

The Bacterial Urban Resistome

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Cities that are densely populated are reservoirs of antibiotic resistant genes (ARGs). The overall presence of all resistance genes in a specific environment is defined as a resistome. Spatial proximity of surfaces and different hygienic conditions leads to the transfer of antibiotic resistant bacteria (ARB) within urban environments. Built environments, public transportation, green spaces, and citizens' behaviors all support persistence and transfer of antimicrobial resistances (AMR).

Keywords: antimicrobial resistances ; urban microbiome ; hospitals ; antibiotic resistant bacteria ; urban resistome ; antibiotic resistance genes

1. Introduction

More than half of the world's population lives in urban areas and, increasingly, within high population-density cities ^{[1][2]}. Some built environments undergo frequent and high-volume human throughput/activity and influenced strongly by their surrounding environments. These may be considered 'unrestricted' buildings (e.g., offices, retail centres, schools, and farms). Other buildings are more 'restricted' in that they permit only limited access and within which operations aimed at reducing/eliminating microbes and/or frequent cleaning take place (e.g., clean-room facilities and intensive care units). Cities also include outdoor environments (recreational parks, ponds, lakes) that can support high human throughput and activity. Such environments, either in the same city or in different ones, are connected via public transport (e.g., metro, buses, air transport), and shared transport (e.g., cars, scooters, bicycles). Waterborne linkage between and within these environments occurs via runoff over impermeable surfaces and wastewater transport within closed and open drainage and sewerage systems. Additional linkages occur via airborne movement of microbes or of materials colonised by them.

One consequence of high human densities within this complex array of built environments, associated outdoor environments, and their infrastructural and human transport links, is that there is substantial opportunity for the transport of antibiotic resistant bacteria (ARB) and the antibiotic resistance genes (ARGs) they contain. The sum of all such genes conferring antibiotic resistance is defined as a 'resistome', and it includes those present in both pathogenic and non-pathogenic microorganisms.

2. Built Environments

People from developed countries spend about 90% of their time indoors ^{[3][4]}. Such environments are extremely heterogeneous in composition, experience widely different air circulation patterns and may harbour dust or other organic detritus.

Humans and pets contribute to the microbial community of these environments, as each individual can transfer its microbial fingerprint to indoor spaces ^[5]. Thus, because of the varied composition and activities within, built environments are potential hotspots for microbial exchange and spread ^{[6][7]}.

To attempt a comprehensive review of AMR in hospitals would diverge from the purposes. Because, however, literature shows hospitals are reservoirs and incubators for new ARG variants ^[8], researchers performed a systematic research of the frequency of reports regarding the top 50 ARG subtypes in hospitals (and their respective antibiotic families) as described by Zhuang et al. ^[9], to determine their role as sources for urban resistomes. Data extracted from PubMed publications from 1990 to the present showed that the families of β -lactams and *mecA* genes (involved in methicillin resistance) ^[10] were the most reported. Potential escape routes from hospitals include wastewater effluents, biohazard operators, patients, and visitors ^[11]. Another study showed that even dust from indoor hospital samples exhibited a complex resistome profile, with an average ARG concentration of 0.00042 copies/16S rRNA gene. This also found that the outpatient hall was one of the main ARG transmission sources, permitting distribution of ARGs to other departments ^[12]. Among ARGs identified, this reported: *aadE*, *ARRAM*, *mecA*, *aad(9)*, *brcA*, *tetL*, *InuB*, *tet40*, *tetZ*, *tetA*, *tetK*, *norA*, *CE*,

aadD, *qacA*, *vgaA*, *tetK*, *tetH*, *tetG*, *mexT*, *OXA*, *aph(3')*, *mefA*, *bleO*, and *CfxA2*. Hospital air-conditioners can distribute the resistome over time and accumulate ARGs whilst transporting them within departments [12]. Genes present in dust were also shown to change with season: for example, 86 ARGs subtypes were detected in winter, whilst only 11 occurred in summer.

Cleaning chemicals can also affect microbial communities and associations between antimicrobials and bacterial resistomes [13]. Increased confinement and cleaning are associated with a loss of microbial diversity [14]. In this context, Mahnert et al. [14] compared 'human-driven' microbial resistomes present on surfaces in clinical settings and in other built environments. Their metagenomic approach showed that environments with increased confinement and cleaning were associated with genomes enriched with functions related to virulence, disease, defence, stress response, and resistance against five classes of antibiotics. In contrast, unrestricted buildings were characterized by a higher diversity of bacteria associated with the outdoor environment and processed food [14]. Confinement and cleaning were associated with a shift from Gram-positive bacteria, such as Actinobacteria and Firmicutes, to Gram-negative, such as Proteobacteria and that the loss of microbial diversity correlated with an increase in resistance [14].

Other examples showed the distribution of ARGs on floor surfaces in different types of environments. Gupta et al. (2019) sampled the carpet and vinyl floors from medical, veterinary, and office buildings in both high- and low-traffic areas [15]. Results showed a widespread distribution of *tetQ* and *sul1* resistant genes in all sample areas, while carbapenemase encoding genes from *Klebsiella pneumoniae* were only detected from the high-traffic surfaces of medical facilities. Most indoor environments harboured ARGs, veterinary samples had higher concentrations of *tetQ* and *sul1*, and carbapenem resistance was only observed in the medical centre. Moreover, most floor surfaces also showed the presence of dog- and bird-specific faecal bacteria.

Another less commonly considered aspect is the "thanato-resistome" associated with locations and practices related to the funeral industry. Gwenzi [16] suggested that all such environments should be considered as potential reservoirs of ARGs and may present health risks for funeral workers. It is likely that human cadavers harbour resistant microbes and/or ARGs so, consequently, all wastes derived from thanatopraxy (e.g., embalming fluids) should also be considered as potentially contaminated. In addition, decomposition of buried human bodies and discharge of wastewater from thanatopraxy care facilities may eventually contribute to the spreading of resistance in the urban environment [16].

Schools are environments of particular interest, since direct (i.e., human-to-human) and indirect (i.e., object-mediated) contact derived transmissions of bacteria are common. Although schools constitute high-risk environment for infections, literature regarding metagenome shotgun analysis of resistomes in this environment is missing. More investigations are needed.

3. Transportation

3.1. Air Transport

Airports and aeroplanes are a major causal influence upon ARGs and ARB dispersal. Every year billions of passengers are transported between countries and continents. ARGs have been detected in airport wastewater treatment plants (WWTP). The role of aeroplanes, and particularly their toilet sewage, as a source of ARGs has been investigated. Two studies agreed that aeroplane-borne sewage can effectively contribute to the fast and global spread of antibiotic resistance [17][18].

Shotgun sequencing of toilet waste from 18 international aeroplane flights arriving in Copenhagen, Denmark, from nine cities and three different world regions showed that 0.06% of all DNA sequencing reads were assigned to resistance genes and that the most abundant genes encoded resistance against tetracyclines, macrolides, and β -lactams. The relative abundance of *sul1* (sulfonamide) and *tetM* (tetracycline) resistance genes were significantly increased in aeroplane samples compared to the airport's WWTP inlets. Median relative abundances between the two sample origins (aeroplane sewage versus airport's WWTP) differed by factors of 5 (*sul1*) and 18 (*tetM*), respectively. Flights from South Asia showed significantly higher abundance and diversity of *bla*_{CTX-M} genes compared to those from North America. Detection of antibiotic resistances was also associated with the presence of *Salmonella enterica* (higher from South Asia), and *Clostridium difficile* in samples from North America [18]. However, functional-taxonomical tests were not performed; therefore, it is not possible to associate resistances with specific pathogens. When, however, aeroplane-borne sewage was tested for ARGs in terms of diversity and quantity, resistances against fluorochinolones, third-generation cephalosporins, and aminoglycosides were particularly associated with *Escherichia coli* isolated from the sewage [17]. Comparison with municipal sewage also showed that the aeroplane sewage had more mobile ARG elements, with higher

relative abundances. The study [17] also stressed that ARG-concentrated aeroplane sewage is discharged into airport WWTPs so both WWTP influents and effluents should be monitored for their ARG/ARB profiles.

Screening of military aviators showed the presence of methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA infections are significantly more frequent among members of the military than in the general population and some types of MRSA related to military personnel evolves separately from nosocomially acquired MRSA [19]. Attention was raised because, community-acquired, non-healthcare associated MRSA-based resistance might be transferred to dense urban populations [20].

3.2. Resistomes in Trains and Metro

Of all public transport types worldwide, trains and metro are probably those with the highest number of passengers. In the largest cities, several million people use these transport methods daily. Because of confined spaces, poor air circulation and prolonged skin-surface contact within coaches (e.g., handrails), this kind of public transport influences passengers' skin microbiota [21], contributing to the diffusion of resistant strains as well [22][23]. Like aeroplanes, trains also transport people long distances, between urban areas and across international borders.

Resistance patterns of airborne bacteria in the Shanghai (China) metro were analysed finding *Staphylococcus* strains carrying *mecA* and *qac* resistance genes, which confer resistance against methicillin and lactams antibiotics, respectively [24]. Frequencies were compared with those from hospital samples and from control samples taken from parks. Frequencies of detection of resistance genes in metro and hospital samples were comparable but both were higher than in park samples. Similar results were also obtained in two studies regarding several surfaces types in railway stations in Guangzhou (China) [25][26], where about 75% of *Staphylococcus* isolates were from multidrug resistant (MDR) strains.

As with aeroplane transport, wastewater produced in trains can contribute to the spreading of resistance genes. Wei and colleagues [27] investigated the efficiency of a pilot-scale system in removing ARGs and ARB in wastewater collected from multiple units of high-speed trains in Beijing (China). They showed that the abundance of ARGs and mobile genetic elements was similar to that of untreated hospital wastewater and higher than that of domestic wastewater.

3.3. Shared Transportation

Use of shared transport is increasing in popularity, especially in larger cities. Citizens can both save money and reduce overall emissions of greenhouse gases by sharing bicycles, cars, and scooters. Sharing vehicles, however, increases the risk of transferring ARGs by indirect host-to-host transfer. This risk occurs via prolonged contact with surfaces, such as handles and seats. In 2019 in Chengdu (China), there were 1.23 million shared bicycles used for more than 2 million daily rides. Resistant Gram-positive bacteria were isolated from bicycles or riders, and hosted resistances against clinically important antibiotics including linezolid, fosfomycin, and vancomycin, with a significant quantity of these isolates showing multidrug resistance. MRSA strains were also isolated and whole genome sequencing further detected the presence of *fosB*, *fusB*, and *lnu(G)* in *S. aureus* and *optrA* in enterococci, in addition to other genes. Bacterial transmission across geographical-distinct locations (both bicycles and riders) was demonstrated by genetically closely-related bacteria [28].

Another study aimed to address the risk of public shared bicycles transferring resistant strains of *Staphylococcus epidermidis* within a population. Antimicrobial susceptibility and molecular testing were performed to classify the *Staphylococcus* species, resistance patterns, presence of *mecA* gene, and clonal lineage. Overall, 49% of screened staphylococci were *mecA*-positive with a high diversity of staphylococcal cassette chromosome *mec* (SCC*mec*) elements [29]. Such variability of SCC*mec* could be associated with a high variability within *Staphylococcus* species, showing a strong propensity to dissemination [29].

Another bacterial family commonly isolated from shared bicycles are the Enterobacteriaceae. Sampling 2117 shared bicycles at 240 metro stations in Beijing showed a total of 444 non-duplicate Enterobacteriaceae isolated from 418 samples at 166 stations. In this case, the isolates were resistant to amikacin (0.7%), ceftazidime (0.7%), ciprofloxacin (0.5%), colistin (3.6%), doxycycline (5.4%), gentamicin (1.3%), florfenicol (2.5%), fosfomycin (6.3%), and meropenem (0.5%). Moreover, 31.5% were resistant to sulfamethoxazole-trimethoprim. Three ceftazidime-resistant *E. coli* isolates were positive for *bla*_{CTX-M-199} and two were positive for carbapenemase-producing gene *bla*_{NDM-5} [30]. In another study investigating hand-bicycles in China, *Bacillus* spp. Were also found to be resistant to bacitracin and sulfamethoxazole [31].

Multivariate logistic regression of data regarding resistant bacteria sampled from shared bicycles at metro stations near hospitals revealed that variable 'secondary/tertiary non-profit hospital nearby' was significantly ($p < 0.05$) associated with

isolation of Enterobacteriaceae from the shared bicycles [30]. This indicates that these resistances may have a common origin in hospitals [30].

Such data suggest the use of shared bicycles increases risk of ARGs/ARB dissemination and, equally, suggest the need for an effective disinfection strategy.

4. Urban Green Spaces and Parks

Green spaces provide important ecosystem services and improve citizens' physical and mental well-being and development [32][33][34][35]. Hence, most epidemiological studies of urban green exposure focus on their availability and health benefits, few studies examined unwanted side effects, such as pollen allergies, vector-borne diseases [36], or their role as potential reservoirs of antibiotic resistance.

Soil samples taken anywhere on the planet contain antibiotic resistant microorganisms (ARMs), therefore this is also true for soil sample obtained from urban parks. It has been reported that urban park soil microbiomes are both rich in biosynthetic diversity and distinct from non-urban samples in their biosynthetic gene composition [37]. Anthropogenic ARGs/ARM can enter these urban ecosystems via several pathways: faecal shedding by animals (e.g., domestic dogs and wild animals, especially mammals and birds), irrigation with reclaimed water and atmospheric deposition.

Such diversity is further encouraged via horizontal gene transfer (HGT) mediated by mobile genetic elements that facilitate the transfer of ARGs within and between related and unrelated bacterial species. As a consequence, such ARGs have the potential to become widespread within microbial communities in domestic and feral animal populations. Worsley-Tonks et al. [38] reported that faecal samples and rectal swabs of stray dogs had twice as many unique ARGs compared to owned dog samples, which was partly driven by a greater richness of beta-lactamase genes conferring resistance to penicillin and cephalosporin. Other urban wild animals, such as foxes, are more likely to be exposed to AMR bacteria and resistance drivers from food waste, garbage, sewage, wastewater, and consumption of contaminated prey than those living in remote areas. Mo et al. [39] found that the total occurrence of AMR in *E. coli* from faecal swabs of foxes in areas with high population density was significantly high.

Wild birds, such as geese, swans, and gulls, are frequent visitors to many urban parks, particularly those with ponds and lakes. Many migratory birds also come into close contact with humans in urban areas where they feed on terrestrial grasses found in public parks and sports grounds. These wild birds may also play a role in transporting antibiotic resistance to urban green spaces via faecal shedding [40][41][42][43].

ARGs in recreational urban water bodies are also an issue. Urban ponds are utilised in a variety of ways; some are used for recreation, others receive flood relief water from rivers or store urban runoff [40]. Sewage leaking into recreational water is a serious issue, second only to hospitals in terms of ARGs diversity. With an average absolute abundance of 1.38×10^7 copies/mL ARGs and 4.19×10^6 copies/mL mobile genetic elements, urban ponds can be considered as ARGs hotspots [44].

Water scarcity is increasingly a challenge for industrial and urban development, especially in arid and semi-arid regions. To ensure a sustainable water supply, water-reuse and water management concepts have been proposed by a number of researchers requiring the integration of grey infrastructures (water supply and wastewater treatment) with green infrastructures, such as parks and public green spaces [45][46]. For example, in Australia during the period 2009–2010, average state-wide use of recycled water for urban irrigation was 27.2% whilst the nation-wide average of total recycled water produced was 14%. In Madrid, Spain, irrigating urban park turf with reclaimed water has led to grass biomass increase [47] and, in terms of micronutrient content, the reclaimed water used was adequate for irrigation [48].

Reclaimed water may contain ARB that could be transferred to the urban environment via irrigation. Limayem et al. [49] detected the presence of drug resistance in both pathogenic and non-pathogenic bacterial strains in reclaimed water samples, where isolated *Escherichia*, *Klebsiella*, and *Acinetobacter* displayed resistance to chloramphenicol, ciprofloxacin, daptomycin, erythromycin, gentamycin, kanamycin, streptomycin, lincomycin, linezolid, nitrofurantoin, penicillin, quinupristin/dalfopristin, tetracycline, tigecycline, tylosin tartrate, and vancomycin. *Pseudomonas* was resistant to ciprofloxacin, erythromycin, daptomycin, lincomycin, linezolid, nitrofurantoin, and tigecycline. Moreover, *Streptococcus* and *Staphylococcus* were resistant to daptomycin, kanamycin, lincomycin, linezolid, nitrofurantoin, penicillin, quinupristin/dalfopristin, tylosin tartrate, and vancomycin [49].

The irrigation of urban parks with treated wastewater significantly increased the abundance and diversity of various antibiotic resistance genes (resistances to β -lactam were the most prevalent ARG type), although significant increase in

horizontal gene transfer was not observed [50]. The potential for such transfers exists, however, Yan et al. [51] reported that diverse ARGs and mobile genetic elements, including six transposon-transposase genes, class 1 integron genes (*int1* and *cint1*) were present in both urban park grass phyllosphere and soil. Such genes indicated the potential for horizontal gene transfer of soil ARGs.

These cases demonstrate that although there is increasing interest in the use of tertiary wastewater from WWTP for various applications, primarily agricultural and landscape irrigation, there may be very real risks associated with such uses in terms of enhanced resistome profiles within recipient environments.

Atmospheric deposition of industrial pollutants may also play an important role in shaping ARG profiles. In a study of ARGs abundance in bioaerosol and particulate matter (PM_{2.5}) under different rain conditions, ARGs were detected in 8 out of 21 rain events [52], suggesting that wet and dry deposition could contribute to urban green space/park resistomes. Further studies should be focused upon differentiating between normal, natural resistance patterns, and ARGs that may have been introduced from other urban routes (such as hospitals).

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