Microbial contamination of air filters of air conditioning system of urban buses

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ABSTRACT

The use of HVAC in urban buses in developed countries increases the comfort and indoor air quality in the means of ground transportation. The microbial contamination was studied on outlet and inlet surfaces of 5 air filters removed from the urban buses HVAC during regular maintenance. To acquire samples from both the outlet and the inlet sides of the filters, dry swabbing technique was used. Cultivation was performed on different selective or selective-diagnostic agars, to cultivate viable bacteria. To identify the bacterial species, Gram stain and immerse microscopy was used. Selected colonies underwent the proteomic study (MALDI-TOF) as well. After identification, bacteria were quantified. The bacteria of the genus *Bacillus – Bacillus cereus, Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, Bacillus flexus* prevailed on both inlet and outlet surfaces of the filters. The members of genera *Staphylococcus, Brevibacillus, Peribacillus or Paenibacillus* were also identified. The quantification of colony forming units showed low contamination of the outlet surfaces of filters 1 and 2. The contamination of inlet and outlet sides of filters 3, 4, and 5 was comparable, revealed nearly the same contamination of inlet and outlet surfaces. In the case of filters 3, 4 and 5 we recommend more frequent filter changing or more efficient filter choice.

KEYWORDS

air conditioning system – air filters – bacterial contamination – HVAC – urban buses

SOUHRN

Obitková D., Čereiová C., Mráz M., Pavlík E.: Mikrobiální kontaminace vzduchových filtrů klimatizačního systému městských autobusů

Cíl: Používání filtroventrilačních systémů v městských autobusech ve vyspělých zemích zvyšuje komfort a kvalitu vnitřního ovzduší v prostředcích pozemní dopravy. Mikrobiální kontaminace byla studována na výstupních a vstupních plochách 5 vzduchových filtrů vyjmutých z klimatizačního systému městských autobusů při pravidelné údržbě.
Materiál a metodika: K získání vzorků z výstupní i vstupní strany filtrů byla použita technika suchého stěru. Kultivace byla provedena na různých selektivních nebo selektivně-diagnostických půdách pro kultivaci životaschopných bakterií. K identifikaci bakteriálních druhů bylo použito barvení podle Grama a imerzní mikroskopie. Vybrané kolonie byly rovněž podrobeny proteomické studii. Po identifikaci byly bakterie kvantifikovány.
Výsledky: Na vstupním i výstupním povrchu filtrů převažovaly bakterie rodu *Bacillus – Bacillus cereus, Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, Bacillus flexus*. Identifikovány byli také bakterie rodů *Staphylococcus, Brevibacillus, Peribacillus* a *Paenibacillus*. Kvantifikace ukázala nízkou kontaminaci výstupních povrchů filtrů 1 a 2. Kontaminace vstupní a výstupní strany filtrů 3, 4 a 5 a odhalila téměř stejnou kontaminaci vstupních a výstupních ploch.
Závěry: Podle nalezených výsledků doporučujeme buď častější výměnu filtrů, nebo volbu filtrů s nižší porozitou.

KLÍČOVÁ SLOVA

bakteriální kontaminace – klimatizační system – městský autobus – vzduchový filtr

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INTRODUCTION

Infectious diseases, their transmission, and spread have posed a threat to human health from ancient times to the present. The wide range of routes of infectious diseases transmission, especially those caused by airborne pathogens, must be considered. In the bus interior, the airborne pathogens can be transmitted by droplets, droplet nuclei or bioaerosols [1, 2]. The bus can transport about 40 seated passengers and a similar number of standing passengers. Although urban trips are relatively short, public transport has been the center of discussion due to the crowding of people, the large passenger exchange rate. The modern bus units

normally include Heat Ventilation Air Conditioning (HVAC) systems with very low air renewal ratios. Moreover, on many buses the windows cannot be opened. The HVAC system is equipped with efficient air filters. In the Czech Republic, the air filters intended for ground transportation meet the ISO16890 e($PM_{2.5}$), min ≥ 50%, which can collect combustion particles, ultrafine dust and bacteria. Considering different species and genera of bacteria, they may have different sizes and shapes. The pathogens as *Staphylococci*, *Streptococci* or *Neiseria* can be significantly smaller than 32.5 µm, *Staphylococcus aureus* – 0.5–1.5 µm [3] *Streptococcus pneumoniae* – 0.5–1.25 µm [4] N*eisseria* meningitidis – 0.6–1 µm. The fungi as Aspergillus niger -2.0–3.0 µm [5] with the diameter close to the collecting parameters of the $PM_{2.5}$ air filters. Despite the sizes and shapes or capsules, the microbes are collected in the same way as particulate matter as dust or pollen [6]. In the past, the concentration of particulate matter PM_{10} and $PM_{2.5}$ was investigated [7]. Currently, in the times of the SARS CoV-2 pandemic, the air quality and bioaerosols spread in the indoor air of transport media are broadly discussed. The main topic of the discussion is represented by the influence and benefits of HVAC. Currently the studies are focused especially on SARS CoV-2 which can represent the behavior of small particles in the air conditioning system. It was reported repeatedly that the HVAC can contribute to virus spread in the bus. The study from Barcelona, Spain included samples from buses and subway – 82 samples (58 surface swabs, 9 air conditioning (a/c) filters, 3 a/c dust, 12 ambient air). Using an RT-PCR technique for SARS-CoV-2, thirty samples (36%) had evidence for at least one of the three tested viral RNA targets. Interestingly, the surfaces were more contaminated than the air. In addition, there were higher concentrations of viral RNA in buses compared to trains [8]. Zhang et al. investigated potential transmission mechanisms on an urban bus. The bus was equipped with one aerosol generator, to mimic an infected passenger. They identified that the flow carrying aerosols was predominantly controlled by the bus HVAC, uniformly distributing aerosol throughout the bus [9]. A similar situation was described in the coach bus. Shen et al. reported 24 out of 68 passengers positively tested on SARS CoV- 2 after 100 minutes in the coach bus where the air conditioner was set to heating and indoor recirculation [10]. Edwards et al. evaluated COVID-19 control measures including ventilation by open windows and HVAC system use in the model of school bus and transit bus. In the school bus the ventilation and air circulation provided by open windows resulted in reductions in the overall particle count, an average of 84% on school bus and 50% on transit bus. When considering usage of HVAC with MERV 13 air filter, the effectiveness of removing aerosol particles increased significantly in the transit bus. The resulting particle counts with the air filters resulted in an average of 93.95% improvement with aerosols dispersed from a middle location during bus in-motion testing [11]. Studying the air exchange in the urban bus, another study has brought evidence that window open during the on-the road testing decreases the bioaerosol amounts in the indoor air of urban bus significantly [12]. The European Center for Disease Control and Prevention and the European Commission recommended decreasing the bioaerosol concentration in the ground transportation by opening the windows in order to provide larger amount of fresh air and air flow rate increasing [13, 14].

Some studies focused on infection transmission in the buses, both urban od transit, show different results taking in regard air conditioning system. The urban public transport has a great advantage represented by fast passengers' exchange; many passengers stop with door opening which contributes to enhanced air circulation. The air quality control could be an issue for transit and coach buses which imitate more enclosed air-conditioned space with a significant role of HVAC in airborne infection transmission.

In this study we have decided to investigate microbial contamination on an outlet and inlet surface of the air filters of urban buses operating in selected city in the Czech Republic. The use of HVAC and potential microbial contamination of air filters and possible bacteria recirculation inspired our research group to investigate the bacterial contamination of the air filters of HVAC of urban buses. The air filters were removed from urban bus air conditioning system at the very end of their lifespan during HVAC maintenance. The main objective of the study was to evaluate the microbial contamination of inlet and outlet surface of the filtration medium separately. The study focused on pathogenic bacteria, opportunistic pathogens and bacteria commonly present in the air, dust and soil. The investigation of microbial contamination of urban bus air filters can contribute to understanding of potential risk microbes spread in the bus including suggestions on how to improve the filtration capacity of air conditioning system air filters. The cleanliness and safety of indoor air of the means of transport is desirable and necessary especially in the times of highly contagious infections spread.

MATERIAL AND METHODS

Chemicals

Chemicals were obtained from standard suppliers (P-Lab, CZ, Penta, CZ) and were of the highest purity – gram stain kit Carl Roth, acetone. Sterile saline solution (0.9% NaCl Braun, Germany) served as a sampling solution. MALDI matrix alpha-Cyano-4-hydroxycinnamic acid (Biovendor CZ), dilution solution Bruker standard solvent (Merck CZ) was used for mass spectrometry.

Material

The dry swabs were taken by polyester swabs (Inset Ltd. CZ). Then the cultivation was performed on standard solid cultivation plates – Petri plates (diameter 9 cm) – blood agar, blood agar with 5% NaCl, Mueller-Hinton, Sabouraud, Endo's, McConkey agars (Biovendor CZ). The filters serving in bus passenger cabin air conditioning system were obtained during regular filter exchange within regular service interval. Removed aseptically, the filters were transported in plastic bags to the laboratory. Stored at laboratory temperature, the filters were investigated after two months of storage. All work and experiments were performed in biohazard box class 2 (Schoeller CZ). Both inlet and outlet surface of the filters were swabbed.

Bacteriological methods

The dry swabs were inoculated directly and cultivated on solid cultivation media at 36±1°C in aerobic atmosphere for 24 hours. Also, the diagnostic media as Endo and chromogenic agars were used. The 24 hours bacterial cultures were obtained. The standard microbiological techniques were used to isolate pure cultures. Gram stain was performed as follows – crystal violet 90 s, iodine 60 s, acetone 15 s and carbol fuchsin 90 s. Immerse microscopy in 1000x (Labomed 400, SvenBiolabs CZ) magnification was then performed for bacteria identification.

Mass spectrometry

To precise bacterial identification, the Bruker MALDI TOF Biotyper (Bruker, Germany) mass spectrometer was employed. The specimens of 24 hours colonies were placed to the spots of the target plate. After drying, the addition of matrix followed. Then the mass spectrometry assessment was started. The microbiology software automates the process of acquiring the mass spectra. The obtained spectra are matched against the extensive reference library. Then the result is scored. The comparison of the sample and library data gives the number of congruent mass spectrum peaks. The maximum number is 1000, the minimum is 200. The calculation uses logarithmic scale. Log_{10} 1000 = 3, so the maximum score is 3. The minimum score for reliable detection lies between numbers 2 and 3.

RESULTS

Five filters from bus passengers' cabin air conditioning system were investigated. The busses served in city public transportation in the Czech Republic. The service intervals for filter exchange are as follows – the new filter is installed after winter pause in May, then the exchange comes in July, and the last change of the filter is performed at the end of September. The filters for the investigation were removed in July and September. The filters are made of polyester non-woven textile supplied as footage 12 mm of width. The filter material meets the requirements of ISO 16890 $PM_{2.5}$. This kind of filter captures particles of diameter 0.3–2.5 µm with 50% effectiveness. The city buses investigated specimen have the air conditioning unit situated at the rooftop. The cooling medium is driven by the compressor connected to the motor of the bus. The evaporator is situated at the rooftop as well. The hot air is sucked from the cabin of the bus passes through the air filter situated in the ceiling of the bus and continues to the evaporator where there is cooled. The cool air comes back to the cabin of the passengers' part of the bus via special vents. The movement of the air is provided by four pairs of fans situated near to the evaporator.

The surfaces of inlet and outlet side of all filters were swabbed by dry polyester swabs and directly inoculated in blood agar and Mueller Hinton agar plates. After 24hour cultivation, the colonies were counted thoroughly, and the cultivation led to gain pure cultures of particular bacteria. At the very beginning the pure colonies were identified by immerse microscopy. Especially *Bacillus cereus, Bacillus subtilis*, *Bacillus licheniformis, Bacillus flexus, Bacillus thuringiensis* and *Bacillus pumilus* were identified. The remainder of bacteria was identified by MALDI-TOF mass spectrometry. Table 1 represents the dimensions of all investigated filters.

Table 1. The dimensions of the filters

Number of the filter	Width [cm]	Length [cm]	Area [cm^2]
1	47	158.5	7449.5
2	47	159	7473
3	47	158.5	7449.5
4	47	158.5	7449.5
5	31	77	2387

The tables 2–6 summarizes numbers of identified colonies in inlet and outlet surfacer of investigated filters. Majority of identified bacteria are not pathogenic for humans with healthy immune system. In filters 1 and 2, potentially pathogenic *S. epidermidis* and *S. warneri* were identified.

Quantification of selected bacteria in CFU/mL revealed mild contamination of the filters. No pathogens were identified.

Table 2. Number of colonies on inlet and outlet surfaces of filter 1

Identified bacteria	Number of colonies INLET	Number of colonies OUTLET
Bacillus pumilus	2	0
Bacillus subtilis	5	0
Bacillus licheniformis	2	0
Bacillus cereus	5	0
Staphylococcus epidermidis	1	1

Table 3. Number of colonies on inlet and outlet surfaces of filter 2

Identified bacteria	Number of colonies INLET	Number of colonies OUTLET
Bacillus flexus	2	1
Bacillus subtilis	3	1
Staphylococcus warneri	0	1
Bacillus megaterium	1	0
Bacillus licheniformis	1	0

Table 4. Number of colonies on inlet and outlet surfaces of filter 3

Identified bacteria	Number of colonies INLET	Number of colonies OUTLET
Peribacillus simplex	1	1
Bacillus subtilis	5	1
Priestia megaterium	1	1
Paenibacillus woosongensis	1	1
Brevibacillus borstelensis	1	0
Bacillus cereus	3	1
Bacillus licheniformis	2	1
Bacillus pumilus	1	1
Peribacillus muralis	1	0
Priestia endophytica	1	0
Alkalihalobacillus clausii	1	0
Bacillus flexus	0	1
Paenibacillus tylopili	0	1
Micrococcus luteus	0	1
Neobacillus niacini	0	1
Lysinibacillus halotolerans	0	1
Sporosarcina newyorkensis	0	1
Burkholderia glumae	0	1
Paraburkholderia xenovorans	0	1

Table 5. Number of colonies on inlet and outlet surfaces of filter 4

Identified bacteria	Number of colonies INLET	Number of colonies OUTLET
Bacillus licheniformis	5	4
Bacillus cereus	4	3
Paenibacillus glucanolyticus	1	0
Staphylococcus warneri	1	1
Staphylococcus epidermidis	1	1
Micrococcus luteus	1	0
Brevibacillus borstelensis	4	3
Gracilibacillus dipsosauri	0	1
Bacillus subtilis	15	12
Peribacillus simplex	1	1
Bacillus megaterium	5	4
Bacillus thuringiensis	0	1

Table 6. Number of colonies on inlet and outlet surfaces of filter 5

Identified bacteria	Number of colonies INLET	Number of colonies OUTLET
Bacillus subtilis	10	3
Bacillud licheniformis	5	3
Bacillus cereus	3	1
Bacillus flexus	2	2
Bacillus pumilus	1	1
Peribacillus muralis	1	0
Burkholderia ambifaria	1	0
Cytobacillus ocenisediminis	0	1
Cytobacillus horneckiae	0	1
Brevibacillus borstelensis	1	1
Aspergilus niger	3	0

Table 7. Quantification of selected bacteria in the filters represented as CFU/mL

Bacterium	Inlet [CFU/mL]	Outlet [CFU/mL]	Efficiency [%]
Bacillus flexus	2	1.5	25
Alkalihalobacillus clausii	0	0.5	0
Bacillus pumilus	0.5	0	100
Burkholderia glumae	0	0.5	0
Bacillus subtilis	4	1	75
Bacillus licheniformis	1.5	0.5	67.7
Bacillus cereus	1	0	100

DISCUSSION

A wide range of different bacteria was detected. The bacterial species ranking among the members of the genus *Bacillus, Brevibacillus, Peribacillus, Burkholderia* or *Cytobacillus* are environmentally ubiquitous. They can live in dust, soil and air, so their presence on the inlet surface of the air filters on the bus is obvious. Their ability to produce spores gives them the potential to survive in very unfavorable conditions and their resistance to the effect of the environment can preserve their viability on dry air filters for long time. It was suggested that *Bacillus atrophaeus* survived on the surface of a HEPA filter for 210 days without any loss of vitality [15]. The bacteria from the genus *Bacillus* are relatively large but the results show that they penetrate the filter. *Bacillus cereus* is a large rod-like bacterium – the vegetative cells are 0.5 by 1.2 to 2.5 by 10 μm and occur singly or in chains [16]. It is considered as a potential pathogen; it is known most frequently as the cause of food poisoning. The described infections affect the eyes – endophtalmitis [17], skin – wound infections, brain – meningoencephalitis [18]. These infections typically occur in immuno-compromised people. Nevertheless, *Bacillus cereus* caused meningoencephalitis in a person with a healthy immune system [19]. *Bacillus licheniformis* serves as a natural decomposer, living in the soil and the spores could be present in the dust. *B. licheniformis* is primarily pathogenic for insects, can be used as a component of probiotics but it was reported as a cause of food poisoning as well [20]. Although *B. licheniformis* is considered nonpathogenic for humans, it can be responsible for infections of eyes and recurrent sepsis [21]. On the outlet surface of filter 4, *Bacillus thuringiensis* was identified. It was also identified as a potential pathogen for humans. It can cause pulmonary infections in individuals suffering from neutropenia [22]. Identified bacteria ubiquitous in the ambient air can be in higher concentration in the indoor air of the bus and moreover, the bacteria identified on the outlet surface of the filter may recirculate back to the passenger area of the bus. The urban buses are characteristic with fast passenger exchange because of the high frequency of stops with door opening and relatively short time of stay in bus cabin. Most immunocompetent passengers are not at risk of infection caused by described bacteria. Only people with naturally weakened immune system function such as elderly people and small infants have to be aware of some risk.

Technically, the $PM_{2.5}$ filters are installed to the HVAC system to protect mainly the evaporator from dust, pollen and other particulate matter contamination and obstruction. The air filters intended for particulate matter filtration perform well. The question which still remains is how to improve the passengers' protection from bioaerosols and airborne pathogens transmission. The economic burden of more frequent filter change or finer filters use is obvious. This topic may be more profound when considering transit and coach buses in which the HVAC system is set on recirculation inside the passenger's cabin more frequently.

The contamination of both surfaces of the air filters of urban buses was not high. The quantification revealed that the microbial burden is low. When considering the removal of the air filters in the ambient air, the bacterial contamination may also originate in incorrect handling of the filters or omitting the aseptic conditions. Manipulation with the filters without protective gloves may cause contamination of *Staphylococcus epidermidis* and *Staphylococcus warneri*. These bacteria are commensals of human skin and improper manipulation with the filter can cause undesirable contamination. The *Staphylococci* was identified in four filters – on the filter 3 and 4 on inlet and outlet surface comparably. So, we think that undesirable contamination could be avoided. In filters 3 and 4, the *Staphylococci* were identified on both surfaces of the filters. Even there, we should take into regard the possibility of recirculation because these bacteria are potential human pathogens. *Staphylococcus epidermidis* can cause various infections of blood stream, endocarditis or wounds [23]. *Staphylococcus warneri* is also potential pathogen and was reported as a urinary tract infection cause. Despite the described cases are mainly examples of nosocomial infections, the recirculation of these bacteria may seem a problem for sensitive persons. Moreover, both species are capable to produce biofilms which can contaminate the airways of the air conditioning system and enhance the bacteria recirculation [24].

Low bacterial contamination and absence of human pathogens may be caused by the summer season when the filters were removed from the urban buses air conditioning system. In the summer season people usually do not suffer from respiratory diseases and usually use public transport less frequently because of vacations. Even though the urban buses may be sometimes crowded and the concentration of bioaerosols rises, we did not detect any pathogenic bacteria. Absence of pathogens may be caused by several facts – the pathogenic bacteria are sensitive to ultraviolet radiation and dry conditions of the air of summer season. As reported previously, the pathogens can survive on the filter surface for a very short time [15]. So, we did not detect any viable pathogens. Secondly, the urban buses can have the windows open, also the doors can be open quite frequently. Then, the air circulates faster, and the indoor air of the bus cabin is diluted by the fresh air coming from outside. It was reported that the opening windows can significantly lower the bioaerosols concentration.

CONCLUSION

In our experiment we tested bacterial contamination of inlet and outlet surfaces of five air filters removed from urban bus air conditioning system during regular maintenance. The investigation revealed contamination especially by environmental bacteria, mainly the genera *Bacillus, Brevibcillus, Peribacillus or Burkholderia* were detected. Especially *B. Cereus*, *B. licheniformis* and *B. thuringiensis* should be expected as a cause of human disease in sensitive persons. Moreover, *Staphylococcus epidermidis* and *Staphylococcus warneri* could be potential threat. The contamination of both inlet and outlet surfaces of the air filters seems to be proportional. This fact suggests possible recirculation of detected bacteria back to the passenger's cabin of the bus. Based on our research, we recommend changing the air filters more frequently or choosing air filters PM_1 to enhance the urban bus indoor air quality.

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