Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supplemental Material

Sources and Transport of Antibiotic Resistance Genes in Air

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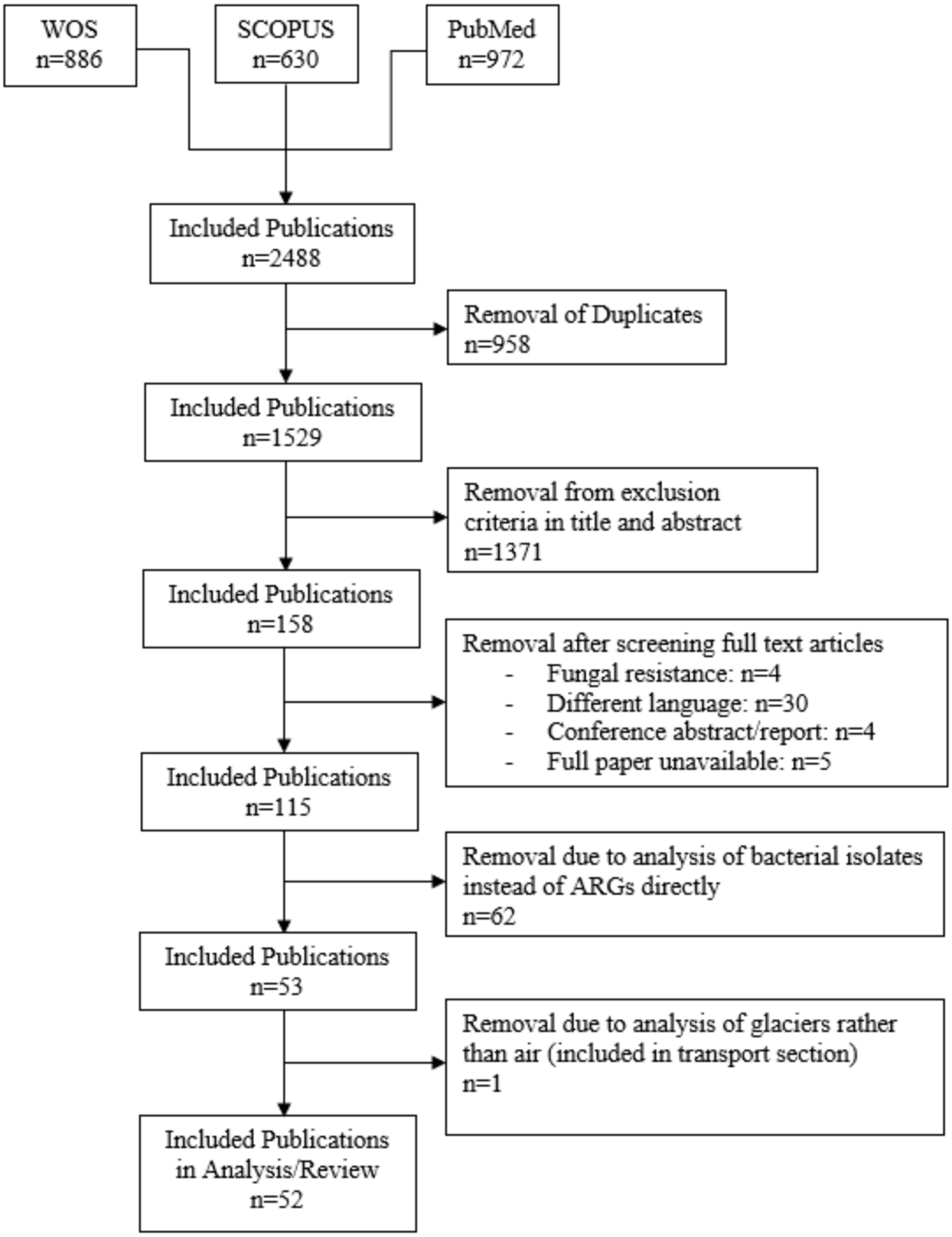

Figure S1: Selection methodology for articles/studies included in the systematic review. Papers were scrutinized through multiple rounds and eventually 54 papers were included in the review, along with one paper that was used in the subsection on air transport.

Table S1: Search Criteria for the literature search on the different databases

Database	Search Criteria
WOS	TS=(("air" OR "airborne" OR "air-borne" OR bioaerosol* OR atmospher*) AND (antimicrobial* OR antibiotic* OR antibacterial*) NEAR/2 (resist*))
SCOPUS	TITLE-ABS ({air} OR {airborne} OR {air-borne} OR bioaerosol* OR atmospher*) AND TITLE-ABS (antimicrobial* OR antibiotic* OR antibacterial* PRE/2 resist*)
PubMed	(air[Title/Abstract] OR airborne[Title/Abstract] OR air-borne[Title/Abstract] OR bioaerosol[Title/Abstract] OR atmospher*[Title/Abstract]) AND (antimicrobial*[Title/Abstract] OR antibiotic*[Title/Abstract] OR antibacterial*[Title/Abstract] AND resist*[Title/Abstract])

Table S2: Key urban air ARG studies identified in this review

(Ref) Location	Sample Type	Sample Fraction	ARGs / MGEs	Dominant ARGs	Analysis method (units)
(37) 26 cities in China, 3 in North America, and 1 in Europe	Urban	Fresh snow	Resistance to all major classes of antibiotics (285 primer sets), transposase genes (8 primer sets) / *intI*, and *cintI*	aminoglycosides, β-lactams, and multidrug resistance. *blaCTX-M-04, blaCMY2-02, aadA1, aadA2-02*, and *vanC-03*	HT-qPCR (gc/L)
(25, 29) Beijing, China	Urban air	$PM_{2.5}$ and PM_{10}	Tetracycline, β-lactam, aminoglycoside, chloramphenicol, polypeptide, multidrug, lincosamide, macrolide, streptogramin, and sulfonamide resistance classes	Polluted days: *tetW, aph(3")-Ib,* and *lnuA*; Clean days: *bacA, BL2cbro, lnuA,* and *macB;* Antibiotic-polluted environments: *sul2, aph(6)-Id, aph(3")-Ib*, and quinolones (qnr) and β-lactams	Metagenomics (gc/16S)
(26) Xi'an, China	Urban air	$PM_{2.5}$	β-lactams, quinolones, macrolides, sulfonamides, tetracyclines, and aminoglycosides / *tnpA, intI1*	*qepA and blaTEM*	HT-qPCR (gc/16S)
(26) 19 cities in 13 countries and 6 continents	Automobile air conditioner filters	Total suspended particles (TSP)			HT-qPCR (gc/16S)
(41) Beijing, China	Urban air (clean and polluted)	$PM_{2.5}$	β-Lactam, sulfonamide, tetracycline, and macrolide resistance. / *intI1, tnpA*	Clean days: *blaTEM, tetW, sul3, tetQ, tetM, ermB, tet32,* and *tetO*; Pulluted days: *blaTEM, NDM-1, ermB, sul1, tetM, qepA, sul1, ermC.* / *tnpA* and *intI1* varied greatly, with *tnpA* abundant during both events, and *intI1* only abundant in February pollution event.	qPCR (gc/16S)
(28) 4 cities in California, USA	Air, soil, and water from public parks	TSP	*sul1* and *blashv*	*blashv* 2 orders of magnitude higher than *sul1*, both detected	qPCR (gc/16S, gc/L)
(33) La Paz, Bolivia	Urban surface waters	TSP	tetracyclines (*tetA*), fluoroquinolines (*qnrB*), and β-lactams (*bla*TEM) / *intI1*	*blaTEM* and *intI1* highest, followed by *tetA* and *qnrB*	ddpcr. gc m^{-3} and frequency of detection
(30) Beijing Normal University, China	Urban air	$PM_{2.5}$ and ambient air	Total ARGs, then qPCR for *tetM, tetO, tetW, ermB, ermQ, mphE,* and *aph(3')-IIIa*	*ermB, tetW,* and *mphE.*	Metagenomics (% total DNA). qPCR (gc m^{-3})
(35) Tianjin, China	University campus	$PM_{2.5}$ and PM_{10}	~60 ARGs. (Aminoglycoside, β-lactam, cloramphenicol, MLSB, multidrug, sulfonamide, tetracycline, vancomycin, colistin) / *intI1, tnpA*	*intI1, strB, aac(6')-Ib, sul2, aadA1, aadA2, bacA, qacEdelta1, tnpA,* β-*lactams (ampC-02, blaIMP-01, blaSHV-01, and blaCTX-M)*	HT-qPCR (gc/16S)

(34) Beijing, China	Urban megacity air	$PM_{2.5}$ and PM_{10}	Total ARGs	*blaTEM*, MFS, RND, Erm 23S ribosomal RNA methyltransferase, *tet* RPP, *LNU*, *sul*, *ABC-F, CAT*, and *ANT*	Metagenomics. reads per kilobase per million reads (RPKM)
(32) Bolu, Turkey	Urban air	PM_{10}	*qnrS, sul1, mecA,* and *ctx-m-32 / intI1*	*MecA, Sul1, IntI1, qnrS* in order of abundance	qPCR (gc/16S, gc m^{-3})
(31) Harbin, China	Urban (hazy PM >75ug/m3 non-hazy PM < 75ug/m3)	$PM_{2.5}$ and PM_{10}	*ermB, ermG, mexF, tetW, qnrS, aadd, sul1*, and *blactx-M1*) / *intI1*	*sul1, intI 1, aadd* (hazy), and *qnrS* (hazy). Hazy days higher than non hazy, but *intI1* higher on non hazy	qPCR (gc/16S and gc m^{-3})
(38) Handan City, China	Urban Air	$PM_{2.5}$	*sul1, sul2, tetA, tetQ, qnrB, qnrS, ermB, ermC, ampC,* and *aacC2* / *intI1*	*Sul1, sul2, tetA, ampC, and intI1* all seasons. Winter: *tetQ, qnrB, qnrS, ermB, ermC, aacC2* Spring: *tetQ* Autumn: *tetQ, qnrS, ermC*	qPCR (gc/16S and gc m^{-3})
(42) Xiangxiang, China	University Campus	$PM_{2.5}$ and TSP	*ermC, sul1, sul2, tetW,* and *blaTEM / intI1*	*ermC, sul1, sul2, blaTEM,* and *intI1* on excellent and moderate pollution days. *tetW* only on excellent air quality days	qPCR (gc/16S and gc m^{-3})
(90) Handan City, China	University Campus	$PM_{2.5}$	*sul1, sul2, tetQ, tetA, qepA, qnrA, qnrB, qnrS, ermB, ermC, OXA-1, OXA-10, CMY-2, ampC, DHA-1, SHV-1,* and *aacC2 / intI1*	*intI1, sul1, sul2, qepA*, and *ampC* for all conditions. During rain*, tetQ, tetA, qnrB, qnrS*, and *OXA-1*	qPCR (gc/16S and gc m^{-3})

Table S3: Key rural air ARG studies identified in this review

(Ref) Location	Sample Type (# of sites)	Sample Fraction	ARGs / MGEs	Dominant ARGs	Analysis method (units)
(61) Six provinces in South Korea	Commercial pig farms (7)	TSP	six TcR genes (RPP class: *tetO*, *tetQ* and *tetW*; EFP class: *tetB*, *tetH* and *tetZ)*	*RPP (tetO, tetQ* and *tetW)* higher than *EFP (tetB, tetH*, and *tetZ),* but all high concentration	qPCR (gc m^{-3})
(59) Eastern Canada	Swine barns (18)	TSP	*tet* genes	*TetA/tetC*, *tetG* were detected in the bioaerosols of all 18 studied buildings.	PCR (Frequency)
(23) Lubbock, Texas	Cattle feed yards (10)	TSP	*TetM, TetO, TetQ, TetW, TetB,* and *TetL*	*TetQ* and *TetW*	qPCR (gc/16S)
(62) Saskatchewan	Cage-housed (CH) (15) and floor-housed (FH) (15) poultry operations	TSP	qPCR for tetG. Endpoint PCR for *bcrR, ermA, ermB*, and *tetA/C*.	In order of frequency. FH: bcrR, ermB, tetA/C, ermA, tetG CH: ermB, bcrR, tetA/C, and tetG	qPCR (gc m^{-3}). Endpoint PCR (Frequency)
(56) Vion, Boxtel, the Netherlands	Dutch pig slaughterhouse (1)	TSP	*tetW, ermB*	All air samples tested positive for *tetW* and *ermB*, except cutting room and deboning room	qPCR (gc/16S, frequency)

(57) Beijing, China	Biogas plant of a commercial swine farm (1)	TSP	six quinolone, seven sulfonamide, twelve tetracycline, eight macrolide, seven β-Lactamase, four vancomycin, and one polymyxin / *intI1* and *tnpA*	*blaTEM* (order of magnitude higher than rest combined), *tetC, tetK, qnrD, tetQ, sul3, sul1, qnrS*	HT-qPCR (gc/16S)
(63) Guizhou province, China	Swine confinement buildings (SCBs) weaning piglets (WP), finishing pigs (FP), farrowing sows (FS), gestating sows (GS), and breeding boars (BB) (3 of each building)	TSP	Total ARGs	aminoglycosides, aminocoumarin, mupirocin, elfamycin, fluoroquinolone, pleuromutilin, rifampin, and lincosamide. *alaS*. *APH3-IIIa*	Metagenomics (gc/total DNA)
(55) The Netherlands	Residential sites near livestock (61)	PM_{10}	*tetW, mecA*	*mecA* - 88%, *tetW* - 95%. tetW higher concentration	qPCR (gc m^{-3}, Frequency)
(66) Quebec, Canada	Swine confinement buildings (10)	Total dust	*blaCTX-M-1, mcr-1*	*blaCTX-M-1* and *mcr-1* found in 6 out of 10 SCBs up to 100s of copies/m3	qPCR (gc m^{-3}, Frequency)
(60) South Korea	Swine confinement buildings (3)	TSP	ribosomal protection proteins (RPP) class: *tetO, tetQ*, and *tetW*; Efflux class: *tetB, tetH*, and *tetZ*	*tetO, tetQ*, and *tetW* higher than *tetB, tetH*, and *tetZ*	qPCR (gc m^{-3}, Frequency)
(46) Pearl River Estuaries, China	Rural/river	TSP	*blaCTX-M, blaTEM, mecA*, *aadA, aadE aacA/aphD, str, sat*, *tetA, tetB, tetC, tetD, tetG, tetL, tetM, tetO, tetQ, tetS, tetT, tetX, tetW*, *ereA, ermB, ermC, ermT, lnuA, lnuB, vatE, mefA*, *sul1, sul2, sul3, acrA, mexF. /* integrons (*IntI1, IntI2,* and *IntI3)*, Insertion sequences (*IS26, IS613* and *ISCR3*), plasmids (*traA* and *trbC*) and transposons (*merA* and *tnpA)*	*tetA* and *tetB*, *sat*, and MGEs.	qPCR (gc m^{-3}, gc/16S)
(58) Illinois and Indiana	Pig (8), turkey (3), and poultry (3) confinement buildings	TSP	*TetB, TetH, TetZ, TetO, TetQ* and *TetW*	RPP (*tetO, tetQ,* and *tetW*) higher than EFP (*tetB, tetH*, and *tetZ*), but all high concentration. None in offices.	qPCR (gc/ng DNA extracted)
(64) California, USA	Conventional (3) and Organic (3) beef cattle farms	Particles > 1.6um	*bla*TEM*, bla SHV, sul1, ermB*, and *ermF*	Conventional: *sul1* and *blaSHV* (*ermB* and *blaTEM* detected but not quantified) Organic: *sul1* and *ermF*	qPCR (gc m^{-3}, Frequency)
(52) Beijing, China	Concentrated animal-feeding operations (CAFOs) layer (4) and broiler (4)	TSP	*tetL* and *tetW*	*tetL* and *tetW*	qPCR (gc/16S)
(67) Beijing, China	Composting site	TSP	*intI1* and 22 subtypes of ARGs	*intI1*	droplet digital PCR (gc/16S, gc m^{-3})
(53) Texas, US	Beef cattle feedyards (5)	TSP	*tetW, tetQ, tetO, tetM, tetL*, and *tetB*	*tetW, tetQ,* and *tetM*	qPCR (Copies)
(65) 9 European Countries	Broiler farms (174) Farrow-to-finish Pig Farms (159)	TSP	*tetW, ermB, aph(3')-III* and *sul2*	From highest to lowest abundance: *ermB, tetW, sul2, aph3-III*	qPCR (gc/16S)
(54) Weinan city, China	Swine Farm	$PM_{2.5}$ and TSP	*tetM, tetG, tetO, sul1, sul2, qnrA, ermA, ermB,* and *strA* / *intI1, IS613,* and *Tp614*	Winter and Summer*: strA, ermB, ermA, tetM, tetO*.	qPCR (gc m^{-3})

				Winter: *qnrA, sul1, sul2, tetG, intI1, IS613,* and *Tp614*	

Table S4: Significant hospital air ARG studies discussed in this review

(Ref) Location	Sample Type (# of sites)	Sample Fraction	ARGs / MGEs	Dominant ARGs	Analysis method (units)
(71) QC, Canada	Recently occupied hospital rooms	TSP, tap water	*vanA, vanB, tetA, tetC, tetG, ermA, ermB, ermC, ermF, ermT, ermX*	*ermX* (10/10), *erm(F)* (9/10), and *tetG* (6/10)	Endpoint PCR (Frequency)
(72) Toronto, ON, Canada	Hospital air	Filter Dust	*aac(6')-aph(2''), ermA, mecA,* and *vanA*	*mecA* (15/15), *aac(6')-aph(2'')* (10/15), and *ermA* (6/15)	Endpoint PCR (Frequency)
(69) Eastern and Southern China	Urban hospitals (5)	TSP	*blaCTX-M* and *mecA*	*blaCTX-M* and *mecA* both abundant (1000 - 100,000 gc/ng DNA). *mecA* > *blaCTX-M*	qPCR (gc/ng DNA)
(70) Leicster, England	Hospital	Exhaled Aerosols	*AmpC, blaTEM, CfxA, FOX-5, PBP2X, ErmB, mefA, tetM, tetA and AcrA-05*	*mefA, tetM, ErmB*, and *blaTEM*	qPCR (Frequency, gc/mask)
(68) Beijing, China	Hospital	Air Filter Dust	Total ARGs. qPCR for *blaNDM, qnrA, sul2, vanA, and tetW / intI1*	*intI1* and *tetW* for qPCR. Most commonly shared: *aadD, CE, tetK, tetA, tetZ* and *norA, lnuA, bacA, sul1, mext, marR,* chloramphenicol exporter, *ompR, aadA, ermB, aph(3")-I*	Metagenomics and qPCR (gc/16S)
(76) Guangzhou, China	Hospital	$PM_{2.5}$	Total ARGs	*aadA, aadD, aadE, ant(9)-I, aph(3''')-iii, bacA, class-a, ugd, ermB, ermC, ermT, lnuA, mphC, msrA, emrB, qacA, mfs_transporter, mexB, mexF, mtrA, abc_transporter, norA, rpoB2, sul1, tet(k), tetL, tetM, tetO,* and *tetW*	Metagenomics (gc/16S)
(73) China	Hospital	$PM_{2.5}$ and PM_{10}	Aminoglycoside, beta-lactam, fluoroquinolone/quinolone/florfenicol/chloramphenicol/amphenicol (FCA), MLSB), sulfonamide, tetracycline, vancomycin, multidrug	aminoglycoside and multidrug	HT-qPCR (gc/16S)

Table S5: Key wastewater air ARG studies identified in this review

(Ref) Location	Sample Type	Sample Fraction	ARGs / MGEs	Dominant ARGs	Analysis method (units)
(81) South Carolina, USA	WWTP air and sludge	TSP	vancomycin, tetracyclines, fluoroquinolone, aminoglycoside, macrolide-lincosamide-streptogramin B, and multidrug resistance classifications	B-lactam with serine-urilizing hydrolases. *ermB, ermC, tetA, tetB, aadA1, qnrB5, mefA, msrA*	qPCR (gc/ng DNA)
(80) Seoul, South Korea	WWTPs	TSP	Total ARGs	*sul1, sul2, ermB, ermG, ermF, tetA, tetG, tetQ, tetW, intI1*	Metagenomics (gc/16S)
(79) China	WWTP	Submicron aerosols (SAs)	Total ARGs	*BacA*, RPP (*tetO, tetQ*, and *tetW*), EFP (*tetB, tetH,* and *tetZ*), *aph, aac, sul, ant, vat*.	Metagenomics (gc/16S)
(78) Beijing, China	WWTP	TSP	*sul1, sul2, sul3, tetA, tetC, tetO, tetW, intI1, intI2,* and *intI3*	*sul2* and *intI1*	PCR (Frequency)

Table S6: Key landfill/waste air ARG studies identified in this review

(Ref) Location	Sample Type	Sample Fraction	ARGs / MGEs	Dominant ARGs	Analysis method (units)
(83) Changzhou, China	Municipal solid waste system	$PM_{2.5}$ and PM_{10}	*sul*, *tet*, quinolone, aminoglycoside, macrolide, B-lactam, and *intI1*.	*intI1, blaTEM-1, sul1, sul2, sul3, tetA, tetM, ermB*	qPCR (gc m^{-3})

Table S7: Significant indoor air ARG studies identified in this review

(Ref) Location	Sample Type	Sample Fraction	ARGs / MGEs	Dominant ARGs	Analysis method (units)
(85) Queensland University of Technology	Indoor air	TSP	*ermA, ermB, ermF, vanA, vatD, tetA, tetC, tetG, tetM, tetO, tetP, tetQ, tetS, tetT, tetW*	*tetA/C* most, then *ermB* and v*anD*	Endpoint PCR (Frequency)
(87) Tianjin, China	Laboratory	TSP	*tetM, tetG, tetC, tetO*	*tetM*, with biology lab > chemical lab	qPCR (gc m^{-3})
(89) Hong Kong	Indoor air. kindergarten	Filter dust	tetracyclines, macrolides, sulfonamides, B-lactams, aminoglycides, and amphenicols	Air: *sul1, sul2, ermF*, and *intI1*	qPCR (gc/16S)

Table S8: Key air ARG studies with multiple sources identified in this review

(Ref) Location	Sample Type (# of sites)	Sample Fraction	ARGs / MGEs	Dominant ARGs	Analysis method (units)
(45) Beijing, China	Urban air (site 1: 61, site 2: 44)	$PM_{2.5}$	*ermB, tetW, qnrS, lnuA, bla*TEM 1, and *sul1 / intI1, tnpA02,* and *tnpA-04*	*lnuA*	qPCR (gc/16S)
(45) Pearl River Delta, China	Air (industrial: 46, urban: 48, and rural 18)			*lnuA* and *sul1*	
(45) Yangtze River Delta, China	Air (industrial: 122, urban: 52, and rural 65)			*ermB* Urban air: *bla*TEM	
(21) Pearl River Delta, China	Air (industrial: 46, urban: 48, and rural 18)	$PM_{2.5}$	*ermB, tetW, qnrS, lnuA, blaTEM 1,* and *sul1 / intI1, tnpA02*, and *tnpA-04*	*ermB , tetW*	qPCR (gc m^{-3}, gc/16S)
(22) Tianjin, China	WWTP, bathroom, lab, hospital, outdoor	TSP	*tetM, tetG, tetC, tetO, tetA, tetQ, tetW, sul1, sul2, sul3, ermA, ermB, ermC, blaOXA-1, blaDHA-1, blaAMPc, blaTEM-1, blaCMY-2, qnrS*	*blaTEM-1, blaAmpc, blaOXA-1,* and *tetM*	qPCR (gc m^{-3})
(47) Zhuhai, China	Urban air	TSP	Total ARGs	*bacA, mexF, lnuA, mexW, aadE*	Metagenomics (gc/16S)
	Chicken farm			*aph(3')-I, sul1* for CF. *aadE* and *tetW* for CF-A. *mexF* both.	
	Pig farm			*aadE, ermT*, and *vatE*. *aad(9)* in PF, *mexF* in PF-A	
	WWTP air			*mexF, amrB* and *mexW*	
(86) Colorado	Indoor air (two CAFOs (one swine, one dairy), two clinics, and a homeless shelter)	PM_{10}	*tetW* and *tetX*, *intI1*	*tetW, tetX*, and *intI1* (only in CAFOs)	qPCR (gc m^{-3}, Frequency)
	Outdoor air (urban, semiurban, livestock agriculture, and alpine forest)			*tetW* (low)	
(40) Israel	Floor-housed poultry facility	PM_{10}, TSP	*intI1, qnRS, sul1, ctx-m-32*, and *mecA*	*intI1, mecA,* and *ctx-m-32 gene*	qPCR (gc/16S)
	Urban			intI1 and mecA	
(27) China	Urban hospital	$PM_{2.5}$ and PM_{10}	Total ARGs	*bacA*, chloramphenicol acetyltransferase (*cat*) and *lnuA*	Metagenomics (gc/16S)
	Urban community			chloramphenicol exporter gene, *sul1*, and *bacA*.	
	Suburban communities			chloramphenicol exporter gene, *sul1,* and *aadE*.	
(74) Ningbo City, China	Hospitals (3)	Filter Dust		*bl3_cpha, ermX, lnuA, mexF*	HT-qPCR (gc/16S)

	farms (chicken, duck, swine), urban (3), villages (3)		Targeted 285 ARGs from all major classes. 177 ARGs were detected between all sample types: Hospital (146 ARGs), farm (154 ARGs), city (103 ARGs), and village (85 ARGs)	*tetG, tetQ, tetL, tetM, tetO, tetK, tetX, mexF, ermB, ermT, ermF, pikR1, ermX, lnuA, intI, CintI, ant3ia*	
	City Indoor			*ermX, vanxd*	
	Village Indoor			*bl3_cpha, lnuA, mexF, intI*	
(88) Shenzhen, China	Indoor air wet market	TSP	*tetC, tetG, sul2,* and *ermC*	*sul2, ermC, tetG, tetC*	qPCR (gc/ng DNA, gc m^{-3})
	Live poultry air			*tetG, sul2, ermC, tetC*	
	Outdoor air			*sul2, ermC, tetC, tetG*	
(84) Tianjin, China	University campus indoor	TSP	120 primer pairs based on most common ARGs. 73 ARGs and 5 MGEs detected	B-lactam, multidrug, and tetracycline resistance. Indoor > Outdoor	HT-qPCR (gc/16S)
	Campus outdoor			multidrug, aminoglycoside, and tetracycline resistance.	
(82) Hong Kong	Coastal	$PM_{2.5}$	Total ARGs	Descending order, Multidrug, peptide, MLS, aminoglycoside, tetracycline, B-lactam, rifamycin, glycopeptide. *tetA, bacA, mtrA, mexF*, and *cpxR* indicator ARGs.	Metagenomics (gc/16S)
	Urban				
	WWTP				

Table S9: Significant miscellaneous air ARG studies identified in this review

(Ref) Location	Location Type	Sample Fraction	ARGs/MGEs	Dominant ARGs	Sampling method
(96) Worldwide	Glaciers/snow/ice	Snow and Ice	94 subtypes	*aac(3), strA2, blaIMP, strA1*.	Endpoint PCR (Frequency)
(39) Rehovot, Israel	Eastern Mediterranean dust storms	$<PM_{10}$	*qnrS, sul1 / intI1*	*intI1*, *qnrS,* and *sul1* genes two-orders of magnitude lower during dust-storms. *sul1* most abundant	qPCR (gc/16S)