METHODOLOGY FOR TESTING BIOLOGICAL CONTAMINATION OF CABIN FILTERS AT TEST STATION SIMULATING PASSENGER CAR VENTILATION

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Abstract. Due to the biological hazards posed by the diversity of microflora in cabin filters, experimental testing requires a method that eliminates human involvement in preparing biological cultures. Awareness of the limitations of conducting experimental tests on bacteria growing in passenger car cabin filters contributed to the development of a test station for multiplying bacteria found in vehicle air conditioning systems. A fragment of the ventilation system of a Toyota Auris passenger car, together with the original fan and cabin filter mounting space, was used to construct the station. The rest of the station was manufactured using 3D printing technology based on a 3D scan of the selected ventilation system. The proposed measurement system is equipped with digital probes for measuring air temperature and humidity, as well as volumetric flow rate. The redesigned inlet and outlet manifolds allow the installation of a second (clean) filter and the operation of the station in both closed- and open-circuit configurations. Using the constructed station, tests were conducted to assess the impact of contaminated filters on clean filters. Subsequently, the presence of antibiotic-resistant bacteria was considered for activated carbon filters and antibacterial filters. The minimum inhibitory concentration for selected strains of bacteria isolated from the filters was assessed. It was found that cabin filters, after prolonged use, are not only heavily contaminated with bacteria and fungi but also provide a habitat for many antibiotic-resistant bacteria. A lower bacterial content was observed in antibacterial filters, but this was not associated with a proportionally lower content of antibiotic-resistant microorganisms. The developed position is used for subsequent stages of research on the threat posed by excessive air pollution in vehicle passenger compartments.

Keywords: antibiotic resistance, microorganisms, reverse engineering, vehicle interior air quality.

Introduction

We currently spend a relatively large amount of time in various types of vehicles. A 2016 study titled “Our Lives in Cars,” conducted by Citroën and CSA Research, found that Europeans spend an average of four years and one month of their lives in a car [1]. According to a German study, we spend an average of 45 minutes a day in a vehicle [2].

The vehicles we use are overwhelmingly equipped with ventilation and air conditioning systems. In addition to the undoubted benefits of ensuring adequate travel comfort, these systems can unfortunately be a significant source of contamination, including microbiological contamination. Secondary emission of microorganisms accumulated in filters and components of the biofilm covering the duct walls leads to indoor air contamination, which sometimes poses a serious health risk [3-8]. It has been found that the air in air-conditioned passenger cars contains 25 bacterial species, most commonly from the genera Staphylococcus, Kocuria, and Micrococcus, as well as Bacillus [4]. The latter genus was represented, among others, by species such as B. subtilis and B. thuringiensis, which are among the agents causing allergic alveolitis. In the same study, 30 fungal species representing 18 different genera were also isolated.

User exposure to bioaerosols emitted by the vehicle’s air conditioning system is particularly associated with the respirable fraction (below 1.1 μm), which tends to penetrate deeply into the respiratory system [9; 10]. It has been shown that this fraction accounted for over 50% of the bacteria and fungi present in the air inside vehicles. A correlation was also observed between the number of bacteria and the concentration of PM 2.5 [2; 3]. Furthermore, microorganisms growing within a vehicle’s ventilation and air conditioning system can release volatile organic compounds into the air, which can cause unpleasant odours [12]. It has been observed that very intense emission of microorganisms accumulated in the filter occurs within the first five minutes after the air conditioning is turned on [9; 12].

Locations conducive to the proliferation of microorganisms within a vehicle’s air conditioning system include not only air filters but also, for example, air coolers, air conditioning ducts (especially those with small diameters), or surfaces contaminated with accumulated dust, which provide ideal sites for biofilm formation [3; 13].

The relatively long service life of air filters in automotive air conditioning systems under diverse, variable environmental conditions means that the microflora inhabiting them is characterized by high diversity [8]. Over 400 bacterial species were detected in dust from air filters, including opportunistic pathogens of the genera Acinetobacter, Pseudomonas, Stenotrophomonas, and Bacillus. Eighteen fungal species with potential allergenic activity were also found, including representatives of the genera Alternaria, Cladosporium, Penicillium, and Aspergillus [12]. In another study [14], 21 filters from automotive air conditioning systems were examined for fungal contamination, and 17 genera of fungi were isolated, including Penicillium, Fusarium, and Aspergillus. The latter genus was represented by potentially pathogenic strains, including A. flavus, A. niger, A. fumigatus, A. ochraceus, and A. clavatus [8]. In a separate study of 73 air conditioning filters from various vehicle types, using culture methods and real-time qPCR techniques, the presence of molds such as Aspergillus niger, A. fumigatus, and A. versicolor was demonstrated [9]. In another study of filters from 19 truck cabins and 28 passenger cars, azole-resistant fungi of the genera Penicillium, Cladosporium, and Aspergillus were identified.

Microbiological contamination was also examined in 12 automotive air conditioning systems from six different manufacturers [15]. Mixed communities of bacteria and fungi, as well as occasional protozoa, were found in biofilms collected from various system components. The bacterium Methylobacterium mesophilicum [16] was isolated from aluminum heat exchangers in 10 systems, while the mold Penicillium viridicatum was detected in four cases. Biofilm forming inside automotive HVAC systems can also serve as a source of air contamination with Legionella bacteria. It has been reported that over 30% of cabin filters from various types of cars contained Legionella pneumophila [7]. In one case, condensate from a malfunctioning automotive air conditioning system was identified as a likely source of this bacterium. The potential presence of Legionella bacteria in automotive air conditioning systems has also been highlighted [17].

It is worth noting that several new bacterial strains specific to this environment have been identified, such as Spirosoma aerolatum [18], Mucilaginibacter carri [19], Spirosoma carri [20], Sphingomonas carri [21], Deinococcus metallilatus and Deinococcus carri [22], Deinococcus aluminii [23], Spirosoma metallum [24], Methylobacterium currus [25], Nocardioides currus [26], Nakamurella aerolata [27], and Flexivirga aerilata [28].

Most studies on microbial contamination of ventilation and air conditioning systems focus on quantitative assessments and general qualitative analysis. However, the prevalence of drug-resistant bacteria in this environment remains largely unexplored. There is evidence supporting the possibility of the spread of drug resistance in the air environment [29-31]. Therefore, in this study, in addition to a quantitative analysis of the microflora inhabiting filters in passenger car air conditioning systems, an attempt was made to assess the presence of bacteria resistant to selected, commonly used antibiotics.

Materials and methods

Conducting experimental studies under laboratory conditions requires the most accurate possible replication of the real-world environment, while ensuring the repeatability of measurements and control of boundary conditions. Tests of vehicle cabin filter contaminants may involve biological, chemical, and mechanical contaminants. The designed test rig allows for the observation of all these types of contaminants; however, this paper presents results pertaining exclusively to biological contaminants.

The test bench is configured to operate in both open and closed loops. The core component is a section of the actual ventilation system from a 2015 Toyota Auris passenger car, including the mounting space for the original cabin filter and a centrifugal fan that forces airflow through the system. Additional intake and exhaust ducts have been attached to the base module, which simplify measurements and allow for the installation of additional filtration elements and sensors.

The intake duct is designed to allow for the installation of an additional, clean air filter. The outlet duct is used to change the direction of airflow and to house probes for measuring air velocity, temperature, and humidity. The cross-sections of the inlet and outlet ducts, responsible for changing the direction of flow, were designed in the Siemens NX CAD system and then manufactured using 3D printing technology (Anycubic Chiron printer). The remaining duct sections were constructed using straight sections of aluminum ventilation pipes. The use of 3D printing to manufacture custom duct

sections allowed for arbitrary shaping of the geometry (size and shape of cross-sections, bending radii) while maintaining the specified flow cross-sectional area.

a) b)

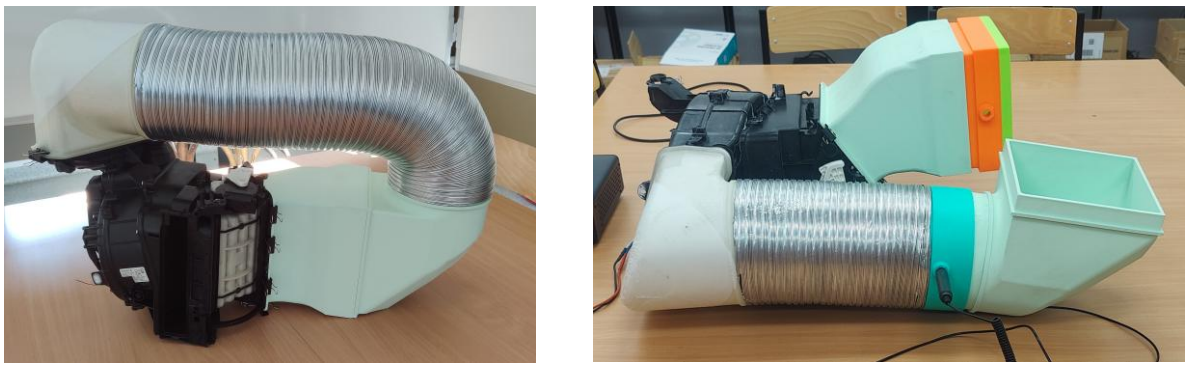

Fig. 1. **Prototype test rig for cabin air filters:** a – closed-loop configuration, b – open-loop configuration

In the closed-loop configuration, the unit consists of: a module with a fan and a filter mount sourced from the Toyota Auris ventilation system, a 3D-printed intake duct with the option to install an additional filter, a 3D-printed exhaust duct connected by a flexible circular hose, and a mounting ring for measurement probes. The geometry of the intake and exhaust ducts was recreated using reverse engineering techniques – the ventilation system was 3D scanned to obtain a model reflecting the actual shapes of the ducts.

The modular design of the test bench allows variations in the measurement probes used, including both the number and types. Ultimately, a configuration was selected featuring flow velocity, temperature, and relative humidity sensors at the end of a straight section of the circular duct. At the same time, the modular design allows for the preparation of adapters to mount ventilation modules from other vehicles without interfering with the measurement system.

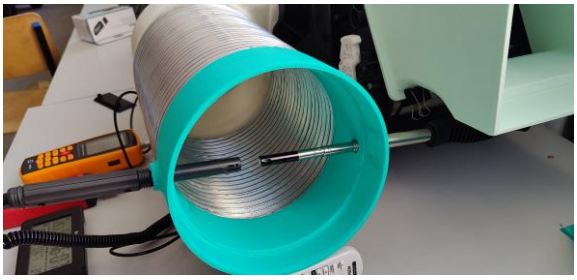

Fig. 2. **Probes for measuring airflow parameters installed in the exhaust duct**

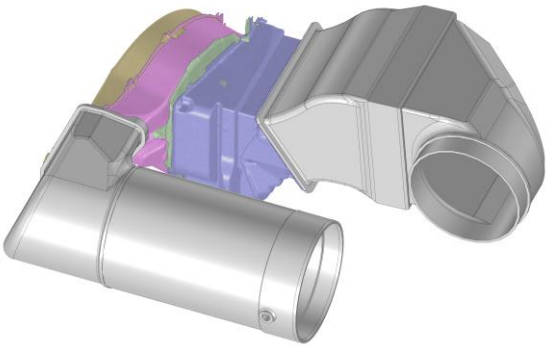

Fig. 3. **CAD geometric model of the station (the scanned parts of the filter housing are highlighted in color)**

The need to change the flow configuration stemmed from the requirement to conduct research both in an open circuit – in which a contaminated filter is purged with uncontaminated air drawn from the environment – and in a closed circuit, in which air circulates exclusively within the system, without a significant inflow from the outside. This operating mode allows for both the simulation of typical operating conditions of the supply air system and controlled experiments on the emission of microorganisms from contaminated filters.

To prepare a numerical model of the test rig for CFD analysis, 3D scans of the interior of the ducts in the ventilation system under study were performed. The system was disassembled into individual components, and each was then scanned using an Artec Eva handheld scanner, yielding point clouds converted into triangular meshes and saved in STL format. The triangular mesh can be converted into full 3D CAD geometry using dedicated software (e.g., Geomagic Wrap), but this is not necessary for

creating a computational mesh for numerical simulations. It is sufficient to use a preprocessor (e.g., Altair HyperMesh) to combine the geometry developed in the CAD system with the triangular mesh obtained during 3D scanning and prepare a homogeneous computational geometry.

Digital thermo-hygrometric probes were used to measure air temperature and relative humidity in the ducts upstream and downstream of the filter, while the volumetric flow rate was determined using a duct anemometer in conjunction with a differential pressure transducer. The selection of sensors and their installation method were based on solutions used in the evaluation of cabin filters and HVAC system filters. Prior to the actual tests, the repeatability of the measurement system readings was verified at several fixed flow rates and humidity levels by comparing the results with reference values obtained from an independent measurement setup. This procedure was consistent with practices used, among others, in the validation of sterile filters and filters used in medical respiratory systems.

Fig. 4. **Cabin filter mounting frame attached to the test stand**

Tests for the emission of biological contaminants were conducted on new carbon filters mounted in filter frames. Before testing began, each filter was inoculated with defined contaminants containing antibiotic-resistant bacteria. After a sample stabilization period, the planned test program was carried out, covering two values for temperature, humidity, and airflow velocity in the duct. Bacterial emissions were measured using the AES Laboratoire SAMPL'AIR™ Lite portable microbiological air sampler, which draws air through a sterilizable stainless steel grille and maintains a constant flow rate of 100 l/min with an accuracy of ± 5%. The collected air samples were subjected to further processing and microbiological analysis under laboratory conditions at the Department of Biology, Faculty of Environmental Engineering, Warsaw University of Technology.

The material for the study consisted of standard cabin filters removed from Toyota passenger cars, dedicated to the Auris, Corolla, Aygo, Yaris and Rav4 models, respectively. A total of 8 filters previously operated in the vehicles for 6-8 months were tested. The tests were conducted during the spring period. Filter samples were taken aseptically immediately after disassembly from the cars and placed in sterile containers, which were transported to the laboratory within 24 hours and subjected to microbiological analysis.

To obtain samples for testing, 10 g and 1 g filter fragments were shaken for 0.5 h in a 0.1% solution of sterile sodium pyrophosphate at 120 rpm to suspend the dust in the filter along with the microorganisms. The resulting suspension was then quantitatively tested by the Koch plate method, using surface culture of samples on appropriate culture media. The basic range of microbiological analyses included the determination of the total number of bacteria growing at 26 °C, the total number of bacteria capable of growing at 37 °C, the number of mannitol-positive staphylococci and the abundance of molds. The total number of bacteria was determined on nutrient agar medium, after 48 h incubation. Staphylococci counts were determined on agar medium according to Chapman after 48 h at 37 °C, while mold counts were determined on Rose Bengal and chloramphenicol medium after 5 days of incubation at 26 °C. The number of microorganisms was calculated to obtain the result as the number of $CFU \cdot g^{-1}$ of the filter.

Five commonly used antibiotics were selected for bacterial resistance studies: penicillin, nitrofurantoin, doxycycline, rifampicin and gentamicin. A sterile solution of the appropriate antibiotic was added to the nutrient agar medium at 100 mg·L^{-1}, and surface cultures of the suspensions obtained from the individual filters were performed. The cultures were incubated at 26 °C for 48 h and after this time the number of bacterial colonies capable of growth in the presence of the antibiotic was determined. The result was reported as the number of CFU·g^{-1} of the filter.

Results and discussion

Quantitative tests of the abundance of microorganisms in the air inside the vehicle, represent the simplest, albeit not very precise method of assessing the condition of the ventilation and air conditioning system. They usually show that the average abundance of microorganisms does not exceed a few hundred CFU·m^{-3} for bacteria and a dozen-odd CFU·m^{-3} for fungi [2; 11]. The concentration of bacteria in the air taken before disinfection of the air conditioning system was 75-2000 CFU·m^{-3}, while that of fungi was 40-500 CFU·m^{-3} [5]. Studies of the air conditioning system itself already show higher values. For example, Jo and Lee (2008) found that bacterial counts in the car air conditioning system reached 2550 CFU·m^{-3}. Zaman et al. [32] reported that bacterial concentrations in dust accumulated in car cabin filters reached 4·10^{6} for bacteria and 5·10^{5} CFU·g^{-1} for fungi. Li et al. [12] found in dust samples from car air conditioners from 30 vehicles that the bacterial content reached 2.6·10^{4} CFU·g^{-1}, while the fungal content reached 1.3·10^{4} CFU·g^{-1}. Li et al. [12] found that dust accumulated in a filter from a car air conditioner contained up to 2.6·10^{4} CFU·mg^{-1} for bacteria and reached 1.3·10^{3} CFU·mg^{-1} for fungi.

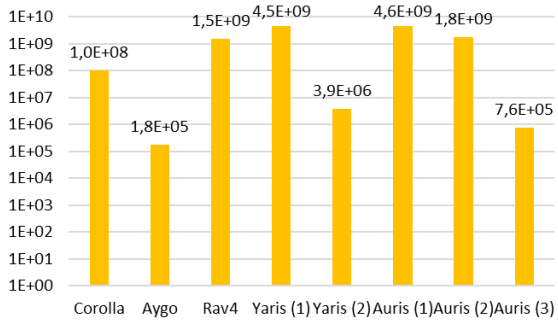

Fig. 5. **Bacteria growing in 26 °C in filters from car air-conditioning systems**

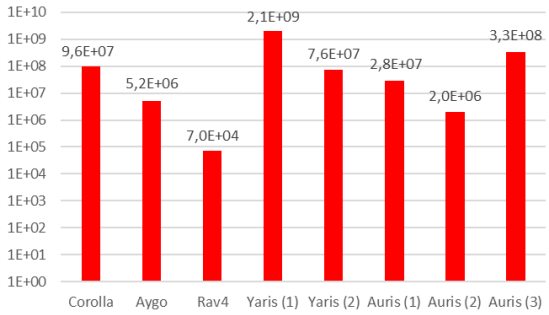

Fig. 6. **Bacteria growing in 37 °C in filters from car air-conditioning systems**

The research conducted in this study showed that the abundance of microorganisms in filters from a vehicle air conditioning system could actually be much higher. The test results cited above referred to the bacterial content per mass of dust alone. In the present study, the number of microorganisms was reported per unit weight of the entire filter material, yet values were significantly higher than those reported by other authors. The method used to rinse out the filters introduced into suspension not only microorganisms present in the dust but also those associated with the filter material itself. As a result, the present study found that the abundance of bacteria capable of growth at 26 °C ranged from reached to CFU·g^{-1} of filter (Fig. 5). The abundance of bacteria capable of growing at 37 °C was similarly shaped (Fig. 6), which may confirm that among the microorganisms present in the filter material, there are very numerous bacteria capable of growing at human body temperature. This is important because potentially pathogenic microorganisms may be among them. It is argued that the conditions within the ducts of an automotive air conditioning system are not conducive to the proliferation of mesophilic bacteria [33], which have an optimal growth temperature close to 37 °C. However, it is also reported [34] that air conditioning systems may harbor biofilm-forming pathogenic bacteria, including Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Bacteria representing the Legionella genus may also be present [35]. Sources of opportunistic pathogens present in the installation may include road dust or water from puddles accumulating on the road [7].

As noted in the present study, in the case of three vehicles, bacterial counts obtained during incubation at 37 °C were 1-3 orders of magnitude higher than bacteria growing at lower of the temperatures used. In addition, in 6 of the samples tested, mannitol-positive staphylococci were found, which can be an indicator of potential air pollution by pathogenic microorganisms - their counts reached

up to 10^5 CFU·g^{-1} of filter (Fig.7). On the other hand, the abundance of moulds in the tested filter materials was at the level of 10^4-10^5 CFU·g^{-1} (Fig. 8).

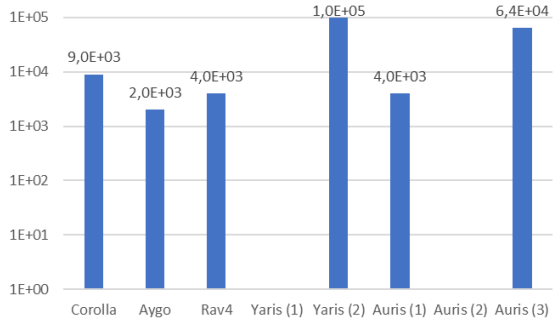

Fig. 7. **Mannitol-positive staphylococci in filters from car air-conditioning systems**

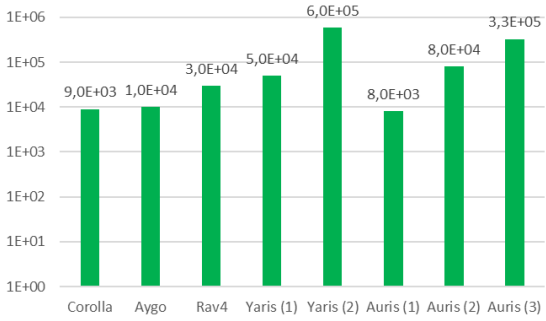

Fig. 8. **Moulds in filters from car air-conditioning systems**

A quantitative study showed that the degree of microbial contamination did not depend on the vehicle model or the type of filter used in the air conditioning system, for either bacteria or fungi. Similar observations were reported [33] in a study of bioaerosol concentrations in indoor car air. Surprisingly, no dependence of total aerosol concentration on the time elapsed since the last service of the air conditioning system was noted. On the other hand, [4] observed a relationship between the number of kilometers driven and the concentration of bacterial and fungal aerosols inside the vehicle. In the present study, the duration of vehicle operation was similar, although data on vehicle mileage during the period when air conditioning was used were missing. The influence of other factors related to vehicle operation cannot be excluded either. It has been found, for example, that using an automobile heater can reduce the concentration of microorganisms in the vehicle's interior air [12; 21; 36], which may also be relevant to microorganisms deposited within the system. The concentration of microorganisms inside vehicles also exhibits seasonal variations [10], with the spring period adopted in the present study being considered close to year-round averages.

An assessment of potential health risks based on general data on microbial contamination of cabin filters in automotive air conditioning does not appear to be complete. A phenomenon that plays an increasingly important role in the case of microbial contamination is the possibility that microorganisms resistant to commonly used pharmaceuticals may be present in a given environmental element.

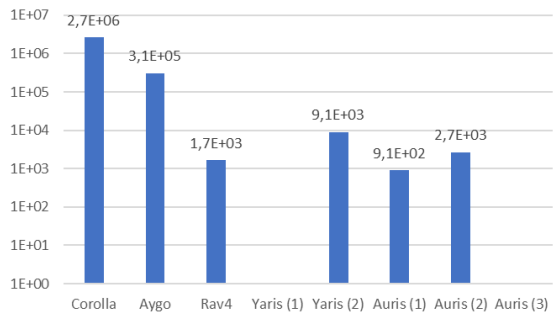

Fig. 9. **Penicillin-resistant bacteria in filters from car air-conditioning systems**

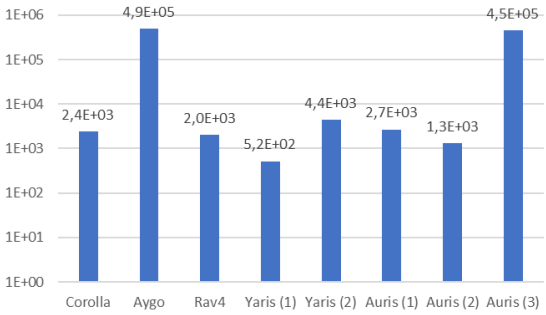

Fig. 10. **Nitrofurantoin-resistant bacteria in filters from car air-conditioning systems**

Microbial resistance to antibiotics has become a worldwide challenge in recent years [37]. The prevalence of antibiotic-resistant pathogens across all currently approved classes of these pharmaceuticals is now being reported [38]. The transfer of drug-resistant traits can occur via various environments, including ambient air [31; 37]. Also of note is the possibility of transfer of drug resistance features to potentially pathogenic microorganisms [39], as well as the accumulation of genes responsible for resistance to various pharmaceuticals in the cells of naturally occurring microorganisms in the environment, which can then serve as their reservoir and source of secondary transfer and spread [37].

The available data on the possibility of airborne (aerosol) transmission of drug resistance primarily focus on living quarters and livestock facilities [29; 30]. However, the possibility of transmission of microorganisms carrying drug resistance genes via bioaerosols is important wherever secondary contact with drug-resistant microflora may occur by inhalation [31].

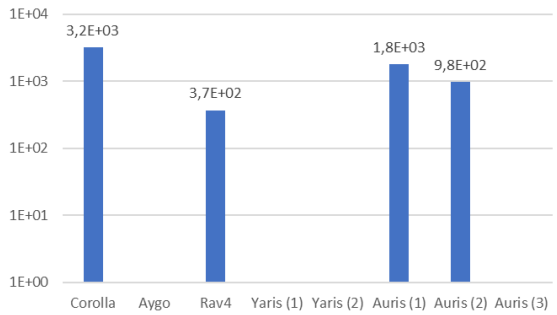

Fig. 11. **Rifampicin-resistant bacteria in filters from car air-conditioning systems**

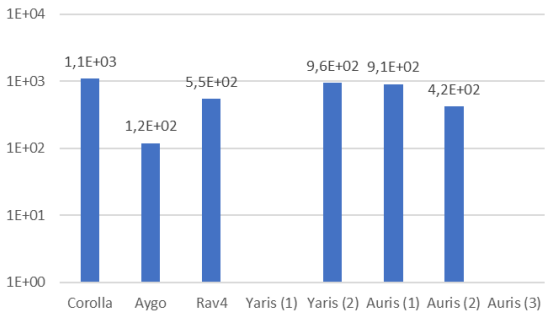

Fig. 12. **Doxycycline-resistant bacteria in filters from car air-conditioning systems**

There are only very few reports on the possibility of this type of contamination in automobile filters [36]. Microbiological tests on dust samples from cabin filters in Lahore, Pakistan, showed that the bacterial isolates obtained were resistant to ampicillin and cefotaxime. Given the specificity of the study region, the available information may not reflect European conditions, but it nevertheless suggests the possibility of drug-resistant bacteria in air-conditioning systems. Hence, the results obtained within the framework of the present study seem particularly relevant, as they unequivocally confirm not only the presence but also the widespread occurrence in filters of microflora resistant to commonly used antibiotics, characterized by different mechanisms of action on microorganisms.

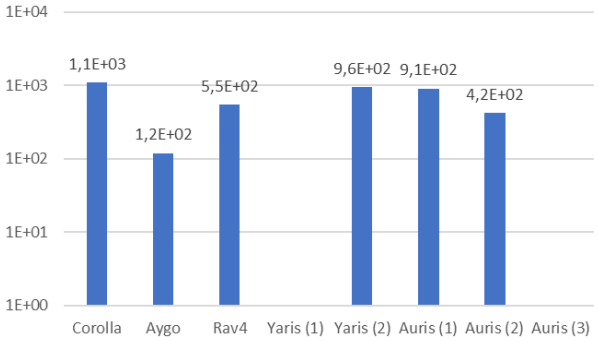

Fig. 13. **Gentamicin-resistant bacteria in filters from car air-conditioning systems**

It should be noted that the presence of bacteria resistant to at least one antibiotic was found in all the tested filters. In three of the tested filters, the microflora contained bacteria resistant to all tested antibiotics, while their abundance reached up to 10^5-10^6 CFU·g^{-1} (Fig. 9-13). Given the significant abundance in the tested filters of bacteria capable of growing at 37 °C, as well as the possibility of transferring drug-resistant traits between bacterial cells, the issue of potential health risks should be considered.

Conclusions

The study demonstrated that the degree of microbial contamination of used cabin air filters from passenger cars can be significantly higher than values typically reported in the literature, even when results are expressed per gram of complete filter material rather than per gram of accumulated dust alone. The rinsing method applied in this work enabled the recovery of microorganisms from dust deposits and the filter matrix itself, resulting in bacterial abundances of 10^5-10^9CFU·g^{-1} of filter for bacteria capable of growing at 26 °C and similar values for bacteria growing at 37 °C.

Quantitative analysis did not reveal a clear dependence of microbial load (both bacterial and fungal) on the vehicle model or the nominal filter type used, across the set of cars and operating conditions

investigated in this work. This suggests that other factors, such as actual usage patterns, environmental conditions, maintenance practices, or seasonal variability, exert a stronger influence on filter contamination levels than the specific vehicle model alone.

The most important finding of the present study is the consistent detection of numerous bacteria resistant to commonly used antibiotics in all tested filters. In three filters, microorganisms resistant to all five antibiotics considered in this work (penicillin, nitrofurantoin, doxycycline, rifampicin, and gentamicin) were detected, with abundances ranging from 10^5-10^6 CFU·g^{-1} of filter material. These results indicate that automotive air-conditioning systems can act as reservoirs for antibiotic-resistant bacteria and may contribute to user exposure via bioaerosols and to the environmental spread of antibiotic resistance determinants.

The test station developed for this study, based on a real fragment of a vehicle ventilation system and extended using reverse-engineered 3D-printed components, proved to be a useful tool for controlled investigations of biological contamination under both open- and closed-loop operating conditions. Its modular design, the ability to install additional filters and sensors, and its compatibility with CFD-based numerical modeling provide a basis for future studies focused on emission dynamics, the influence of operating parameters (flow rate, humidity, temperature), and the efficiency of various filtration and disinfection strategies.

From a practical perspective, the findings highlight the need to consider cabin filters not only as mechanical dust collectors but also as potential hotspots for the accumulation and proliferation of antibiotic-resistant microorganisms. This underlines the importance of appropriate maintenance intervals, the development of filter materials with improved antimicrobial performance verified under realistic operating conditions, and the inclusion of microbiological and antibiotic-resistance considerations in guidelines for vehicle HVAC system design and service.

Future research should address, in particular, the dynamics of release of antibiotic-resistant bacteria from filters to the cabin air under transient operating conditions, the role of different environmental scenarios (season, urban vs. rural traffic, pollution level), and the effectiveness of alternative filter designs or disinfection procedures in reducing both total microbial loads and the burden of antibiotic resistance.

Author contributions

All authors contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

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