP4	27.7	27.9	6.8	6.4	388	388	774	775
STP5	29.6	28.4	7.6	8.3	738	730	1150	1140
STP6	29.6	29.8	7.6	8.4	738	647	1150	1010
STP7	29.1	30.3	6.4	6.6	400	402	799	805
STP8	29.1	31.1	6.4	6.6	400	395	799	793
STP9	30.8	30.7	6.7	6.7	438	498	877	996
STP10	30.5	31.1	6.6	7.0	966	716	1933	1438
STP11	31.1	31.2	6.5	6.8	435	455	873	911
STP12	30.7	30.7	6.8	6.9	587	642	1283	1163

Table S4: Water quality (chemical and biochemical) parameters for the influent and effluent of the STPs.

Name of the STP	BOD (mg/l)		COD (mg/l)		NH ₃ (mg/l)		NO ⁻³ (mg/l)		PO ₄ ⁻³ (mg/l)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
STP1	144	15	400	96	44	24	4	12	2.7	2.1
STP2	179	8	415	41	44	18	4	10.2	2.4	1.9
STP3	126	18	400	161	126	85	14	31	2.9	2.4
STP4	145	7	455	44	129	12	13	42	2.4	2.1
STP5	157	11	461	87	35	18	17	38	2.6	1
STP6	157	19	461	53	35	22	17	10	2.6	1.4
STP7	162	31	374	119	32	27	14	22	5	2.6
STP8	162	33	374	80	32	25	14	26	5	3
STP9	158	20	432	52	42	27	7.48	10.2	1.85	1.1
STP10	290	28	934	93	52	44	7.8	10.4	2.1	1.1
STP11	208	4	580	38	28	18	5	17	1.4	2.6
STP12	159	11	456	53	33	19	7	18.5	1.2	0.6

Table S5: Log abundance of coliforms, ARB, ARGs, blaNDM-1, blaCTX, blaOXA, blaTEM, 16S rRNA and integron genes in different unit operations of 12 STPs.

	TC	FC	ESBL	KPC	<i>int1</i>	<i>int2</i>	<i>int3</i>	<i>bla</i> _{NDM-1}	<i>bla</i> _{CTX}	<i>bla</i> _{OXA}	<i>bla</i> _{TEM}	16S rRNA
STP1_Inlet	6.60	5.70	5.94	6.01	8.97	6.13	8.91	6.70	7.54	5.84	9.49	11.45
STP1_PST	5.69	4.16	5.4	5.80	9.19	6.60	8.57	6.63	7.89	5.87	9.74	10.24
STP1_SA	6.09	5.75	5.99	5.89	9.88	6.14	8.54	7.47	7.75	5.26	9.54	10.42
STP1_FST	5.15	4.91	5.14	4.64	8.75	5.87	6.53	5.35	6.51	5.60	9.48	10.97
STP2_Inlet	6.65	5.66	6.14	6.32	9.25	6.26	9.04	6.45	77.77	6.18	9.38	11.20
STP2_PST	5.50	4.30	5.57	5.81	9.36	6.28	8.98	6.30	7.89	5.88	9.81	10.87
STP2_D	5.94	5.71	6.15	5.86	9.57	6.07	7.54	7.19	7.45	4.98	9.25	10.10
STP2_FST	6.24	5.24	5.36	5.40	8.50	6.12	6.76	5.60	6.16	6.22	8.74	10.87
STP2_C	3.77	3.74	3.59	3.11	8.30	5.78	6.81	5.47	6.73	4.05	6.75	10.74
STP3_Inlet	6.66	5.65	6.18	5.91	9.37	6.54	9.58	6.62	7.82	6.18	9.56	10.98
STP3_SA	5.74	5.24	6.38	5.76	8.88	6.19	8.67	7.35	7.03	5.66	9.76	10.24
STP3_FST	5.98	4.98	5.98	4.49	8.92	5.92	7.98	6.87	7.35	5.11	9.24	11.24
STP4_Inlet	6.53	5.58	6.37	6.28	9.52	6.70	9.84	6.64	7.91	6.33	9.68	10.45
STP4_SA	5.40	5.77	5.66	5.50	9.42	6.76	8.54	7.16	7.83	5.14	9.85	9.87
STP4_FST	5.74	4.62	4.64	4.74	8.91	5.25	7.93	5.57	6.48	5.50	9.64	10.24
STP5_Inlet	6.73	5.72	7.29	7.21	9.46	7.22	8.53	6.53	8.06	6.68	9.61	9.89
STP5_PST	5.36	4.39	6.42	6.49	9.34	6.82	8.25	6.76	7.92	6.46	9.85	9.74
STP5_SA	5.54	4.98	5.74	5.92	9.44	6.56	8.21	7.61	7.58	4.89	9.61	10.21
STP5_FST	5.01	4.22	4.28	5.48	8.95	7.52	8.68	5.63	7.82	6.11	9.22	10.01
STP6_Inlet	6.24	5.24	7.24	6.97	9.49	7.10	8.24	6.12	8.12	6.12	9.13	11.24
STP6_D	6.24	5.73	6.24	6.24	9.51	6.51	8.8	7.60	7.41	5.40	9.47	10.98
STP6_FST	6.12	5.88	5.24	5.84	9.63	6.37	9.65	7.53	7.61	5.82	9.60	9.97
STP7_Inlet	6.51	5.56	5.89	5.96	9.33	7.60	7.77	5.98	8.24	6.96	8.9	11.45
STP7_PST	5.47	4.56	5.40	5.89	9.46	6.83	8.42	6.38	8.33	6.93	9.47	10.87
STP7_SA	5.96	5.12	6.27	6.03	9.98	5.39	8.87	7.64	6.98	5.81	9.81	9.87
STP7_FST	6.20	5.87	5.95	5.98	9.01	7.28	9.48	6.70	7.97	6.18	8.31	10.21
STP8_Inlet	6.55	5.65	6.57	6.13	9.36	7.03	8.40	5.95	8.13	6.84	8.83	11.54
STP8_PST	5.40	4.58	5.36	5.93	9.29	6.54	8.25	6.18	8.18	6.69	6.09	10.14
STP8_SA	5.61	5.76	5.83	5.54	9.87	6.20	7.70	7.25	7.30	5.81	8.85	9.24

STP8_FST	5.50	4.95	5.46	4.78	9.90	6.48	8.77	6.81	7.70	6.94	8.70	10.24
STP9_Inlet	6.68	5.50	7.25	7.29	9.27	6.66	7.75	6.28	7.75	6.39	9.51	12.01
STP9_PST	5.66	4.39	5.78	6.09	9.38	6.74	8.09	6.98	7.86	6.37	8.91	10.98
STP9_SA	6.21	5.74	5.95	6.47	9.74	5.69	7.43	7.33	7.58	5.81	9.97	9.78
STP9_FST	6.11	4.98	5.41	5.49	9.01	6.97	8.61	6.28	7.84	6.16	9.71	10.21
STP10_Inlet	6.24	5.89	6.74	7.22	9.24	6.72	7.24	6.18	7.24	6.27	9.32	11.24
STP10_PST	5.54	4.60	5.69	6.12	9.44	6.95	7.92	6.97	7.88	6.30	9.68	9.87
STP10_D	6.12	5.95	5.87	6.24	9.89	5.63	7.24	6.98	7.29	5.57	9.90	10.21
STP10_FST	6.02	5.15	6.63	6.83	8.94	7.67	8.24	6.44	7.93	6.85	9.57	11.04
STP11_Inlet	6.56	5.64	6.08	6.24	9.64	6.90	7.26	6.78	7.77	6.48	8.88	11.48
STP11_PST	5.47	4.48	5.23	5.76	9.29	6.57	8.12	6.89	7.76	6.32	8.62	10.74
STP11_SA	5.35	5.18	5.99	5.37	9.44	6.94	7.89	7.24	6.83	5.47	8.97	10.24
STP11_FST	5.69	4.84	5.87	4.85	9.7	6.32	6.6	7.65	7.56	6.43	8.49	9.87
STP12_Inlet	6.64	5.65	6.13	6.21	9.45	6.71	6.68	6.25	7.81	6.48	8.89	11.92
STP12_PST	5.56	4.56	5.28	5.96	9.37	6.61	8.23	6.15	7.99	6.27	8.47	10.04
STP12_D	5.54	5.33	5.95	5.98	9.89	6.03	8.59	7.58	8.12	6.01	9.31	9.78
STP12_FST	5.98	5.76	5.98	5.85	9.24	6.86	9.07	6.81	7.94	6.51	9.12	10.14
STP12_C	3.85	3.89	3.61	3.61	8.12	5.67	7.12	5.25	6.49	4.23	6.83	10.70

(* Coliforms and ARB in CFU/ml and ARGs and integrons in No. of copies/ml)

Table S6: Log abundance of coliforms, ARB, ARGs and integrons in different unit operations of a 30 MGD treatment plant.

	Influent	PST	Aeration basin	FST	3 ^o treatment unit	PS	FS	ADS
Total Coliform	5.98	4.24	4.92	4.87	3.25	5.56	6.38	4.23
Faecal Coliform	5.65	3.81	3.97	3.88	3.06	5.06	6.41	4.12
ESBL resistant bacteria	5.50	4.71	4.89	4.11	3.52	5.45	6.52	4.32
KPC resistant bacteria	5.37	5.91	4.34	4.48	3.15	4.23	5.61	3.21
bla_{NDM-1}	5.84	6.27	7.46	5.20	5.05	5.62	6.10	4.98
bla_{CTX}	7.88	7.43	7.94	7.89	6.69	7.23	7.68	6.87
bla_{OXA}	5.54	5.12	5.00	4.92	4.93	4.95	5.13	4.57
bla_{TEM}	8.82	8.78	8.06	7.44	7.56	8.47	8.90	8.01
Int1	8.36	8.53	8.60	7.99	7.10	8.57	8.94	7.56
Int2	5.83	5.45	5.22	4.98	5.10	4.92	5.44	4.42
Int3	7.17	7.20	6.75	6.70	6.68	6.31	7.27	5.98
16S rRNA	11.60	10.21	10.93	11.11	10.78	9.76	9.81	10.10

(* Coliforms and ARB in CFU/ml and ARGs and integrons in No. of copies/ml, MGD- Million Gallons per Day, PS- sludge collected from PST, FS-sludge collected from FST, ADS-sludge collected from AD)