Impact of air-conditioning system disinfection on microbial contamination of passenger cars

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Abstract

An automobile air-conditioning (AC) system can be a source of microbiological contamination in a driver's car. The aim of this study was to assess the level of microbial contamination before and after servicing of the automobile AC system, based on qualitative and quantitative analyses of bacteria and fungi isolated from the air, and to check the efficiency of different AC system cleaning methods in the removal of microbial contaminants. The study was carried out in 35 randomly selected cars, equipped with AC systems. In each car tested, viable bioaerosol samples were taken using a single-stage MAS impactor before and after servicing the AC systems. Four methods were used to disinfect the air-conditioning installations in the vehicles examined: ozonisation, application of chemical disinfectant using ultrasounds, manual application of chemical disinfectant foam and simultaneous ozonisation and application of chemical disinfectant foam. The bacterial and fungal concentrations in air samples before AC servicing in cars were between 7.5×10^1 cfu/m^3 and 2.0×10^3 cfu/m^3 and 4.0×10^1 cfu/m^3 and 5.0×10^2 cfu/m^3, respectively. Regardless of the disinfection method, after the AC service, a decrease in microbial concentrations was observed. The highest disinfection efficiency was observed for two methods: ozonisation combined with manual application of the chemical disinfectant into the air-conditioning system and spraying of the chemical disinfectant using ultrasounds.

On the basis of the results obtained, it can be concluded that air-conditioning systems in cars should be regularly disinfected to eliminate microbiological contaminants, which may be the cause of adverse health effects on humans.

Keywords Air-conditioning · Contamination · Bacteria · Fungi · Cars

Introduction

The total number of cars in the world in 2015 was 947 million passenger cars and 335 million commercial vehicles. There are 256 million passenger cars registered in Europe alone. Each year, the number of cars is increasing and it is projected that it will reach 2 billion by around 2030. These numbers also make the automotive sector very important to the global economy. For example, about 12.6 million people, i.e. 5.7% of European Union workers, are employed in this sector (European Vehicle Market Statistics 2016).

Cars are our number one source of mobility, and road transport is the most common means of transport in the world. Over 70% of journeys around the world are private car or taxi journeys. Every day, thousands of people spend many hours in different types of road transport vehicles (e.g. private cars, taxis, buses or trucks). Professional drivers (e.g. taxi and truck drivers) spend on average about 8 h a day in their vehicles (Holmer et al. 1995; Jo and Lee 2008; Vonberg et al. 2010; Wu et al. 2016).

To improve our travel comfort, cars are equipped with air-conditioning (AC) installations. The first air-conditioning system installed into a vehicle took place in 1933 in the USA. Since then, an increase in the number of cars equipped with AC systems has been systematically observed. Cars equipped with an AC system accounted for less than 50% of these produced before the year 2000, while this percentage is significantly higher among new models (in 2013 it was already 74%).

The main task of an automotive AC system is to ensure the appropriate quality of the air inside the car. The most important air parameters, which can be regulated in the air-conditioned interior, are temperature and

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relative humidity. A well-functioning air-conditioning system guarantees driver and passenger comfort by, for example, providing cold air during the summer, as well as protection from particle pollution of the atmospheric air. The use of an AC system also usually results in a decrease in the presence of microbial contaminants inside the vehicle (Vonberg et al. 2010; Lee and Jo 2005; Li et al. 2013). In practice, modern automotive air-conditioning installations become quite often an active source of emission of harmful biological agents (mainly bacteria and fungi). This development of microbes is a result of surface contamination of the AC system, strongly supported by the increase in air humidity caused by the small diameter of air-conditioning cords, air cleaners, air refrigerators etc. Some microbial contaminants are able to easily create a biofilm in the air-conditioning ducts and as such can be distributed around the other installation elements, as well as within the car interior itself (Simmons et al. 1997, 1999).

The longer the automotive AC installation is used, the more likely it will become a source of adverse air pollution of microbial origin in the car cabin.

Exposure to bioaerosols may lead to adverse health effects, ranging from simple irritations, through allergic reactions, to infectious diseases or toxic response (Douwes et al. 2003; Flannigan and Miller 1994; Sykes et al. 2007; Jodeh et al. 2018). Numerous studies prove that exposure to high concentrations of microorganisms in the air is often linked to asthma, rhinitis and pneumonia. Bioaerosols might also present a risk to human health since they can be a source of harmful structures and substances exhibiting immunological reactivity (allergens, mycotoxins, endotoxins and other microbial cell fragments) in the air (Fiegel et al. 2006; Kumar et al. 1984).

Proper servicing of automotive air-conditioning installations requires systematic maintenance. It is advised to regularly replace the cabin filters, clean and disinfect the air ducts, evaporator and air inlets of the motor-car body and control the permeability of the condensation extracting canal from the air-conditioning installation evaporator.

Various methods are used to clean air-conditioning installations: chemical (use of disinfectants), physical (ozonisation) and chemicophysical (use of disinfectants combined with ultrasounds). Regular cleaning of air-conditioning installations significantly influences the maintenance process and, therefore, the indoor air quality.

The aim of this study was to assess the level of microbial contamination before and after servicing of the automobile AC system, based on qualitative and quantitative analyses of bacteria and fungi isolated from the air, and to check the efficiency of different AC system cleaning methods in the removal of microbial contaminants.

Methods

Characteristics of disinfection methods for automotive air-conditioning systems

In all of the vehicles examined, the tightness of the air-conditioning installation was checked, the coolant level was inspected and topped up, cabin filters were replaced and the dehumidifier and evaporator were inspected before the specific disinfection method was applied.

Four methods were used to disinfect the AC installations in the vehicles examined:

1. Ozonisation, involving the application of concentrated ozone (14 g/h) in the vehicle cab, starting the engine and turning on the air-conditioner in a closed cycle mode. The ozonisation process ran for 20 min in every car, and after approx. 15–20 min, the vehicles were ventilated. An ozone generator was used for ozonisation.
2. Application of a chemical disinfectant containing 2-propanol (1–2.5%) inside the vehicle cab using ultrasounds; after 30 min of disinfection, the vehicles were ventilated for 15 min.
3. Manual application (spraying chemical disinfectant containing 2-propanol, in a foam form (50–70%)) through the air inlets in the dashboard for 30 min.
4. Ozonisation combined with the application of a chemical disinfectant foam.

Car characteristics

Thirty-five randomly selected vehicles equipped with air-conditioning systems were examined and divided into 4 groups, including the vehicles with AC systems that were disinfected with ozone (1/O–10/O), ultrasonically distributed chemical disinfectant (1/U–10/U), with manually distributed chemical disinfectant (1/CH–10/CH) and using a combination of two methods, i.e. ozonisation and manual application of disinfectant (1/O/CH–5/O/CH).

The examined vehicles had two types of AC systems installed:

- Manual single-zone air-conditioner, controlled directly by the driver; the temperature regulation was performed by mixing hot air (from the air heater) with cold air; the driver was unable to precisely set the temperature inside the car.
- Automatic air-conditioner, controlled by an electronic system using readings from the car sensors (e.g. external and internal temperature sensors, solar sensor); depending on the internal conditions, the ventilation is usually pointed either on the windscreen, side windows or the legs; to avoid installation overload, 20% of the air comes from

the outside and the rest comes from a closed cycle (so-called recirculation); the automatic AC systems were equipped with a multisegment heater that provided both the driver and passenger with climate control (dual and four-zone) (26).

During the experiments, the indoor temperature was set for 23 °C in all of the vehicles examined.

Detailed characteristics of the examined vehicles are provided as supplemental documentation (Table S1).

Sampling strategies

Air samples were collected before and after disinfection of the AC systems in the cars under study. At the same time, in order to determine 'outdoor background', the atmospheric bioaerosol was collected outside the car cabin.

The viable (understood here as culturable) bioaerosol samples were taken using a single-stage MAS impactor (model 100 Eco, Merck, Germany). The flow rates, as well as sampling times, were 100 L/min and 1.5 min, respectively.

During the tests, the impactor was placed within a zone of about 0.5 m from the vent/supply air outlets located next to the steering wheel, and at a height of 1 m above the car's floor, to simulate aspiration from the human breathing zone of the car driver/passenger.

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 (Jensen and Schafer 1998). After incubation, the concentrations of viable microorganisms in the air were calculated as colony-forming units per cubic metre of the air (cfu/m^3).

Taxonomical identification of isolated microorganisms

Species identification was carried out using microscopic and biochemical methods.

The taxonomical origin of isolated pathogens was additionally confirmed using molecular techniques.

Conventional methodologies

At the beginning, the bacteria were identified by Gram staining (111885 Gram-color stain set; Merck KgaA, Germany) (Murray et al. 2013) to determine their morphology and,

finally, by the biochemical analytical profile index (API) tests (bioMérieux, France). Yeast strains were identified by the API (API 20 C Aux) test. Filamentous fungi were initially stained with lactophenol and subsequently identified according to their morphology using several identification keys (Fisher and Cook 1998; Samson et al. 2004; St-Germain and Summerbell 2011).

Molecular methodologies

Bacterial species identification Bacterial DNA was isolated from pure cultures which were grown on TSS plates (trypticase soy agar + 5% sheep blood) using a Qiamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA isolation included preincubation with lysozyme and lysostaphin. The isolated DNA was used as a template in PCR reaction with the following primers set: BAK11w (5′-AGTTTGATCMTGGCTCAG-3′) and BAK2 (5′-GGACTACHAGGGTATCTAAT-3′), which enable the amplification of bacterial 16S rRNA gene fragment corresponding to *E. coli* 16S rRNA gene positions from 10 to 806. The amplified PCR product was purified and sequenced using an ABI3730 Genetic Analyser (Applied Biosystems, Waltham, USA). The sequence was compared with the GenBank database (National Center for Biotechnology Information, U.S. National Library of Medicine, USA) using a BLAST algorithm (Basic Local Alignment Search Tool) (Bosshard et al. 2003; Bosshard et al. 2006).

Fungal species identification Fungal DNA was isolated from pure cultures which were grown on MEA plates (malt extract agar), using a Fungi DNA Mini Kit (Syngen Biotech, Wrocław, Poland), following the manufacturer's instructions. The isolated DNA was used as a template in PCR reaction using an ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primer set which enables the amplification of the ITS (internal transcribed spacer) region of the nuclear ribosomal DNA (rDNA) repeat. The amplified PCR products were purified, sequenced using an ABI3730 Genetic Analyzer (Applied Biosystems) and compared with the GenBank database (National Center for Biotechnology Information) using a BLAST algorithm (Basic Local Alignment Search Tool) (White et al. 1990).

Measurement of microclimate parameters

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

Statistical analysis

The measurement obtained data were statistically processed, on the basis of the Kruskal-Wallis, Wilcoxon and Mann-Whitney tests, as well as Spearman's correlation analysis using the 'STATISTICA data analysis software system' package, version 7.1 (StatSoft, Inc., USA). The p values below 0.05 were adopted as statistically significant.

Results

Quantitative analysis of bacterial and fungal aerosols

The mean concentrations of bacterial and fungal aerosols measured using the MAS impactor in the studied cars and in outdoor background (in the air outside the cars) are presented in Table 1.

Data from this study showed that the observed concentrations of airborne microbiota were low and did not exceed 2×10^3 cfu/m^3.

The present study revealed statistically significant differences in concentrations of microorganisms between tested cars (Kruskal-Wallis test ($p < 0.001$)). The lowest concentrations of bioaerosols were observed in the 5/O car. This vehicle was the newest vehicle with the lowest number of kilometres travelled (demo car in a dealer's showroom). The highest concentrations of bioaerosols were recorded in the 1/O/CH car, i.e. in the vehicle with the highest mileage.

Using the Spearman correlation coefficient with the impact of the mileage, the type of air-conditioning system (1-, 2-, 4-zone and manual), type of car body (SUV, sedan, estate) and type of upholstery (fabric, leather) for concentrations of microorganisms in the air of the tested vehicles were evaluated. The results revealed that the mileage significantly determined the observed concentrations of bacterial and fungal aerosols ($R = 0.71$ at $p < 0.001$). Along with the high number of kilometres travelled, an increase in the concentration of bacterial fungal aerosols in the studied vehicles was observed before the air-conditioning service. There was also a significant effect of the body car type on the concentration of bacteria in the air of the tested vehicles ($p < 0.05$). SUVs have lower bacterial concentrations than sedans and estate cars. No such differences were observed in the concentration of fungi.

It was also found that the type of AC system and the type of upholstery did not significantly determine the observed concentrations of bacterial and fungal aerosols in the tested vehicles.

Independent from the applied disinfection method, the concentrations of bacterial and fungal aerosols measured in cars before the service of the vehicle AC system were significantly higher than those in cars after an AC service (Wilcoxon test ($p < 0.001$)).

The use of ozone as a disinfectant resulted in an average reduction of bacterial and fungal aerosol concentrations of 45.8% and 42.7%, respectively. The distribution of chemical disinfectant in the car cabin using ultrasounds resulted in a decrease in the bacterial aerosol concentrations of about 76% and fungal aerosol concentrations of about 77%. In turn, the manual application of a chemical disinfectant in the AC systems of the tested cars resulted in an average decrease in bacterial and fungal aerosol concentrations of 30.8% and 42.2%, respectively. The use of the ozonisation method combined with the manual application of a chemical disinfectant was the most effective in AC system cleaning, reducing the bacterial and fungal aerosol concentrations in car cabins by 80.8% and by 61.9%, respectively.

Bacterial concentrations in outdoor background were significantly lower (except in cars after an ultrasound service) than those observed in cars before the service of the vehicle air-conditioning system ($p < 0.01$). In turn, fungal outdoor background levels were significantly higher than those observed in the studied cars before and after the service ($p < 0.01$).

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

Qualitative analysis of bacterial and fungal aerosols

All bacterial and fungal strains isolated from the air of examined cars are provided as supplemental documentation (Table S2 and S3).

A total number of 28 bacterial species belonging to 10 genera and 31 fungal species from 18 genera were identified in the air of car cabins before the AC service. Qualitative analysis demonstrated that the most prevalent bacterial species in all the cars studied 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 species were those from *Penicillium*, *Aspergillus* and *Cladosporium* genera.

Using the classifications of harmful biological agents included in Directive 2000/54/EC and Ordinance of Polish Minister of Health, it was ascertained that in the air of car cabins before the AC service, there are species and genera of microorganisms classified into risk group 2: *Bacillus subtilis*, *Bacillus thuringiensis*, *Corynebacterium* spp., *Aspergillus fumigatus* and *Penicillium* spp. (microorganisms can cause disease in humans, but the disease is treatable or preventable). In car cabins where manual

Table 1 Bacterial and fungal concentrations in the studied cars before and after servicing of the vehicle air-conditioning systems. SD—standard deviation

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
Ozonisation				
1/O	270 ± 14.1	115 ± 7.1	120 ± 28.2	65 ± 7.1
2/O	510 ± 42.4	275 ± 77.8	195 ± 7.1	115 ± 21.2
3/O	300 ± 14.1	150 ± 56.6	185 ± 7.1	90 ± 14.1
4/O	375 ± 91.9	170 ± 42.4	160 ± 28.0	80 ± 14.1
5/O	75 ± 21.2	40 ± 14.1	40 ± 28.3	20 ± 14.1
6/O	810 ± 287.0	260 ± 28.2	80 ± 28.0	25 ± 7.0
7/O	990 ± 98.8	600 ± 21.2	505 ± 49.4	320 ± 7.0
8/O	790 ± 254.5	580 ± 212.1	175 ± 35.3	125 ± 35.3
9/O	495 ± 91.2	305 ± 63.6	230 ± 42.4	175 ± 35.3
10/O	320 ± 42.4	220 ± 28.3	160 ± 14.1	110 ± 28.1
Application of a chemical disinfectant substance inside the vehicle cab using ultrasounds				
1/U	280 ± 103.3	80 ± 14.1	195 ± 7.1	25 ± 7.1
2/U	580 ± 42.4	95 ± 7.1	165 ± 21.2	35 ± 7.1
3/U	818 ± 120.2	140 ± 14.1	295 ± 106.1	50 ± 7.1
4/U	310 ± 84.8	120 ± 14.1	115 ± 21.2	40 ± 28.3
5/U	965 ± 148.4	165 ± 7.1	285 ± 120.2	70 ± 14.1
6/U	585 ± 120.2	140 ± 0	225 ± 49.5	65 ± 7.1
7/U	430 ± 84.8	115 ± 35.3	170 ± 42.4	55 ± 21.2
8/U	205 ± 21.2	65 ± 21.2	105 ± 7.1	25 ± 7.1
9/U	835 ± 233.3	145 ± 21.2	315 ± 77.8	75 ± 35.3
10/U	610 ± 84.8	120 ± 42.4	170 ± 1.5	25 ± 14.1
Chemical disinfectant foam				
1/CH	900 ± 424.2	780 ± 98.9	90 ± 113.1	25 ± 21.1
2/CH	670 ± 84.8	430 ± 0	125 ± 35.3	115 ± 21.2
3/CH	230 ± 28.3	150 ± 56.5	385 ± 21.2	155 ± 7.1
4/CH	305 ± 35.3	115 ± 7.1	215 ± 35.3	70 ± 14.1
5/CH	120 ± 42.4	120 ± 28.2	90 ± 42.4	50 ± 0
6/CH	170 ± 28.3	140 ± 0	105 ± 7.1	75 ± 21.2
7/CH	290 ± 42.4	185 ± 21.1	180 ± 14.1	125 ± 35.3
8/CH	460 ± 42.4	310 ± 14.1	250 ± 14.1	185 ± 7.1
9/CH	640 ± 42.4	410 ± 28.2	175 ± 35.3	150 ± 0
10/CH	940 ± 380.1	570 ± 28.2	155 ± 63.6	45 ± 7.1
Ozonisation and application of a chemical disinfectant foam				
1/O/CH	2025 ± 63.3	555 ± 7.1	415 ± 63.3	165 ± 21.1
2/O/CH	800 ± 98.9	295 ± 35.3	320 ± 42.4	100 ± 0
3/O/CH	1800 ± 565.6	290 ± 42.4	435 ± 49.4	185 ± 7.1
4/O/CH	985 ± 120.2	330 ± 14.1	275 ± 63.6	120 ± 28.3
5/O/CH	1045 ± 346.5	200 ± 0	420 ± 98.9	160 ± 14.1
Outdoor background	187 ± 14.8		290 ± 56.6	

application of a chemical disinfectant foam was performed as simultaneous ozonisation and application of a chemical disinfectant foam, *Aspergillus fumigates* were not detected. No presence of *Bacillus subtilis* was found in the cars under study after an AC service.

Each of the tested AC service methods decreased the taxonomical diversity of the car cabin microbiota. Only 16 bacterial species from 6 genera and 13 fungal species from 11 genera were identified in car cabins after ozonisation. In the car cabins after disinfectant application with ultrasounds, 17

bacterial species from 7 genera and 15 fungal species from 9 genera were identified. A total number of 19 bacterial species from 8 genera and 15 fungal species from 8 genera were identified in car cabins after chemical (foam) service of the AC systems. The samples taken after the AC service by means of chemical and ozonisation methods presented 19 species of bacteria from 8 genera and 15 species of fungi from 8 genera.

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

The most numerous groups of microorganisms in the air of the car cabins were Gram-positive cocci (from 40 to 54%), followed by filamentous fungi (from 20 to 33%) and endospore-forming Gram-positive bacilli (from 20 to 29%). Yeasts, nonsporing Gram-positive rods and actinomycetes were the smallest fractions of identified microorganisms in studied cars. The species composition of airborne microbiota in cars before the AC service was similar to that observed in cars after the AC service, except of actinomycetes, which were not detected after AC service using ozonisation and a chemical disinfectant.

Qualitative structure of microbiota in car cabins differed substantially from the microbial composition of the outdoor air, where a domination of filamentous fungi (68%) was clearly visible.

Discussion

Cars are nowadays by far the most common means of transport. People spend a great deal of time in them, so the quality of their indoor air is of great importance to health.

According to the literature, the use of an AC system usually decreases bioaerosol concentrations inside the vehicle. Sowiak et al. (2018) found that operating an AC system makes it possible to decrease fungal microbiota by 68–78%. Similar findings were observed by Wang et al. (2013) and Vonberg et al. (2010), studying bacterial and fungal spores after the air-conditioning or ventilation was turned on.

However, the abovementioned findings indicate that the AC systems or cabin filters may be contaminated with these microorganisms, and their extended use may result in an increase in microbial concentrations inside the driver's car. An AC 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 cars. As already shown, a constant accumulation of various contaminants (e.g. on filter membranes or as a biofilm on vehicle evaporator surface) contributes to higher concentrations of microorganisms in the car cabin (Lee and Jo 2005; Simmons et al. 1997, 1999).

On the basis of the results of our study, it can be concluded that all four methods of servicing of automotive air-conditioning systems were efficient in the reduction of microbial contamination of the air inside the vehicle cabins. The most prominent purification effect was observed for two methods, namely ozonisation combined with the application of a chemical disinfectant foam and spraying of a chemical disinfectant using ultrasounds. Ozone has well-documented antibacterial properties and can be used to inactivate a wide range of microorganisms, including viruses and bacteria, which can be resistant to other disinfectants (Kim et al. 1999). As indicated by Sharna and Hudson (2008), ozone also has bactericidal properties against MRSA strains. Ozone is usually applied to eliminate odours and to reduce and/or remove microorganisms from both air and surfaces in hospital rooms and ventilation ducts in buildings (EPA 1997). This gas can effectively penetrate the hard-to-reach areas of air-conditioning ducts and all other indoor spaces, making it possible to achieve a very high level of disinfection (Otter et al. 2009; Sharna and Hudson 2008; Zoutman et al. 2011). However, as a highly reactive gas, ozone levels should never exceed the level of 0.15 mg/m^3 for an 8-h exposure.

Biocides used for the disinfection of air-conditioning systems usually contain 2-propanol (isopropyl alcohol), i.e. a substance with a wide spectrum of antiviral, bactericidal and

Fig. 1 Percentage distributions of microbial groups in the air of the studied car cabins
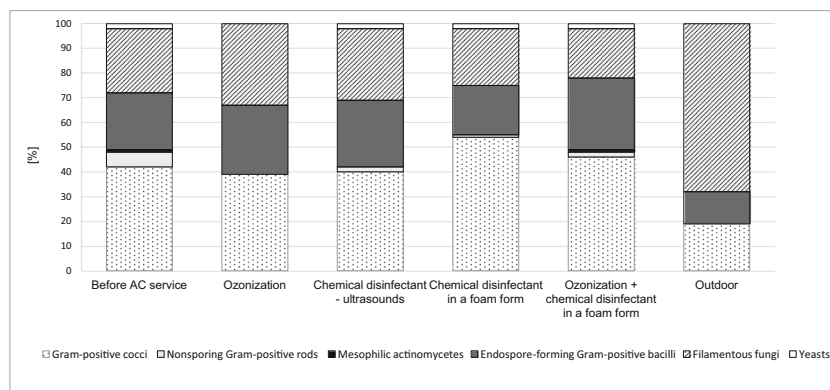
fungicidal activity. As already shown, 2-propanol is more effective against *Staphylococcus aureus* and *Escherichia coli* than ethanol (Burcea et al. 2013). It is easily available, so it is most widely used for ventilation duct cleaning and as a disinfectant in healthcare. Alcohols are most effective when mixed with purified water. This form facilitates their diffusion through the cell membranes of microorganisms. The optimal antimicrobial effect of isopropyl alcohol is in the range of 60 to 90% (McDonnell and Denver Russell 1999; Sultan et al. 2012). The 2-propanol-based chemical disinfectant, when introduced through the vehicle's dashboard, is not always evenly distributed across all components of the car's air-conditioning system. Therefore, this process should be mechanically supported by, for example a source of ultrasounds.

The device used in our study produces high-frequency ultrasounds (1.7 Hz), spraying the aerosol disinfectant. This spraying of the disinfectant, through the inlet and outlet ducts (air and ventilation) of the vehicle, reaches the evaporator of the air-conditioner and cleans all the components of the air-conditioning system in cars. The use of ultrasound to spray the disinfectant in the studied cars was very effective and resulted in a decrease in the microbiological concentration by over 70%.

Air-conditioning service engineers using this type of product must have experience and knowledge of the construction of ventilation systems in various vehicle brands.

Many studies have documented the presence of many types of harmful biological agents in the air in vehicles (Aquino et al. 2018; Lee and Jo 2005; Li et al. 2013; Simmons et al. 1999; Sowiak et al. 2018; Viegas et al. 2018). The current airborne microorganism concentrations inside the studied cars were found to be similar to those published in other reports (Lee and Jo 2005; Li et al. 2013; Nowakowicz-Dębek et al. 2017; Simmons et al. 1999).

The air of the studied cars before the AC service contained bacterial and fungal species belonging to risk group 2; according to European Directive 2000/54, the following were identified: *Bacillus subtilis*, *Bacillus thuringiensis*, *Corynebacterium* spp. and *Aspergillus fumigatus*. This means that car drivers and passengers may be exposed to direct contact with biological agents that are occupational hazards and which are potentially pathogenic.

The cars tested were contaminated with allergenic fungal species (*Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp. and *Alternaria* spp.). The same fungal genera of fungi were isolated from the air inside the vehicles by Nowakowicz-Dębek et al. (2017), Jo and Lee (2008), Sowiak et al. (2018) and Viegas et al. (2018). Fungi and their by-products, such as (1–3)-β-D-glucan, mycotoxins and microbial volatile organic compounds (MVOCs), have been implicated in adverse health reactions and diseases. Expert reviews of reported health problems associated with biological agents concluded, on the basis of many epidemiological studies, that human exposure to high levels of fungal aerosols can result in a variety of adverse health outcomes including respiratory, haematological, immunological and neurological system disorders and/or diseases (Gołofit-Szymczak and Górny 2018, Rylander et al. 2008). The presence of fungi in high concentrations can cause the appearance of allergic reactions and various nonspecific adverse health outcomes such as sick building syndrome (SBS). The moulds of *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Rhizopus* and *Penicillium* species are associated with allergy and respiratory problems (Aquino et al. 2018; Jones et al. 2011; Hwang and Cho 2016; Stephenson et al. 2014; Kumar et al. 1990; Rylander et al. 2008).

On the basis of the results obtained, it can be concluded that air-conditioning systems in cars should be regularly disinfected to eliminate bacterial and fungal contaminants that may be the cause of adverse health effects on humans.

Conclusions

- The tested methods of servicing automotive air-conditioning systems in the cars studied work so efficiently to cause a reduction of microbial contamination of the air inside the vehicle cabin. The highest disinfection efficiency was observed for ozonisation combined with the manual application of a chemical disinfectant into the car air-conditioning system and spraying a chemical disinfectant using ultrasounds.

- The qualitative analysis of the air microbiota in the cars studied indicated the presence of bacterial and fungal microorganisms which, according to the Ordinance of the Minister of Health, are classified into risk group 2. Direct contact with those microorganisms may cause adverse health effects for drivers and passengers alike.

- 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 the *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* genera.

- Vehicle air-conditioning systems should be systematically maintained. The whole system should be regularly disinfected to eliminate microbiological contaminants.

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