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Antibiotic-resistant strains of *Escherichia coli* in urban atmospheric dust aerosols of Moscow city – the potential human health risks

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Abstract

Atmospheric dust aerosols of Moscow city contain significant numbers of particles enriched in phosphorus and other biophillous elements, which likely indicate faecal contamination. A high diversity of *Enterobacteriaceae*, including the opportunistic and potentially pathogenic species isolated from the dust aerosols, confirmed the presence of faeces (*Escherichia coli, Salmonella enterica*, etc.). Therefore, airborne dust is an active transmitter of microbial pollution within the megalopolis. It was found that about 27% of the isolated from dust *E. coli* strains were resistant to widely used antibiotics. The traffic areas were characterized by a significantly higher abundance of antibiotic-resistant *E. coli* strains as compared to the recreational areas of Moscow city (25 *vs.* 7 strains). These fundings clearly demonstrate a high health risk of dust exposure to people in the area of a strong anthropogenic impact. The most adverse properties were observed in wintertime accumulations of dust aerosols because urban soils covered with snow have a low activity as a «bacterial filter».

Keywords: atmospheric solid; anthropogenic impact; phosphorus; faecal contamination; Escherichia coli; Enterobacteriaceae, saprotrophic soil bacterial complex; antibiotic resistance; city environment

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1. Introduction

Monitoring of air pollution by dust is one of the priority aspects of the current ecological research of urban areas, in particular, megalopolises (European Commission. Air Quality Standards; World Air Quality Index Sitemap). Increasingly often dust becomes one of the main pollutants of the air in the world's largest cities including Moscow (Vlasov et al., 2020).

Main components of atmospheric solid aerosols are represented by soil-sediment particles, which are lifted from the ground surface as a result of erosion processes, and also by primary biological aerosol particles, i.e., microorganisms (aeroplankton) such as bacteria, fungal spores, live cells of algae, protozoans, etc. and their remains (Després et al., 2012; Prokof'eva et al., 2017). Diverse pollutants of mineral and organic nature have been found within areas of intensive anthropogenic impact, which include all megalopolises of the world (Goel & Kumar 2015; Karagulian et al., 2015; Luong et al., 2017; Phan et al., 2020). Dust aerosols can carry not only saprotrophic microorganisms, but also opportunistic (potentially pathogenic) and allergenic species of microorganisms (bacteria, yeasts, and mycelial fungi). Some of their strains can be resistant to widely known and commonly used antibiotics. Antibiotic-resistant strains of microorganisms in ambient air generate high risks to the health of immunosuppressed human populations (Arabaghian et al., 2019; Araujo et al., 2021; Davin-Regli & Pagès 2015). Reactions of opportunistic and pathogenic microorganisms to antimicrobial medicines is a widely studied aspect of the influence of environmental factors on their biological characteristics, especially in the strains isolated from clinical materials and characterized by a multiple antibiotic resistance (Rodríguez-Medina et al., 2019). However, biological characteristics of strains of potentially pathogenic microorganisms that live in soils, vegetation and specific man-made substrates within urban ecosystems have been left practically without attention. Although there is a compulsory control over total dust contents in urban air (i.e., fine particulate matter – PM2.5 and particulate matter – PM10) (European Commission. Air Quality Standards), there is still a lack of knowledge about the role of solid particles in the transportation of microorganisms through the atmosphere (Prokof'eva et al., 2021). The present study of cultured saprotrophic bacterial assemblages isolated from urban dust aerosols was aimed at verifying their possible faecal contamination and assessing the resistance of *Escherichia coli* strains to antibiotics that are widely used in medical practice.

2. Materials and Methods

2.1. Study location and sampling

The study was conducted within Moscow city, located in the center of the East European Plain, which is composed of loose fine-textured sediments of predominantly glacial and glaciofluvial origins. The study area is characterized by a moderately continental climate with cold snowy winters and moderately warm summers. Sampling was performed at several sites that differed in terms of land use. Airborne dust samples from two recreational areas were collected in the summer of 2019 (Prokof'eva et al., 2021). Dust aerosols from intensive traffic areas were sampled in September 2020 (summertime accumulations) and March 2021 (wintertime accumulations). The recreational sites were in the orchard of the Leo Tolstoy House-Museum in Khamovniki in the city center (GPS coordinates: 55.7342, 37.5862; sample code D-

1) and the campus (Botanical Garden) of the Lomonosov Moscow State University (GPS: 55.7049, 37.5270; sample code D-2). Airborne deposits were sampled into plastic containers at the height of 80 cm above the soil surface. At the intensive traffic site located at the entrance into the 6-lane tunnel at the crossing of the Third Transport Ring and the Kutuzovsky Avenue, a dust sample (GPS: 55.7383, 37.5373; D-3) was swept from the surface of a 40-cm-high asphalt-concrete block. At the nearby site by the Third Transport Ring, another sample (GPS: 55.7382, 37.5377; 55.7389, 37.5370; D-4) was swept from the road-facing side of a noise-protection screen at heights of 150-200 cm. From the same sites, sweep samples were collected after snow thawed. The tunnel entrance sample (D-5) and the noise-protection screen sample (D-6) were wintertime counterparts of samples D-3 and D-4. The third wintertime dust sample (GPS: 55.7365, 37.5397; D-7) was swept from both sides of 70-cm-high metal barriers along the road (Third Transport Ring) on a 20-m-high bridge above the railway (Kievsky overpass). A total of 7 mixed samples of dust were carried out. Each sample was analyzed in twenty replicates.

2.2. Morphological analyses

The samples were subjected to submicromorphological investigations. The composition of dust aerosols was determined using a JEOL JSM-6610LV scanning electron microscope equipped with an INCAx-act energy dispersive detector.

2.3 Microbiological analyses and species identification

Strains of *Escherichia coli* and other representatives of *Enterobacteriaceae* family were isolated from airborne dust samples using a REBECCA® EB highly selective chromogenic medium (NF Validation EN ISO 16140) and standard microbiological culturing techniques. The isolated strains were identified by modern techniques of molecular genetics based on analyses of variable sequences of the V3-V4 region of the 16SpRNA gene with the subsequent use of the NCBI BLAST database.

2.4. Antibiotic susceptibility of Escherichia coli strains

Antibiotic susceptibility of *E. coli* strains was tested using the Mueller-Hinton agar, which is a standard medium for the disk-diffusion method (Bauer-Kirby test), according to the guidelines of the Global Laboratory Standards for a Healthier World (CLSI) of the USA. Disks with a wide selection of antimicrobial medicines (HiMedia Laboratories Pvt. Ltd., India) were tested: Amoxicillin 10 (AMO), Ampicillin 10 (AMP), Meropenem 10 (MER), Pefloxacin 5 (PEF), Streptomycin 300 (STR), Ticarcillin+Cl 75 (TIC), Fosfomycin 200 (FOS), Ceftibuten 30 (CEF), Ciprofloxacin 10 (CIP). The reference strain of *E. coli* ATCC 2592 was used as a control, which is recommended by the CLSI as well as the Russian Federation clinical recommendations for «Determination of susceptibility of microorganisms to antimicrobial drugs», 2015. A total of 118 strains of *E. coli* from dust were tested. Each strain was tested in three replicates.

2.5. Data analyses

Statistical data processing of the obtained results was carried out using Excel 2010 (Microsoft, USA) and Statistica 8.0 (StatSoft, USA) programs.

3. Results

3.1. Diversity of Enterobacteriaceae

Representatives of the family *Enterobacteriaceae* isolated from atmospheric dust aerosols were classified into 5 genera and 13 species as follows: 5 *Klebsiella* spp., 5 *Enterobacter* spp., 1 *Citrobacter* sp. and 2 species requiring a sanitary standardization – *Escherichia coli* (found in all samples) and *Salmonella enterica* (Table 1).

Table 1. Relative abundances (%) * of *Enterobacteriaceae* spp. isolated from the dust samples

Species/samples	**D-1**	**D-2**	**D-3**	**D-4**	**D-5**	**D-6**	**D-7**
Salmonella enterica	–	–	–	–	–	2.6	–
Escherichia coli	89.2	100.0	24.2	21.5	17.8	19.9	15.7
Enterobacter aerogenes	–	–	–	–	3.2	4.1	–
Enterobacter agglomerans	–	–	45.1	57.4	4.1	3.3	4.5
Enterobacter cloacae	–	–	–	–	2.1	3.1	–
Enterobacter kobei	–	–	–	–	3.7	3.6	3.9
Enterobacter nimipressuralis	–	–	30.7	21.1	4.8	2.8	8.04
Citrobacter europaeus	–	–	–	–	8.2	6.01	–
Klebsiella granulomatis	–	–	–	–	11.2	10.9	26.9
Klebsiella grimontii	–	–	–	–	10.3	11.3	–
Klebsiella oxytoca	10.9	–	–	–	11.1	12.05	–
Klebsiella quasipneumoniae	–	–	–	–	11.1	10.2	40.9
Klebsiella variicola	–	–	–	–	12.5	10.2	–

*A species average percentage of the total number of bacteria in a sample.

As shown in Table 1, *Enterobacteriaceae* had highest levels of diversity, i.e., 13, 12 and 6 species in samples D-6, D-5 and D-7, respectively, which were wintertime dust accumulations from the traffic area. Each of summertime samples D-3 and D-4 from the same traffic sites (counterparts of D-5 and D-6, respectively) contained only 3 species of Enterobacteriaceae. Summertime samples D-1 and D-2 from recreational areas contained 2 and 1 Enterobacteriaceae species, respectively.

However, *Escherichia coli* had relative abundances between 15.7 and 100%, with the highest values in samples D-1 and D-2.

Salmonella enterica was found in only one sample D-6 and characterized by a relative abundance of 2.6% (Table 1).

3.2. Faecal contamination of dust aerosols

Most of the studied samples (summertime dust aerosols from the recreational sites and wintertime – from the traffic areas) contained dried microfragments of faeces, which were identified based on high bulk concentrations of carbon and phosphorus. The P concentrations ranged from decimal fractions to >5% of the total mass of the analyzed substrate (Prokof'eva et al., 2021) (Fig. 1A, B). A SEM-EDS elemental microanalysis of the control sample of dog faeces, which remained on the soil surface for several weeks during the summer, showed the P concentration of about 13% (Fig. 1C).

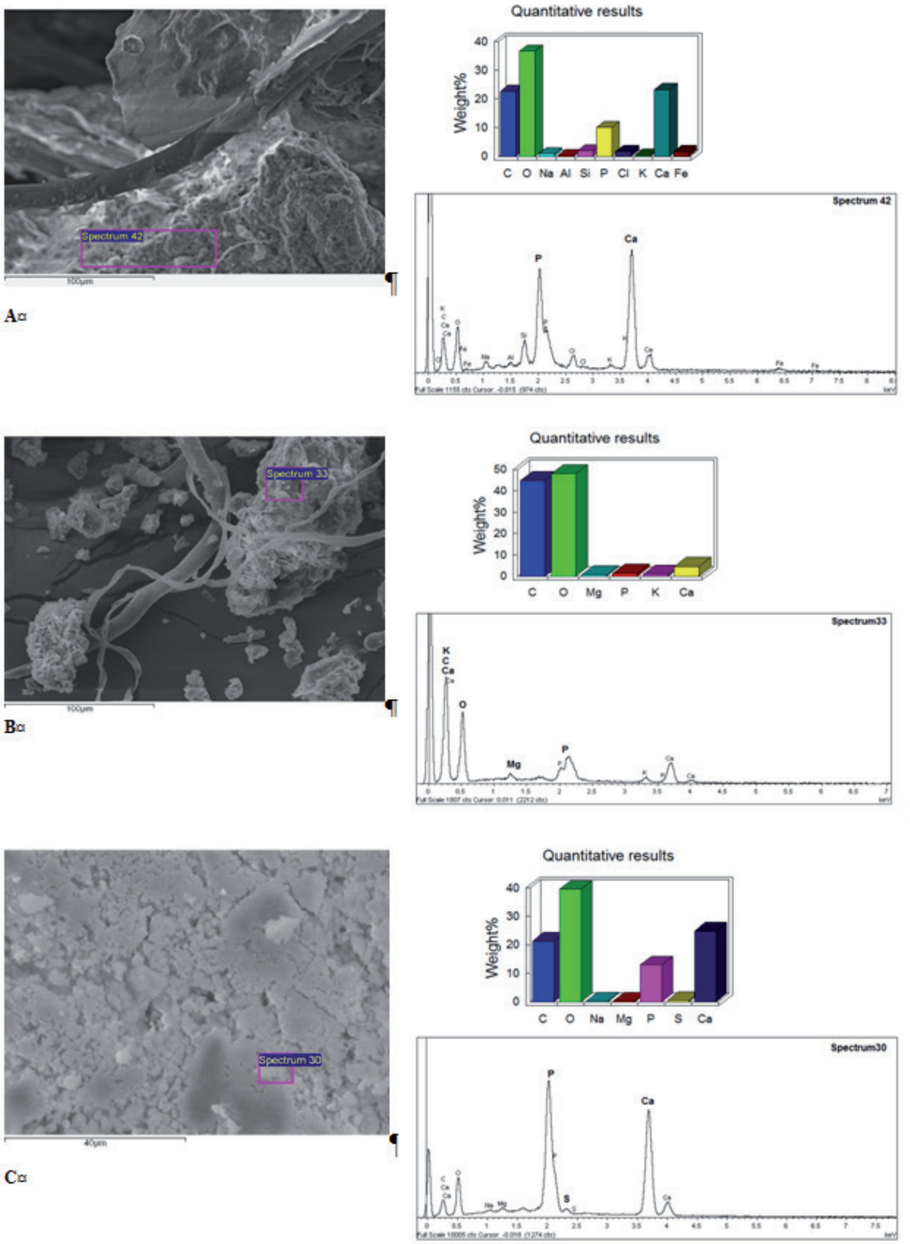

Fig. 1. Microparticles with supposedly a faecal contamination (A and B) and a fragment of dried and decomposing dog excrement collected from the soil surface (C).

3.3. Escherichia coli strains with antibiotic resistance

A total of 118 strains of *E. coli* were analyzed for resistance to the widely used antibiotics. In 32 strains of *E. coli*, one or more values of antibiotic susceptibility were significantly lower than reference values of the control strain (Table 2). Only 7 antibiotic-resistant strains were found in the recreational areas, whereas the majority (25) of such strains originated from the traffic areas.

4. Discussion

4.1. Isolated Enterobacteriaceae species

It is known that the presence of sanitary-standardized bacteria such as certain representatives of the family *Enterobacteriaceae* in soil bacterial communities is an important indication of the faecal contamination of soils (Campos Pinilla et al., 2015). Species of that family are widespread in different parts of the environment including soils, freshwater habitats, sewage, and surfaces of vascular plants. In 2017 the WHO published the list of microorganisms with the highest resistance to antibiotics, where representatives of the family *Enterobacteriaceae* (including *Escherichia, Salmonella, Enterobacter* and *Klebsiella* species) take top positions. In Moscow dust aerosols, the genus *Klebsiella* was represented by five species with relative abundances between 10.8 and 67.8% and the greatest diversity in samples D-5 and D-6 (Table 1). In 2017 the WHO listed *Klebsiella* spp. among most hazardous bacteria due to their resistance to a wide range of antibacterial drugs. All five *Klebsiella* spp. observed by us are regularly isolated from clinical materials and take part in the development of opportunistic infections (Arabaghian et al., 2019; Araujo et al., 2021). Regarding five *Enterobacter* spp. isolated from the dust aerosols, it has been reported that most representatives of this genus can cause opportunistic infections in humans and animals (Davin-Regli & Pagès 2015). Bacteria of the *Citrobacter* genus can be found in both clean and polluted waters, soils and, moreover, some species are normally present in the intestinal microflora of humans. However, this genus also includes opportunistic and potentially pathogenic species (Ranjan & Ranjan 2013).

For example, *Citrobacter europaeus* isolated from the studied samples (D-5 and D-6) has firstly been isolated from faeces of a diarrhoea patient and described in 2017 (Ribeiro et al., 2017). The *Enterobacteriaceae* species isolated by us from dust aerosols included representatives of the sanitary-standard group, i.e., *Escherichia coli* and *Salmonella enterica.* Both species are listed in the WHO environmental quality standards and the Russian sanitary regulations (SanPiN 1.2.3685–21) and their identification methods are included in the international ISO standards. We observed the maximal diversity of *Enterobacteriaceae* in samples of wintertime dust accumulations from the traffic areas (D-6, D-5, and D-7). During winters, under conditions of temperate climate with a clear seasonality and a stable snow cover, urban soils under severe anthropogenic impact usually fail to perform or poorly support their activities as «bacterial filters», which is negatively reflected in the microbial composition of atmospheric dust aerosols.

Table 2. Selected strains of *Escherichia coli* with antibiotic susceptibility (retarded growth, mm) below the reference values*

Antibiotics	AMO	AMP	MER	PEF	STR	TIC	FOS	CEF	CIP
Reference values**	14-17	14-16	14-15	16-21	12-14	22-23	13-15	18-20	16-20
Strain number									
D1-1	16.7	14.3	16.7	16.7	20.3	11.3	26.3	11.7	16.3
D1-7	17.7	14.0	16.7	17.7	20.7	10.7	23.0	12.3	16.3
D1-8	21.0	14.0	17.0	15.7	6.0	8.0	18.3	10.3	20.7
D1-12	14.8	19.0	14.7	16.3	6.7	7.0	27.7	8.7	19.3
D1-19	16.0	16.0	16.3	16.3	6.0	8.0	22.7	11.7	19.3
D2-3	9.0	14.0	9.7	16.7	5.7	6.3	27.3	7.5	20.0
D2-15	16.0	14.0	18.0	16.3	8.3	10.0	19.7	9.7	16.7
D3-4	15.0	17.3	16.3	15.4	6.7	25.3	25.0	20.0	21.7
D3-7	14.0	14.0	15.7	17.0	8.3	18.3	21.0	10.3	22.3
D3-8	14.0	15.3	16.3	18.0	9.7	20.7	16.7	11.3	21.7
D3-9	14.7	14.0	16.7	17.0	14.3	10.3	20.3	18.3	22.7
D3-10	16.0	16.3	13.3	7.3	23.3	23.3	21.7	20.3	25.3
D3-13	17.0	16.0	15.3	20.0	24.0	25.7	25.7	19.3	20.0
D3-15	14.0	14.0	15.3	15.0	6.0	6.0	26.7	19.0	22.7
D3-22	16.0	16.7	14.7	17.0	18.3	19.3	21.0	19.3	23.3
D3-32	16.7	14.0	15.3	18.7	10.0	15.0	20.3	19.0	17.3
D4-1	16.0	15.7	16.0	12.7	16.3	9.7	20.3	9.3	21.0
D4-19	14.0	15.0	17.3	18.0	18.3	23.7	16.0	22.0	25.3
D5-4	22.0	16.3	15.3	17.0	11.0	21.7	23.7	20.3	22.3
D5-12	17.1	16.0	14.2	20.2	14.2	23.4	12.6	20.5	20.4
D5-28	22.0	18.0	16.0	20.7	14.3	22.3	22.7	21.3	16.3
D5-46	19.0	12.0	17.0	20.7	13.7	20.7	22.3	18.7	21.3
D5-56	11.0	16.3	15.3	12.0	17.3	16.0	27.7	13.7	23.0
D6-8	14.0	16.3	16.1	21.4	12.8	22.8	22.8	19.2	18.2
D6-9	12.3	19.0	14.3	13.3	16.3	21.7	24.0	11.7	23.0

*– Values indicating antibiotic resistance are highlighted in gray. ** – Values for the control strain ATCC 2592.

4.2. Faecal contamination of dust aerosols

The search and identification of morphologically recognizable microfragments of faeces in dust aerosols are very disputable. We relied on the presence of significant concentrations of biophilous elements in the studied microfragments. In cases when such concentrations were higher than those in soils, we identified the analyzed microfragments as faeces. It is known that soils have bulk phosphorus concentrations between 0.02-0.5% (Vorob'eva, 2006). Fresh dung of cattle has the P concentration of about 0.3%, which increases to 0.5% after 6-8 months of storage (Vasil'ev & Filippova 1988). Dogs have a high-protein diet, and their excrements were shown to have a very high P concentration (Fig. 1C). Based on P concentrations, some microfragments of dust could have originated from P-enriched soils (Fig. 1 B). However, the main source of P enrichment of Moscow soils is the excrements of domestic pets (Stroganova et al., 1998).

4.3. Antibiotic susceptibility of Escherichia coli

Antibiotic resistance was observed in 27% of the tested strains of *E. coli,* which showed the highest resistance (65.6%) to Ticarcillin-Clavulanate – antibiotic from the penicillin group mixed with clavulanic acid (β-lactamase inhibitor). The majority (25 of 32) antibiotic-resistant *E. coli* strains originated from the traffic areas. Indeed, the persistence of *E. coli* is likely to be activated by the anthropogenic impact, by analogy with previously reported tendencies in mycobacteria, shigella, salmonella and other pathogens (Savilov et al., 2020).

The phenomenon of antibiotic resistance in microorganisms has been reported from all continents. However, its distribution is to a high degree predetermined by policies of antimicrobial drug usage and significantly variable between different regions.

5. Conclusion

The present study showed that the atmospheric dust aerosols of Moscow contain significant numbers of particles enriched in phosphorus and other biophillous elements, which is likely to indicate their faecal contamination.

Significant numbers and a high diversity of *Enterobacteriaceae* including the opportunistic and potentially pathogenic species isolated from the dust aerosols confirmed the presence of faeces. Therefore, airborne dust is an active transmitter of microbial pollution within the megalopolis.

It was found that 27% of the tested *E. coli* strains were resistant to antibiotics. The traffic areas were characterized by a significantly higher total number of antibiotic-resistant *E. coli* strains as compared to the recreational areas of Moscow city (25 vs. 7 strains) which clearly demonstrates a high health risk of dust exposure in the area of a high anthropogenic pressure. The most adverse properties were observed in wintertime accumulations of dust aerosols because urban soils covered with snow have a low activity as a bacterial filter.

The data obtained also allow us to make a preliminary suggestion to avoid the use of antibiotics of the penicillin group within the Moscow region at the present time. In order to prevent the further development of antibiotic resistance in potentially pathogenic bacteria, the use of antibacterial drugs should be clearly substantiated by experimental testing of certain bacterial strains.

The use of local screening with prospective testing of dust aerosols in any urban environment would be a valuable tool in ecological and monitoring assessments and also in the development of recommendations regarding the selection of antimicrobial drugs for the treatment of infections in city populations.

Our study also gives a basis for recommendations concerning the need to dispose of dog waste. Until now, there is a lack of a strict administrative control over domestic pet owners in Moscow.

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