

AUTOMOBILE AIR-CONDITIONING SYSTEMS AS A SOURCE OF MICROBIAL CONTAMINANTS

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Abstract

Automobile air-conditioning (AC) systems may be a source of microbiological contamination of driver's cabs. The study was carried out in 5 randomly selected cars equipped with automatic climate control system. The bacterial and fungal concentrations in air samples before AC servicing in cars were between 75-510cfu/m³ and 40-195cfu/m³, respectively. After AC service, the decrease in microbial concentrations was observed, lowering the contamination levels to 40-275cfu/m³ for bacteria and to 20-115cfu/m³ for fungi. The most prevalent bacterial species in the air of car cabins were Gram-positive cocci (mainly from Staphylococcus and Micrococcus/Kocuria genera) and endospore-forming Gram-positive rods from Bacillus genus. Among the most common fungal representatives were those from Alternaria, Aspergillus, Cladosporium and Penicillium genera. This study showed that to improve the air quality in cars, regular service, including filter replacement, refilling of cooling medium and disinfection of automobile AC system must be done at regular basis.

Key words: automobile, air-conditioning system, fungi, bacteria, biological aerosol

1. INTRODUCTION

In 2015, the number of cars in the world was 1.1 billion, which means that there are 498 cars per 1,000 inhabitants. The number of cars continues to increase and in 2025 it will reach 1.5 billion, and probably 15 years later - 2 billion (European Vehicle Market Statistics 2016). Each day, many people spend a lot of their time inside different vehicles including private cars, taxicabs, public buses or trucks. Professional drivers (such as taxi or truck drivers) spend at their vehicles on average more than 8 hours per day. In cars, like in many other enclosed spaces, there are electronic appliances, such as air conditioners and humidifiers, which are used to maintain a comfortable environment (Holmer et al. 1995; Jo & Lee 2008; Vonberg et al. 2010). However, the use of these appliances in such places is often associated with unwanted proliferation and dissemination of microorganisms.

The conditions in automotive air-conditioning (AC) systems are generally favourable for the growth of harmful biological agents (including bacteria and fungi). The microbial growth on surfaces contaminated with dust particles, like air conditioning conduits, air filters and coolers, is enhanced by high humidity. Improperly maintained automobile AC systems may be a source of microbiological contamination of driver's cabs (Kumar et al. 1990; Lee & Jo 2005; Simmons et al. 1997, 1999; Wang et al. 2013). From biofilms formed in car heaters/air conditioners might be release into the air different microorganisms including environmental strains as well as pathogens such as *Legionella* (Diekmann et al. 2013; Sattar et al. 2016). Exposure to bioaerosols can cause numerous adverse health effects manifested by nose and eye irritation, asthmatic reactions and allergic inflammation (Douwes et al. 2003; Gołofit-Szymczak & Górny 2010; Kumar et al. 1981).

Cleaning procedures of air conditioning systems significantly influence their hygienic quality and, by that, the quality of air inside the cars. The efficiency of these procedures, however, depends on the applied method. Chemical (i.e. the use of different disinfectants), physical (e.g. ozonation) methods as well as their combination (e.g. the use of disinfectant combined with ultrasonication) are usually used for cleaning of air-conditioning systems in cars. The aim of this study was to assess the level of microbial contamination before and after servicing of AC in cars based on qualitative and quantitative analysis of microorganisms isolated from the air samples.

2. MATERIALS AND METHODS

2.1. Cars' characteristics

This study was conducted in 5 randomly selected cars, produced on the EU market, equipped with automatic climate control system. The basic characteristics of studied cars is listed in table 1.

No.	Type of car	Year of production	Car mileage [km]	Type of climate control system
1	Sedan	2012	56.000	Automatic, dual-zone
2	SUV	2011	114.000	Automatic, 4-zone
3	Sedan	2013	87.000	Automatic, dual-zone
4	SUV	2012	98.000	Automatic, 4-zone
5	SUV	2014	200	Automatic, 4-zone

Table 1. Basic characteristics of studied cars.

Automatic climate control system in each car was used for control of temperature, fan speed, air circulation and air distribution. It maintains the selected parameters in the cabin, allowing their separate adjustment according to the following scheme: driver and passenger sides (automatic dual-zone climate control) or the driver, front passenger and back passengers' zones (automatic 4-zone climate control).

2.2. Sampling strategies

Air samples were collected before and after servicing of AC systems in studied cars. AC service included: replacement of cabin air filter, disinfection and refilling of the cooling medium. Disinfection was carried out using ozone. Ozone treatment was performed with ozone generator (with output of 5g/h of ozone). In each of the studied car ozonation was carried out for 30 minutes.

The viable (culturable) bioaerosol samples were taken using single-stage MAS impactor (model 100 *Eco*, Merck, Germany). The flow rate and sampling time were 100L/min and 1.5min, respectively. Standard Petri dishes filled with blood trypticase soy agar and malt extract agar were used for bacterial and fungal sampling, respectively.

During the tests, impactor was placed at a height of 1m above the car's floor to simulate aspiration from the human breathing zone of the car driver/passenger.

2.3. Colony counting and microbiological analyses

Air samples were collected on agar media. Standard Petri dishes filled with blood trypticase soy agar (TSA, 51044, bioMérieux, France) and malt extract agar (MEA, CM-59, Oxoid Ltd., Great Britain) were used for bacterial and fungal sampling, respectively. The collected samples were incubated as follows: bacteria – 1 day at 37°C, followed by 3 days at 22°C and 3 days at 4°C; fungi – 4 days at 30°C followed by 4 days at 22°C. After incubation, the concentrations of viable microorganisms in the air were calculated as colony forming units per one cubic meter of the air (cfu/m³).

Bacterial and yeast strains were identified by Gram staining (111885 Gramcolor stain set; Merck KGaA, Germany) (Fischer & Dott 2003) to determine their morphology and, finally, by the biochemical analytical profile index (API) tests (BioMérieux, France). Filamentous fungi were identified according to their morphology using several identification keys: Fisher and Cook (1998), Murray et al. (2013), Samson et al. (2004) and St-Germain & Summerbell (2011).

2.4. Measurement of microclimate parameters

The environmental conditions (temperature and relative humidity of the air) in studied cars were checked during every sampling session using hytherograph (Omniport 20, E+E Elektronik GmbH, Austria).

2.5. Statistical analysis

The measurement data were statistically elaborated by a single-factor analysis of variance (ANOVA) with post-hoc analysis (Scheffe's test) as well as t-test using the Statistica data analysis software system, version 7.1-2006 (StatSoft, Inc., USA). Probability values were treated as statistically significant values for $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Quantitative analysis of bacterial and fungal aerosols

The mean concentrations of bacterial and fungal aerosols measured using MAS impactor in the studied cars and in outdoor background (in the air outside the cars) are presented in Table 2.

Sample type	Bacterial concentrations [cfu/m ³] ± SD		Fungal concentrations [cfu/m ³] ± SD	
	Before AC service	After AC service	Before AC service	After AC service
Car 1	270 ± 14.1	115 ± 7.1	120 ± 28.2	65 ± 7.1
Car 2	510 ± 42.4	275 ± 77.8	195 ± 7.1	115 ± 21.2
Car 3	300 ± 14.1	150 ± 56.6	185 ± 7.1	90 ± 14.1
Car 4	375 ± 91.9	170 ± 42.4	160 ± 28.0	80 ± 14.1
Car 5	75 ± 21.2	40 ± 14.1	40 ± 28.3	20 ± 14.1
Outdoor background	187 ± 14.8		290 ± 56.6	

Table 2. Bacterial and fungal concentrations in studied cars before and after service of vehicle air conditioning system. SD - standard deviation

Data from this study showed that the observed concentrations of airborne microbiota were low and did not exceed 10³cfu/m³. The noted concentrations of bioaerosols in examined cars were similar to those published in other reports. For example, the average concentrations of microorganisms observed in different Polish cars before service were 214cfu/m³ for bacteria and 265cfu/m³ for fungi (Grzybowski 2011). Data obtained by Vonberg et al. (2010) have shown that mean concentrations of airborne microorganisms in cars with AC system were 200cfu/m³.

The present study revealed statistically significant differences in concentrations of microorganisms between tested cars (ANOVA: $p < 0.05$). The analysis showed that cars number 2 and 5 differed significantly from the other cars (Scheffé test: $p < 0.05$). Car number 2 was the oldest, has the highest mileage among all studied cars and the concentrations of bioaerosols in this car were the highest. It can be assumed that a constant accumulation of various pollutants, could contribute to higher concentrations of microorganisms. Car number 5 was the youngest (demo car in dealer's showroom) and the concentrations of microorganisms observed in this car were the lowest from among the tested vehicles.

The concentration of bacterial and fungal aerosols measured in cars before service of vehicle AC system were significantly higher (2 times on average) than those in cars after AC service (t-test: $p < 0.01$).

Bacterial concentrations in outdoor background were significantly lower (except car no. 5) than those observed in cars before service of vehicle air conditioning system ($p < 0.01$). Fungal outdoor background levels were significantly higher than those observed in studied cars before and after the service ($p < 0.01$).

Comparison of microbial aerosol concentrations between the group of sedan cars and SUV cars did not indicate significant differences between them. For the type of climate control system dual v. 4-zone, these differences were not statistically significant.

Microclimate parameters did not significantly determine the observed concentrations of bioaerosols in all tested cars. This demonstrates that mechanical separation of microbial particles is more important for car cabin contamination than microclimate conditions provided by AC system.

All bacterial and fungal strains isolated from the air of examined cars are listed in Table 3.

Bacteria	Fungi
Gram-positive cocci: <i>Kocuria</i> spp., <i>Micrococcus</i> spp., <i>Staphylococcus epidermidis</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus cohnii</i> , <i>Staphylococcus hominis</i>	Filamentous fungi: <i>Acremonium</i> spp., <i>Alternaria</i> spp., <i>Aspergillus candidus</i> , <i>Aspergillus carbonarius</i> , <i>Aspergillus</i> spp., <i>Chaetomium</i> spp., <i>Cladosporium herbarum</i> , <i>Cladosporium</i> spp., <i>Fusarium</i> spp., <i>Mucor</i> spp., <i>Penicillium brevicompactum</i> , <i>Penicillium citrinum</i> , <i>Penicillium</i> spp., <i>Phoma</i> spp.
Endospore-forming Gram-positive bacilli: <i>Bacillus cereus</i> , <i>Bacillus circulans</i> , <i>Bacillus pumilus</i> , <i>Bacillus sphaericus</i> , <i>Bacillus</i> spp.	Yeasts: <i>Candida albicans</i> , <i>Candida famata</i>

Table 3. Microorganisms identified in the air of the studied cars.

Total number of 11 bacterial species from 4 genera and 16 fungal species from 9 genera were identified. The highest numbers of bacterial species belonged to the following genera: *Micrococcus*, *Staphylococcus* (mainly *S. epidermidis*: 14.7% of the total identified microbiota) and *Bacillus* (mainly *B. pumilus*: 4.5%). These species are usually the most prevalent indoors (Dutkiewicz 1997). Species of the genera *Micrococcus*, *Staphylococcus* and *Bacillus* are the part of the human microbiota. However, among bacteria of the *Staphylococcus* genus, as determined at the studied car interiors microbiota, may appear dangerous opportunistic pathogens (Sattar et al. 2016; Stephenson et al. 2014). Among fungal species, the most frequently isolated strains belonged to *Penicillium* (10.3% of the total identified microbiota), *Acremonium* (4.8%) and *Alternaria* (3.9%) genera. These species are usually the most prevalent in the car interiors (Holme et al. 2010).

The percentage share of particular groups of bacteria and fungi in relation to the whole microbiota identified in the studied cars are presented in Figure 1.

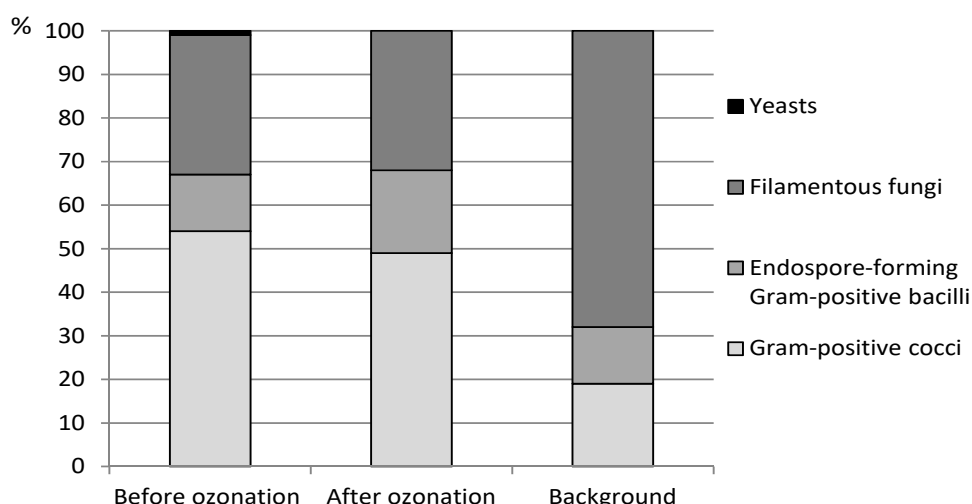


Fig. 1. Percentage distributions of microbial groups in the air at studied cars.

The analysis showed that bacteria constitute 62–75% of the air microbiota in all of the examined cars. The fungi (filamentous fungi and yeasts) formed not more than 29–38% of the whole microbiota. The species composition of airborne microbiota in cars before AC service was similar to that observed in cars after AC service. The most numerous groups of microorganisms in the air of studied cars were Gram-positive cocci (from 54% to 39%, respectively), followed by endospore-forming Gram-positive bacilli (from 13% to 19%, respectively) and filamentous fungi (32%). Yeasts were identified only in air samples taken in the cars before AC service (1%). Qualitative microbial structure in both groups of studied cars, however, differed substantially from a microbial composition of the outdoor air, where a domination of filamentous fungi (68%) was clearly visible. The observed relationships are in a good agreement with data concerning the sources of bioaerosol origin. People are the main active source of bacterial aerosol indoors. The most important emission source for fungal aerosol is outdoor environment (e.g. soil, water, plants etc.) (Gołofit-Szymczak & Górny 2010; Kulkarni et al. 2011).

Jo and Lee (2005), Lee et al. (2013) and Vonberg et al. (2010) examined the influence of AC systems on the levels of airborne bacteria and fungi inside automobiles. So far, more than 400 types of bacterial species were detected in the air inside the cars including opportunistic pathogens from *Acinetobacter*, *Bacillus*, *Pseudomonas* and *Stenotrophomonas* genera and allergenic fungal species. It is well known from epidemiological studies that exposure to these molds has been associated with a variety of adverse health outcomes including respiratory, hematological, immunological, and neurological system disorders and/or diseases (Jones et al. 2011; Kumar et al. 1984; Rylander et al. 2008). Their presence in high concentrations may result in the appearance of allergic reactions and various non-specific adverse health outcomes. Kumar et al. (1990) associated allergic rhinitis and hypersensitivity pneumonitis with fungal exposure, including *Penicillium* species released from automobile AC system.

A properly functioning vehicle air conditioning system ensures the driver and passengers comfort, providing e.g. cool air in summer time, as well as their protection from particulate pollution. The use of AC system usually decreases bioaerosol concentrations inside the vehicle. It has been shown to reduce more than 80% of the total number of microorganisms, including bacterial and fungal spores (Jo & Lee 2008; Lee & Jo 2005). Similarly, the number of fungal spores decreased by 83.3% compared to fungal concentration in cars before service of vehicle air conditioning system (Vonberg et al. 2010).

Vehicle air conditioning system should be systematically maintained. Filters system need to be checked and replaced regularly. The whole system should be regularly disinfected for elimination of biological contamination. A system which has been improperly maintained (long-term operation of filters or lack of systematic disinfection) may be a source of additional unwanted contamination of the

air in the car cabin. Hence, a constant accumulation of various contaminants (e.g. on filter membranes or as a biofilm on vehicle evaporator surface) could contribute to higher concentrations of microorganisms in the cabin (Li et al. 2013; Simmons et al. 1997, 1999; Vonberg et al. 2010).

Among the methods of air conditioning service in cars, the most frequently used are: filter exchange, refilling of the cooling medium and ozonation. Ozone has well-documented antimicrobiological properties and can be used to inactivate a wide range of microorganisms, including viruses and bacteria that may be resistant to other disinfectants. This gas can effectively penetrate contaminated spaces and surfaces of the car allowing to achieve a very high level of disinfection (Otter et al. 2009; Sharna & Hudson 2008; Zoutman et al. 2011).

4. CONCLUSIONS

Automobile air-conditioning (AC) systems may be a source of microbiological contamination of driver's cabs. Average concentrations of microorganisms in air samples before and after AC servicing in cars were $3.6 \times 10^2 \text{ cfu/m}^3$ and $1.8 \times 10^2 \text{ cfu/m}^3$, respectively. Age and mileage of the vehicle have a significant impact on microbial contamination of the cars. The older cars with high mileage are more polluted indoors than younger cars. After air conditioning service (which include replacement of cabin air filter, disinfection using ozone and refilling the cooling medium) decrease in microbial concentrations was observed. This study showed that to improve the air quality in cars, regular service, filling and disinfection of automobile AC system must be done at regular basis.

ACKNOWLEDGMENT

This work was supported by the Polish Ministry of Family, Labour and Social Policy from the Multiannual Program "Improvement of safety and working conditions (2017–2019)" through the grant no. 2.G.09.

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