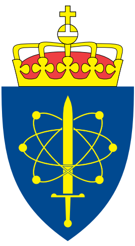

FFI-RAPPORT

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Summary

Monitoring systems for rapid airborne biothreat detection experience performance degradation in environments with a complex, dynamic bioaerosol background. Knowledge about bioaerosol backgrounds may therefore help improve the performance of such systems. Subway stations are enclosed public environments regarded as potential bioterrorism targets.

This study describes for the first time a snapshot of cultivable airborne bacteria (CAB) at a Czech subway station. The airborne bacterial concentration level and diversity was investigated and directly compared with previous observations made at a Norwegian subway station using a similar experimental approach.

The CAB level ranged from 10^1 to 10^2 colony forming units (CFU) m^{-3}, which was within the observed range in Norway. However, the average level was approximately three times lower than in Norway. The diurnal concentration profile showed increased levels during rush hours compared to non-rush hours and decaying evening levels, consistent with the Norwegian profile.

The bacterial genera *Micrococcus*, *Bacillus*, and *Staphylococcus*, previously shown to be abundant in Norway, corresponded to ~80 percent of the CAB at the station. Interestingly, nearly all the observed genera had also been encountered in Norway. The bacterial diversity was investigated using the MALDI Biotyper (MALDI-TOF MS), and its applicability as a rapid, cost-effective classification method for bacterial isolates was confirmed.

This study demonstrated that very similar CAB backgrounds can be encountered at different European stations (Czech Republic and Norway), and that anthropogenic activities (mainly passengers) appear to be a major CAB source.

The outcome of this work has relevance for public and occupational health as well as microbial ecology. In addition, it may potentially be used to improve the performance of biothreat detection systems and contribute to the development of more realistic test methodologies for such systems.

Sammendrag

Overvåkningssystemer for rask påvisning av biologiske trusselstoffer i luft får ofte redusert ytelse ved bruk i miljøer med en naturlig, kompleks og dynamisk bakgrunn av biologiske aerosoler (bioaerosoler). En kjent kilde til slik kompleksitet og dynamikk er naturlige svingninger i konsentrasjonsnivået til forskjellige typer luftbårne mikroorganismer, for eksempel bakterier, sopp, og virus, samt pollen, som oppstår som følge av meteorologiske forhold eller menneskelig aktivitet. Økt kunnskap om bioaerosolbakgrunnen i relevante operasjonsmiljøer kan derfor potensielt bidra til å utvikle påvisningssystemer med forbedret ytelse. T-banestasjoner er ofte innelukkede underjordiske steder og anses som potensielle mål for bioterrorisme.

Denne studien beskriver for første gang et øyeblikksbilde av dyrkbare luftbårne bakterier på en T-banestasjon i Tsjekkia. Konsentrasjonsnivået og diversiteten av luftbårne bakterier ble undersøkt og direkte sammenlignet med lignende resultater fra en tidligere studie. Den sistnevnte studien hadde tilsvarende eksperimentell tilnærming og ble utført på en T-banestasjon i Norge.

Konsentrasjonsnivået av dyrkbare luftbårne bakterier på stasjonen i Tsjekkia var i størrelsesorden 10^1 til 10^2 CFU (colony forming units, kolonidannende enheter) per kubikkmeter luft, noe som var innenfor konsentrasjonsområdet observert i Norge. Den gjennomsnittlige konsentrasjonen var likevel omtrent tre ganger lavere enn det som ble observert i Norge. Døgnprofilen viste økte konsentrasjoner i rushtiden og en gradvis konsentrasjonsreduksjon mot kvelden, noe som var konsistent med døgnprofilen i Norge.

De bakterielle slektene *Micrococcus*, *Bacillus* og *Staphylococcus*, som tidligere er vist å være rikelig tilstede i Norge, utgjorde ~80 prosent av alle dyrkbare luftbårne bakterier på den tsjekkiske stasjonen. En interessant observasjon var at nesten alle slektene som ble observert i Tsjekkia, også hadde blitt observert i Norge. Den bakterielle diversiteten ble undersøkt med MALDI Biotyper (MALDI-TOF MS, massespektrometri). Denne teknikken regnes som en rask og kostnadseffektiv klassifikasjons- og identifikasjonsmetode for bakterielle isolater. Studien bekrefter at denne teknikken er egnet til slike undersøkelser.

Denne studien har demonstrert at svært like konsentrasjonsnivåer og diversitet av dyrkbare luftbårne bakterier kan påtreffes på T-banestasjoner ulike steder i Europa (Tsjekkia og Norge). Det ser også ut til at menneskelig aktivitet (hovedsakelig passasjerer) er en betydelig kilde til dyrkbare luftbårne bakterier.

Utbytte fra dette arbeidet har relevans for folkehelse og arbeidsmiljøhelse samt mikrobiell økologi. Det kan også bli brukt til å forbedre ytelsen til overvåkningssystemer for rask påvisning av biologiske trusselstoffer i luft og bidra til utvikling av mer realistiske testmetoder for slike systemer.

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Preface

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1 Introduction

Subway transportation systems are found in more than 148 cities worldwide and transport about 150 million passengers daily [11]. Subway stations are crowded and confined underground environments which may be regarded as critical infrastructures for metropolitan public transportation and potential bioterrorism targets. The importance of understanding, monitoring and controlling the presence of biological aerosols (bioaerosols) in public and occupational environments has become increasingly recognized in recent years. Early-warning biodetection through the use of automated biological detection, identification and monitoring (BioDIM) systems could improve the protection of facility occupants in the event of a biological incident, e.g. by improving the situational awareness and allowing for timelier implementation of countermeasures, however a recognized limitation of many early-warning BioDIM systems has been their susceptibility to experience performance degradation in environments with a complex, dynamic natural bioaerosol background [4-7]. Bioaerosol background investigations in relevant real life environments may obtain information that could be used to design BioDIM systems which are less susceptible to background-related performance issues and also to develop test and evaluation (T&E) schemes which are better at predicting realistic real life performance [4-7]. The airborne bacterial background, as well as several other bioaerosol and non-biological aerosol background constituents, have been studied at subway stations [7]. However, several questions still remain unanswered, especially regarding the spatiotemporal variability of the background. Airborne bacterial background characteristics from subway stations in different geographic and climatic regions may be used to estimate the level of variability that should be expected between stations. Although a substantial amount of information exists in the literature, meaningful direct comparisons, trend analyses, and extrapolations of the results from previous investigations are however commonly hampered by differences in the aim, scope, and experimental procedures of past efforts [7]. The primary aim of this study was to characterize the airborne bacterial background at a subway station in Prague, Czech Republic. However, in an effort to investigate trends in the airborne bacterial background at stations in different European countries, the results from Prague were directly compared with those from a similar subway station in Oslo, Norway [7-9]. The experimental work was focused on investigating the concentration level and diversity of cultivable airborne bacteria at the station. Total aerosol characteristics including concentration level and size distribution, as well as meteorological conditions including temperature and relative humidity, were however also studied. This study is the first published account to describe the airborne bacterial background at a subway station in the Czech Republic, and also provides novel information about the background variability between stations in different European countries.

2 Materials and methods

2.1 Study location

This study was conducted in the winter season of 2012 at the Muzeum subway station in Prague, Czech Republic. The Prague subway is organized as a Secant system with three lines (Line A-C). The Muzeum station is one of three downtown junction stations and serves as a transfer station between Line A and Line C. The A platform is located at a depth of 34 m below Vinohradská street between the National Museum and the building of Radio Free Europe, while the C platform is located at a depth of 10 m below Wilson Street and Washington Street. The station vestibule is located below Wenceslas Square and connects directly to both platforms. The C platform is accessible via a short staircase and escalator from the vestibule, while a longer escalator runs from the vestibule down to the A platform. Both platforms are also inter-connected via an escalator running from one end of the A platform up to the center of the C platform. The A and C platform tunnels each occupy an air volume of 40,193 m^3, with platform length and width of 108 m and 20.5 m, and 194 m and 10 m, respectively. The neighboring stations to the Muzeum station are Můstek and Náměstí Míru on Line A at a distance of 700 and 800 m, respectively, and Hlavní nádraží and I. P. Pavlova on Line C at a distance of 400 and 700 m, respectively. About 8,500 and 26,000 passengers enter the Muzeum station each day to depart from Line A and Line C, respectively, while about 50,000 and 54,000 passengers transfer from Line A to Line C and Line C to Line A, respectively [14], and which in total sums up to more than 50 million yearly departures. The mechanical ventilation system consists of ceiling-mounted vents in the middle of each platform which exhaust air from or add air to the station depending on the temperature difference between the tunnel network and the outdoor ambient air. The main ventilation mechanism for the station is however natural ventilation arising due to indoor-to-outdoor temperature differences (i.e. thermal stack effects) and the movement of trains in narrow subway tunnels (i.e. train-induced piston effects).

2.2 Bioaerosol collection

Air sampling was performed at one site on the A platform and two sites on the C platform to characterize the concentration level of cultivable airborne bacteria at the station. The sampling sites were located opposite from public entrances at the far end of each platform due to safety regulations and to avoid passenger disturbance. Air sampling was done during the opening hours of the station, continuously for 17 h at the C platform on 28 February 2012 (07:00-00:05) and 14 h at the A platform on 13 March 2012 (07:00-00:05). Air samples were collected at a rate of 100 l min^{-1} for 5 min at the

beginning of every hour using a Spin Air impactor (IUL Instruments, Barcelona, Spain) and Colombia agar plates without blood supplements (Trios, Prague, Czech Republic). The impactor rotates the impaction plates to evenly distribute the particle deposition and which in turn reduce the localized desiccation effects commonly observed with non-rotating impactors. The impactor was positioned on a table with the air intake at 1.3 m above the concourse. The impaction plates were placed in a portable incubator after sampling and incubated at 37°C. The resulting colonies were enumerated after both 24 and 48 h of incubation and the plate counts were used to calculate the concentration level of cultivable airborne bacteria at the station, expressed as colony forming units per cubic meter of air (CFU m^{-3}).

2.3 Total aerosol and meteorological data collection

Monitoring of the total aerosol level and size distribution at the station was done using APS 3321 aerodynamic particle sizers (TSI, MN, USA). The APS 3321 was positioned on the same table as the Spin Air impactor with the intake at the same height above the concourse. The APS 3321 was operated with a 20-s measurement period and recorded size-resolved particle counts in 52 channels, corresponding to an aerodynamic diameter size range from about 0.5 to 20 µm. The total aerosol level was expressed as particles per cubic meter of air (particles m^{-3}) while the size distribution was expressed as count median aerodynamic diameter (CMAD) and geometric standard deviation (GSD). Monitoring of the meteorological conditions at the station was done using Comet S3121 temperature and humidity meters (Comet Systems, Rožnov pod Radhoštěm, Czech Republic). The Comet S3121 was positioned on the same table as the Spin Air impactor with the intake at the same height above the concourse. The Comet S3121 was operated with a 20-s measurement period and the recorded temperature and humidity was expressed as °C and % relative humidity, respectively.

2.4 Bacterial isolation

To assess the cultivable airborne bacterial diversity at the station, random selections of bacterial colonies were isolated from the primary cultivation plates and re-streaked onto Colombia agar plates without blood supplements (Trios) using standard microbiological techniques. The agar plates were incubated at 37°C for 24 h. The sub-culturing process was repeated twice or until pure isolates were obtained.

2.5 MALDI-TOF MS

The bacterial isolates were classified using the MALDI Biotyper microbial identification platform (Bruker Daltonics, Bremen, Germany) and a MicroFlex LT matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) instrument (Bruker Daltonics) according to the recommended standard direct transfer method. Briefly, a single bacterial colony was transferred onto a MSP 96 ground steel target (Bruker Daltonics) as four replicate spots. The α-cyano-4-hydroxycinnamic acid (HCCA) matrix (Bruker Daltonics) was prepared in accordance with the manufacturer's recommendations and overlaid onto each target spot (1 µl). The MALDI Biotyper 2.0 system was run in automatic classification mode and the reference database was the Bruker Taxonomy library (3.1.1.0, containing 3,740 library entries) coupled with the security-relevant add-on library. An updated version of the MALDI Biotyper system (MALDI Biotyper 3.0) with a newer version of the Bruker Taxonomy library (3.3.1.0, containing 4,613 library entries) was used in a subsequent re-analysis of the bacterial isolates to investigate whether the updated reference library impacted the classification results. The MALDI Biotyper system reports classification score values (SV) between 0-3, and which are generally interpreted to suggest; i) probable species identification if SV is ≥2.3, ii) secure genus identification and probable species identification if SV is ≥2.0, iii) probable genus identification if SV is ≥1.7, and iv) no reliable identification if SV is <1.7.

2.6 16S rRNA gene sequencing

A subset of the bacterial isolates was subjected to partial 16S rRNA gene sequencing to confirm the MALDI Biotyper classification results. Nucleic acids were purified from a single bacterial colony using the GenElute Bacterial gDNA kit (Sigma-Aldrich, St. Louis, MO, USA). The purified gDNA was used for PCR amplification of the bacterial 16S rRNA gene with universal bacterial primers originally described by Lane et al. [12]. The PCR reaction (20 µl) consisted of 10 ng template, 1 x PPP Master mix without $MgCl_2$ (Top-Bio, Prague, Czech Republic), 2 mM $MgCl_2$, 200 µM of each dNTP, and 0.5 µM of the forward 27F (5'- GAGTTTGATCMTGGCTCAG-3') and reverse 519R (5'- GWATTACCGCGGCKGCTG-3') primer in ultrapure water. The PCR program consisted of an initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 55°C for 10 s and elongation at 72°C for 90 s. Standard agarose gel electrophoresis was used to verify the amplification of specific PCR products. The PCR products were purified with a MinElute PCR Purification kit (Qiagen, Hilden, Germany), quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and diluted with ultrapure water to a

concentration of 5 ng μl^{-1}. Sequencing of the PCR products was done using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA), according to the recommended standard protocol. The terminated products were cleaned using a DyeEx 2.0 Spin kit (Qiagen), dissolved in Hi-Di formamide (Applied Biosystems) and incubated at 95°C for 2 min. Direct sequencing was performed on a 310 Genetic Analyzer (Applied Biosystems). The sequence trace files were manually checked and the sequences were uploaded to the Classifier and SeqMatch tools at the Ribosomal Database Project (RDP) website (http://rdp.cme.msu.edu, release 10, update 30) [3, 17].

2.7 Statistical analysis

The results were subjected to statistical analyses using SigmaPlot v12.3 (Systat Software, Inc., San Jose, CA). Normality testing was done with the Shapiro-Wilk test and depending on whether the normality and equal variance criteria were fulfilled or not, significance testing was performed with the Student's t-test or the Mann-Whitney rank sum test, respectively. Temporal correlation within and between the airborne bacterial and total aerosol data sets from the Muzeum station, as well as previous data sets from the Nationaltheatret subway station in Oslo, Norway [7-9], were investigated using the Pearson product-moment correlation coefficient. The significance level was set at $p<0.05$ for all statistical tests.

3 Results

3.1 Airborne bacterial level

A total of 52 air samples were collected at the Muzeum subway station to determine the concentration level of airborne bacteria (Table 3.1 and Figure 3.1). The bacterial level at the station ranged from 10^1 to 10^2 CFU m^{-3} (Table 3.1 and Figure 3.1). The level observed on different sampling days (28 February and 13 March) and different locations inside the station (one site at the A platform and two sites at the C platform) showed marginal differences, and the results were therefore combined and reported as average values (Table 3.1 and Figure 3.1). The average concentration level at the station was 120±56 and 163±76 CFU m^{-3} based on a plate incubation time of 24 and 48 h, respectively (Table 3.1 and Figure 3.1). The estimated level increased with 40±9% when the incubation time was extended from 24 to 48 h, however the 24- and 48-h results were found to be associated with a significant temporal correlation (r = 0.994) (Table 3.1 and Figure 3.1).

Table 3.1 Concentration level of airborne bacteria and meteorological conditions at the Muzeum subway station (Prague, Czech Republic, current study) and the Nationaltheatret subway station (Oslo, Norway, published data[9]).

Time (hh:mm)	Muzeum subway station, Prague, Czech Republic				Nationaltheatret subway station, Oslo, Norway[1]		
	Airborne bacterial level (CFU m^{-3})		Temp	Humidity	Airborne bacterial level (CFU m^{-3})	Temp	Humidity
	24-h incubation	48-h incubation	°C	%RH	48-h incubation	°C	%RH
07:00 - 07:05	160 ± 50	249 ± 93	13.2 ± 1.0	53.2 ± 4.4	506 ± 95	7.2 ± 0.3	59.6 ± 4.5
08:00 - 08:05	196 ± 32	270 ± 83	13.9 ± 0.7	53.9 ± 4.1	936 ± 252	7.6 ± 0.2	59.6 ± 4.2
09:00 - 09:05	189 ± 6	249 ± 36	14.1 ± 0.7	54.7 ± 3.7	756 ± 103	7.5 ± 0.3	64.9 ± 2.5
10:00 - 10:05	156 ± 26	205 ± 34	13.9 ± 0.8	54.1 ± 2.0	526 ± 48	7.5 ± 0.4	62.1 ± 6.5
11:00 - 11:05	135 ± 21	190 ± 56	14.0 ± 0.7	54.8 ± 0.5	449 ± 46	7.1 ± 0.3	60.8 ± 6.6
12:00 - 12:05	91 ± 7	132 ± 10	14.0 ± 0.7	55.1 ± 0.7	405 ± 62	7.5 ± 0.4	65.0 ± 5.2
13:00 - 13:05	93 ± 27	133 ± 16	14.1 ± 0.7	56.3 ± 1.1	322 ± 46	7.8 ± 0.1	68.0 ± 3.8
14:00 - 14:05	95 ± 16	140 ± 12	14.2 ± 0.6	57.2 ± 2.0	431 ± 58	7.6 ± 0.5	63.8 ± 5.5
15:00 - 15:05	108 ± 24	149 ± 8	14.1 ± 0.6	58.7 ± 3.1	462 ± 26	7.3 ±0.5	62.7 ± 3.5
16:00 - 16:05	143 ± 38	196 ± 16	14.3 ± 0.7	58.1 ± 2.8	631 ± 46	7.7 ± 0.5	62.2 ± 5.2
17:00 - 17:05	147 ± 20	188 ± 11	14.4 ± 0.7	57.6 ± 2.0	492 ± 110	7.8 ± 0.4	62.6 ± 5.1
18:00 - 18:05	185 ± 26	234 ± 11	14.4 ± 0.7	57.8 ± 2.4	449 ± 94	7.8 ± 0.2	70.3 ± 5.3
19:00 - 19:05	116 ± 11	160 ± 12	14.4 ± 0.6	57.4 ± 2.3	378 ± 40	7.3 ± 0.4	63.4 ± 7.1
20:00 - 20:05	88 ± 10	122 ± 18	14.1 ± 0.6	57.5 ± 2.2	370 ± 25	7.2 ± 0.3	64.4 ± 7.9
21:00 - 21:05	57 ± 19	73 ± 19	14.1 ± 0.6	56.6 ± 2.1	294 ± 38	7.5 ± 0.2	66.5 ± 5.6
22:00 - 22:05	26 ± 2	40 ± 1	13.1 ± 0.1	59.2 ± 0.6	218 ± 31	7.2 ± 0.2	63.1 ± 4.3
23:00 - 23:05	34 ± 6	50 ± 4	13.0 ± 0.1	60.3 ± 0.4	206 ± 17	6.9 ± 0.2	65.3 ± 6.4
00:00 - 00:05	19 ± 1	31 ± 1	13.1 ± 0.1	61.7 ± 0.5	176 ± 54	7.1 ± 0.2	64.7 ± 7.8

CFU; colony forming units, RH; relative humidity

[1] Dybwad, M., G. Skogan, and J. M. Blatny. 2014. Temporal Variability of the Bioaerosol Background at a Subway Station: Concentration Level, Size Distribution, and Diversity of Airborne Bacteria. Applied and environmental microbiology **80**:257-270.

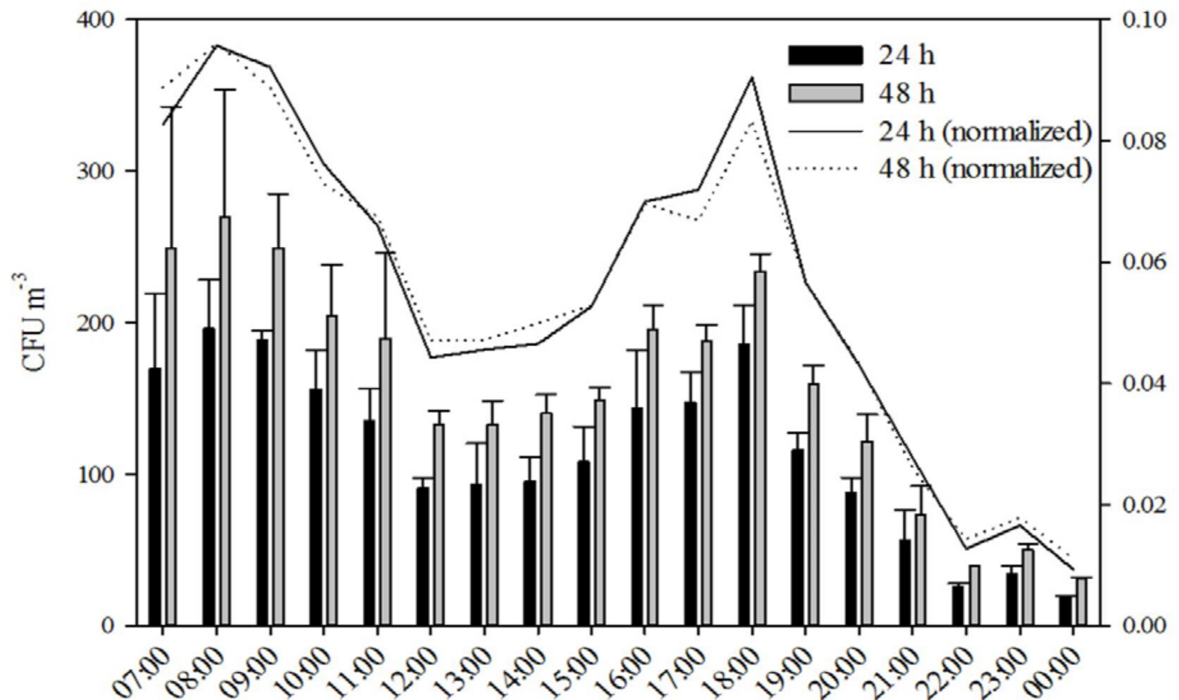

Figure 3.1 *Concentration level of airborne bacteria at the Muzeum subway station. X-axis: Time of day (hh:mm). Left y-axis (bar chart): Estimated airborne bacterial level at the station after 24 h (black) and 48 h (grey) of incubation time, expressed as colony forming units per cubic meter of air (CFU m^{-3}). Right y-axis (line chart): Normalized airborne bacterial level at the station after 24 h (solid) and 48 h (dotted) of incubation time (normalized to measurement period total).*

The results from the Muzeum station were compared to results from previous studies performed at the Nationaltheatret subway station in Oslo, Norway [7-9]. The airborne bacterial level at the Muzeum station was found to be significantly lower (~3-fold) than that reported from the Nationaltheatret station (Table 1 and Figure 2). The temporal concentration profile at the two stations were however significantly correlated (r = 0.872) and showed increased levels during rush hours compared to non-rush hours and decaying evening levels (Table 3.1 and Figure 3.2).

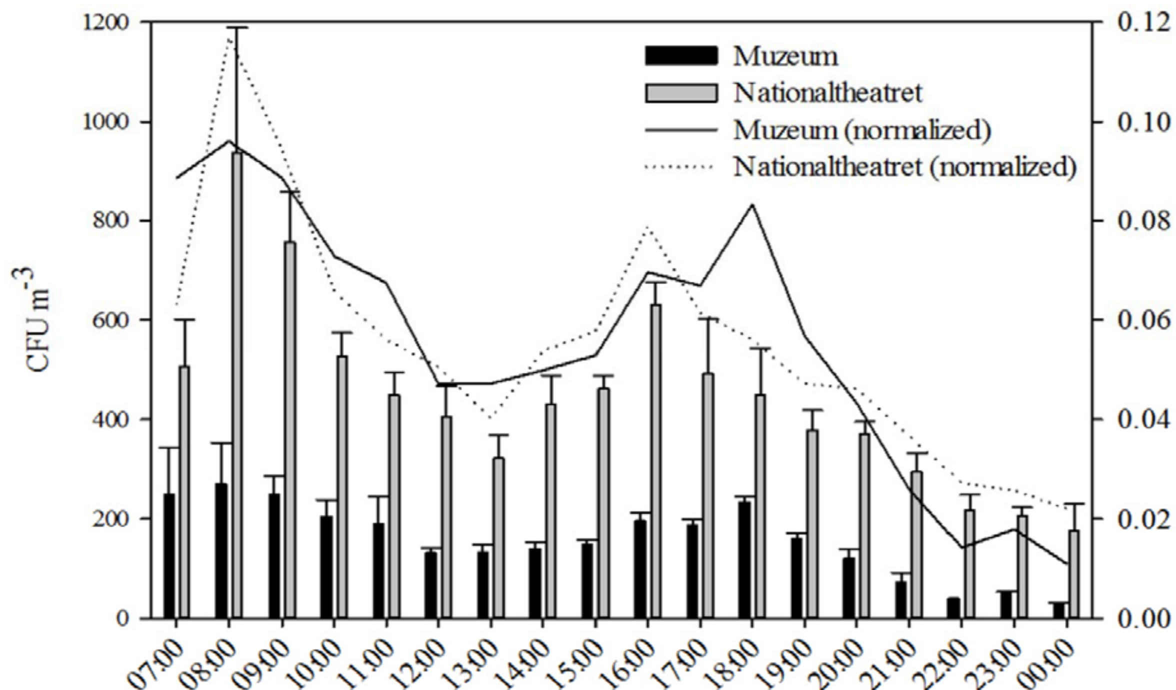

Figure 3.2 *Concentration level of airborne bacteria at the Muzeum subway station (Prague, Czech Republic, current study) and the Nationaltheatret subway station (Oslo, Norway, published data[9]). X-axis: Time of day (hh:mm). Left y-axis (bar chart): Estimated airborne bacterial level after 48 h of incubation time at the Muzeum (black) and Nationaltheatret (grey) station, expressed as colony forming units per cubic meter of air (CFU m^{-3}). Right y-axis (line chart): Normalized airborne bacterial level after 48 h of incubation time at the Muzeum (solid) and Nationaltheatret (dotted) station (normalized to measurement period total).*

3.2 Airborne bacterial diversity

The airborne bacterial diversity at the Muzeum station was investigated by analyzing 196 bacterial isolates with the MALDI Biotyper (MALDI-TOF MS) microbial identification system (Table 3.2). Two versions of the MALDI Biotyper (2.0 and 3.0) coupled with different Bruker Taxonomy reference libraries (3.1.1.0 containing 3,740 entries and 3.3.1.0 containing 4,613 entries, respectively) were used to classify the isolates. Of the total number of isolates, 7% (14/196) failed to generate approvable mass spectra and were not further analyzed. Of the isolates that generated approvable mass spectra (n = 182), 36% (66/182) and 31% (56/182) could not be assigned with a reliable classification by the MALDI Biotyper 2.0 and 3.0, respectively. The MALDI Biotyper 2.0 and 3.0 assigned genus-level classifications to 64% (116/182) and 69% (126/182) of the isolates, respectively, while species-level classifications were assigned to 16% (30/182) and 24% (44/182), respectively. Twelve bacterial genera belonging to three

major phyla were recovered from the air of the Muzeum station (Table 2). When considering the MALDI Biotyper 3.0 results, the observed phylum-level diversity corresponded to 47% *Actinobacteria*, 43% *Firmicutes* and 10% *Proteobacteria* (Table 2). The predominant bacterial genera were *Micrococcus* (40%), *Bacillus* (23%), *Staphylococcus* (17%), *Acinetobacter* (6%) and *Pseudomonas* (4%), and which corresponded to the following predominant bacterial species: *Micrococcus luteus*, *Bacillus* spp. (*B. subtilis, B. pumilus* and *B. licheniformis*), *Staphylococcus* spp. (*S. aureus, S. hominis, S. capitis* and *S. epidermidis*), *Acinetobacter* spp. (*A. lwoffii* and *A. junii*) and *Pseudomonas stutzeri* (Table 3.2).

Table 3.2 Diversity of airborne bacteria at the Muzeum subway station.

Phylum	Genus	Species	MALDI Biotyper 2.0[1]		MALDI Biotyper 3.0[2]	
			N	%	N	%
Actinobacteria	*Micrococcus*	*M. luteus*	46	40	51	40
Firmicutes	*Bacillus*	*B. subtilis, B. pumilus, B. licheniformis*	26	22	29	23
Firmicutes	*Staphylococcus*	*S. aureus, S. hominis, S. capitis, S. epidermidis*	20	17	21	17
Proteobacteria	*Acinetobacter*	*A. lwoffii, A. junii*	6	5	7	6
Proteobacteria	*Pseudomonas*	*P. stutzeri*	5	4	5	4
Actinobacteria	*Corynebacterium*	*C. mucifaciens*	4	3	4	3
Actinobacteria	*Kocuria*	*K. polaris*	3	3	3	2
Firmicutes	*Solibacillus*	*S. silvestris*	2	2	2	2
Firmicutes	*Paenibacillus*	*P. amolyticus*	1	<1	1	<1
Actinobacteria	*Kytococcus*		1	<1	1	<1
Firmicutes	*Anaerococcus*		2	2	0	<1
Firmicutes	*Exiguobacterium*	*E. aurantiacum*	0	<1	2	2
Total			**116**	**100**	**126**	**100**

[1] MALDI Biotyper 2.0 (Bruker Daltonics, Bremen, Germany) with Bruker Taxonomy database v3.1.1.0 (3,740 library entries) and security-relevant add-on database

[2] MALDI Biotyper 3.0 (Bruker Daltonics) with Bruker Taxonomy database v3.3.1.0 (4,613 library entries) and security-relevant add-on database

A subset (*n* = 39) of the bacterial isolates classified to the genus or species level by the MALDI Biotyper was subjected to 16S rRNA gene sequencing to confirm the results (Table 3.3). When the MALDI Biotyper 2.0 and 3.0 classifications were compared to the 16S rRNA gene sequencing-based results, the genus-level consistency was found to be 95% (37/39) and 100% (39/39), respectively, while the species-level consistency was found to be 85% (29/34) and 94% (32/34), respectively (Table 3). In addition to the close agreement between the MALDI Biotyper and 16S rRNA gene sequencing-based results, another interesting observation was the fact that even when the MALDI Biotyper reported classification results with low confidence (e.g. score values below 2.0, i.e. probable genus-level classification), the results were in most cases consistent with the 16S rRNA gene sequencing-based results at the species level (Table 3.3).

Table 3.3 Comparison of MALDI Biotyper (MALDI-TOF MS) and Ribosomal Database Project (16S rRNA gene sequencing) classification results for a selection of airborne bacterial isolates from the Muzeum subway station.

Isolate	Ribosomal Database Project (Classifier and SeqMatch)[1]				MALDI Biotyper 2.0[2]				MALDI Biotyper 3.0[3]			
	Classification	Score	Taxonomic level[4]		Classification	Score	Consistency[5]		Classification	Score	Consistency[5]	
			Genus	Species			Genus	Species			Genus	Species
09/39	*Bacillus pumilus*; DSMZ27; AY456263	1.00	X	X	*Bacillus pumilus*	1.9	X	X	*Bacillus pumilus*	2.1	X	X
09/48	*Pseudomonas stutzeri*; ATCC 17598; AJ006104	1.00	X	X	*Pseudomonas stutzeri*	2.1	X	X	*Pseudomonas stutzeri*	2.2	X	X
09/05	*Bacillus pumilus*; OM-F6; AB020208	0.99	X	X	*Bacillus pumilus*	1.9	X	X	*Bacillus pumilus*	2.1	X	X
09/07	*Solibacillus* sp. R-26868; AM910175	1.00	X		*Solibacillus silvestris*	2.1	X	ND	*Solibacillus silvestris*	2.0	X	ND
09/18	*Bacillus firmus*; D1; AJ491843	1.00	X	X	*Bacillus firmus*	1.9	X	X	*Bacillus firmus*	1.9	X	X
09/24	*Pseudomonas stutzeri*; ATCC 17598; AJ006104	1.00	X	X	*Pseudomonas stutzeri*	2.3	X	X	*Pseudomonas stutzeri*	2.3	X	X
09/28	*Staphylococcus hominis*; L37601	1.00	X	X	*Staphylococcus hominis*	2.1	X	X	*Staphylococcus hominis*	2.1	X	X
22/08	*Bacillus subtilis*; IAM 12118T; AB042061	0.99	X	X	*Bacillus subtilis ssp subtilis*	2.1	X	X	*Bacillus subtilis*	2.1	X	X
22/09	*Bacillus pseudomycoides*; DSM 12442; AM747226	0.99	X	X	*Bacillus pseudomycoides*	1.8	X	X	*Bacillus pseudomycoides*	1.8	X	X
22/10	*Corynebacterium* sp.; nbw965c12c1; GQ041375	1.00	X		*Corynebacterium minutissimum*	1.7	X	ND	*Corynebacterium minutissimum*	1.7	X	ND
22/19	*Bacillus firmus*; D1; AJ491843	0.98	X	X	*Bacillus firmus*	1.7	X	X	*Bacillus firmus*	1.7	X	X
22/21	*Micrococcus luteus*; Ballarat; AJ409096	1.00	X	X	*Micrococcus luteus*	1.9	X	X	*Micrococcus luteus*	1.9	X	X
22/23	*Staphylococcus haemolyticus*; ATCC 29970; D83367	0.99	X	X	*Staphylococcus haemolyticus*	1.8	X	X	*Staphylococcus haemolyticus*	1.8	X	X
22/27	*Staphylococcus haemolyticus*; ES-13a2; FN393797	0.99	X	X	*Staphylococcus haemolyticus*	1.9	X	X	*Staphylococcus haemolyticus*	2.0	X	X
22/33	*Corynebacterium mucifaciens*; 97-0160; AF537601	1.00	X	X	*Corynebacterium mucifaciens*	1.8	X	X	*Corynebacterium mucifaciens*	1.9	X	X
22/34	*Bacillus pumilus*; EI-25-8; AJ494728	1.00	X	X	*Bacillus pseudofirmus*	2.1	X		*Bacillus pumilus*	2.0	X	X
22/35	*Bacillus pseudomycoides*; DSM 12442; AM747226	1.00	X	X	*Bacillus pseudomycoides*	1.9	X	X	*Bacillus pseudomycoides*	1.9	X	X
22/38	*Paenibacillus amylolyticus*; JCM 9906; AB073190	0.99	X	X	*Paenibacillus amylolyticus*	2.3	X	X	*Paenibacillus amylolyticus*	2.3	X	X
22/44	*Bacillus simplex* (T); DSM 1321T; AJ439078	0.99	X	X	*Bacillus muralis*	1.8	X		*Bacillus muralis*	1.8	X	
22/46	*Bacillus cereus*; ATCC53522; AF290551	1.00	X	X	*Bacillus cereus (sensu lato)*	1.7	X	X	*Bacillus cereus (sensu lato)*	1.7	X	X
22/47	*Staphylococcus epidermidis*; GH 654; X75944	1.00	X	X	*Staphylococcus epidermidis*	1.9	X	X	*Staphylococcus epidermidis*	1.9	X	X
22/67	*Bacillus pumilus*; DSMZ27; AY456263	0.99	X	X	*Anaerococcus* sp.	2.0			*Bacillus pumilus*	2.0	X	X
22/69	*Staphylococcus hominis* (T); DSM 20328; X66101	1.00	X	X	*Staphylococcus hominis*	2.0	X	X	*Staphylococcus hominis*	2.0	X	X
22/72	*Kocuria rosea*; ATCC 187T; Y11330	1.00	X	X	*Kocuria polaris*	2.0	X		*Kocuria polaris*	2.0	X	
28/11	*Staphylococcus* sp.; JPL-S2_E19; FJ957503	0.98	X		*Staphylococcus epidermidis*	1.9	X	ND	*Staphylococcus epidermidis*	1.9	X	ND
28/12	*Staphylococcus* sp.; BF0002D116; AM697605	0.92	X		*Staphylococcus haemolyticus*	1.7	X	ND	*Staphylococcus capitis*	1.7	X	ND
28/15	*Acinetobacter lwoffii*; BA49; FJ263923	0.94	X	X	*Acinetobacter lwoffii*	1.9	X	X	*Acinetobacter lwoffii*	1.9	X	X
28/16	*Bacillus licheniformis*; R-13585; AJ582722	0.98	X	X	*Bacillus licheniformis*	2.0	X	X	*Bacillus licheniformis*	2.0	X	X
28/17	*Micrococcus luteus* (T); DSM 20030; AJ536198	0.95	X	X	*Micrococcus luteus*	1.9	X	X	*Micrococcus luteus*	1.9	X	X
28/18	*Bacillus pumilus*; DSMZ27; AY456263	0.99	X	X	*Anaerococcus* sp.	1.9			*Bacillus pumilus*	1.9	X	X
28/19	*Micrococcus luteus*; HN2-11; AF057289	0.98	X	X	*Micrococcus luteus*	1.8	X	X	*Micrococcus luteus*	2.0	X	X
28/2	*Micrococcus luteus*; Ballarat; AJ409096	0.98	X	X	*Micrococcus luteus*	2.2	X	X	*Micrococcus luteus*	2.2	X	X
28/25	*Micrococcus luteus* (T); DSM 20030; AJ536198	0.97	X	X	*Micrococcus luteus*	1.9	X	X	*Micrococcus luteus*	1.9	X	X
28/26	*Acinetobacter* sp.; nbw1185f06c1; GQ080234	0.96	X		*Acinetobacter lwoffii*	2.2	X	ND	*Acinetobacter lwoffii*	2.2	X	ND
28/27	*Micrococcus luteus*; Ballarat; AJ409096	0.98	X	X	*Micrococcus luteus*	1.9	X	X	*Micrococcus luteus*	1.9	X	X
28/28	*Micrococcus luteus*; Ballarat; AJ409096	0.96	X	X	*Micrococcus luteus*	1.9	X	X	*Micrococcus luteus*	2.1	X	X
28/29	*Micrococcus luteus*; Ballarat; AJ409096	0.95	X	X	*Micrococcus luteus*	1.9	X	X	*Micrococcus luteus*	1.9	X	X
28/5	*Micrococcus luteus* (T); DSM 20030; AJ536198	0.98	X	X	*Micrococcus luteus*	1.9	X	X	*Micrococcus luteus*	2.0	X	X
28/6	*Micrococcus luteus*; Ballarat; AJ409096	0.98	X	X	*Micrococcus luteus*	2.0	X	X	*Micrococcus luteus*	2.1	X	X
Total			**100% (39/39)**	**87% (34/39)**			**95% (37/39)**	**85% (29/34)**			**100% (39/39)**	**94% (32/34)**

ND; not determined

[1] Ribosomal Database Project (RDP), Classifier and SeqMatch tools, release 10, update 30, http://rdp.cme.msu.edu/.

[2] MALDI Biotyper 2.0 (Bruker Daltonics, Bremen, Germany) with Bruker Taxonomy database v3.1.1.0 (3,740 library entries) and security-relevant add-on database.

[3] MALDI Biotyper 3.0 (Bruker Daltonics) with Bruker Taxonomy database v3.3.1.0 (4,613 library entries) and security-relevant add-on database.

[4] Taxonomic level (genus±species) observed among the top-ranked RDP SeqMatch database hits.

[5] Consistency (genus±species) observed between the MALDI Biotyper and the RDP SeqMatch classification results. Species-level consistency could not be determined if RDP SeqMatch reported genus-level classifications only.

The airborne bacterial diversity observed at the Muzeum station was compared with previous results from the Nationaltheatret station [7-9]. The results showed that the MALDI Biotyper 3.0 was able to classify a similar fraction of the analyzed isolates to the genus level at both stations (Muzeum: 69% [126/182] and Nationaltheatret: 71% [1,293/1,832]). Conversely however the results revealed that the MALDI Biotyper was not able to reliably classify ~30% of the airborne bacterial isolates recovered from either station. All the bacterial genera observed at the Muzeum station had almost exclusively also been encountered at the Nationaltheatret station (Table 4). Several bacterial genera (e.g. *Micrococcus*, *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Kocuria* and *Paenibacillus*) were consistently observed at both stations irrespectively of the air sampling procedure, growth medium formulation and incubation temperature, while others (e.g. *Rhodococcus*, *Acinetobacter*, *Arthrobacter*, *Dermacoccus*, *Corynebacterium*, *Microbacterium*, *Moraxella* and *Streptomyces*) were observed less consistently although sometimes at a high abundance (Table 3.4).

Table 3.4 Comparison of the diversity of airborne bacteria at the Muzeum subway station (Prague, Czech Republic, current study) and the Nationaltheatret subway station (Oslo, Norway, published data[9]).

Genus	Muzeum subway station, Prague, Czech Republic		Nationaltheatret subway station, Oslo, Norway[1]				Nationaltheatret subway station, Oslo, Norway[2]			
	Sampler: SpinAir impactor Growth medium: Columbia agar Incubation time/temp: 48 h / 37°C		Sampler: SASS 3100 electret filter Growth medium: Trypicase soy agar Incubation time/temp: 48 h / 30°C		Sampler: SASS 3100 electret filter Growth medium: Reasoner's 2a agar Incubation time/temp: 48 h / 30°C		Sampler: Andersen impactor Growth medium: Reasoner's 2a agar Incubation time/temp: 48 h / 30°C		Sampler: SASS 3100 electret filter Growth medium: Reasoner's 2a agar Incubation time/temp: 48 h / 30°C	
	%	N	%	N	%	N	%	N	%	N
Micrococcus	40	51	42	52	26	29	33	371	31	39
Bacillus	23	29	11	14	19	21	3	38	12	15
Staphylococcus	17	21	23	29	17	19	5	55	14	18
Pseudomonas	4	5	6	8	2	2	4	40	2	2
Kocuria	2	3	<1	1	3	3	4	46	4	5
Paenibacillus	<1	1	2	2	2	2	<1	2	<1	1
Streptomyces			2	2	5	6	<1	4	2	2
Acinetobacter	6	7					1	17	<1	1
Corynebacterium	3	4	2	2			<1	2		
Microbacterium			4	5	3	3	<1	9		
Dermacoccus					5	5	4	49	5	6
Rhodococcus					4	4	18	202	22	28
Arthrobacter					3	3	13	144	3	4
Lysinibacillus					<1	1	<1	2	<1	1
Exiguobacterium	2	2			<1	1				
Solibacillus	2	2								
Brevundimonas			2	2			1	13		
Pantoea			<1	1	<1	1				
Paracoccus					3	3	<1	3		
Dietzia					<1	1	<1	6		
Roseomonas					<1	1	<1	8		
Rothia					<1	1	<1	1		
Moraxella							8	94	<1	1
Massilia							<1	5	<1	1
Kytococcus	<1	1								
Planococcus			2	2						
Erwinia			<1	1						
Serratia			<1	1						
Weissella			<1	1						
Enhydrobacter			<1	1						
Comamonas					<1	1				
Gordonia					<1	1				
Janibacter					<1	1				
Paenisporosarcina					<1	1				
Agrococcus							<1	6		
Aerococcus							<1	5		
Sphingomonas							<1	5		
Brevibacterium							<1	2		
Brevibacillus							<1	1		
Chryseobacterium							<1	1		
Enterococcus							<1	1		
Macrococcus							<1	1		
Ochrobactrum							<1	1		
Pseudoclavibacter							<1	1		
Psychrobacillus							<1	1		
Rhizobium							<1	1		
Sphingobacterium							<1	1		
Sphingobium							<1	1		
Stenotrophomonas							<1	1		
Streptococcus							<1	1		
Agromyces									<1	1
Curtobacterium									<1	1
Total		126		124		110		1141		126

[1] Dybwad, M., P. E. Granum, P. Bruheim, and J. M. Blatny. 2012. Characterization of airborne bacteria at an underground subway station. Applied and environmental microbiology 78:1917-1929.

[2] Dybwad, M., G. Skogan, and J. M. Blatny. 2014. Temporal Variability of the Bioaerosol Background at a Subway Station: Concentration Level, Size Distribution, and Diversity of Airborne Bacteria. Applied and environmental microbiology 80:257-270.

3.3 Total aerosol level and size distribution

The concentration level and size distribution of total aerosols was monitored at the Muzeum station (Table 5). The average total aerosol level (0.5 to 20 μm) at the station was $6.5x10^7 \pm 1.7x10^7$ particles m^{-3} with CMAD of 0.79±0.05 μm and GSD of 1.57±0.07. To investigate the temporal correlation between the total aerosol level and the airborne bacterial level from morning to midnight at the Muzeum station and the Nationaltheatret station [7-9], three different particle size ranges were extracted from the measured range (0.5 to 20 μm), as follows: (i) particles of ≥0.5 μm (i.e. total), (ii) particles of ≥1 μm and (iii) particles of ≥3 μm (Table 3.5). At the Muzeum station, the total aerosol levels for the different size ranges showed significant temporal correlation with each other (r = 0.821-0.935), and with the airborne bacterial level (r = 0.768-0.921) (Figure 3.3). These results were in contrast to the results from the Nationaltheatret station where the total aerosol levels for the same size ranges showed significant temporal correlation with each other (r = 0.677-0.943), but not with the airborne bacterial level (r = 0.03-0.05) (Figure 3.3). The average total aerosol level (0.5-20 μm) at the Muzeum station was significantly higher (~2-fold) than the level observed at the Nationaltheatret station, while the size distribution was not significantly different at the two stations (Table 3.5 and Figure 3.3). The average total aerosol level between 1-20 μm at the Muzeum station was similarly found to be significantly higher (~2-fold) than the level observed at the Nationaltheatret station, while the average level between 3-20 μm was not significantly different at the two stations (Table 3.5 and Figure 3.3).

Table 3.5 Concentration level of total aerosols in different size ranges (0.5-20, 1-20 and 3-20 µm) at the Muzeum subway station (Prague, Czech Republic, current study) and the Nationaltheatret subway station (Oslo, Norway, published data[9]).

Time (hh:mm)	Total aerosol level (particles m^{-3})									
	Muzeum subway station, Prague, Czech Republic					Nationaltheatret subway station, Oslo, Norway[2]				
	0.5-20 µm	1.0-20 µm	3.0-20 µm	CMAD (µm)[1]	GSD[1]	0.5-20 µm	1.0-20 µm	3.0-20 µm	CMAD (µm)[1]	GSD[1]
07:00 - 07:05	$9.0 \times 10^{7} \pm 4.3 \times 10^{6}$	$3.1 \times 10^{7} \pm 3.2 \times 10^{6}$	$1.4 \times 10^{6} \pm 2.8 \times 10^{5}$	0.86 ± 0.01	1.55 ± 0.02	$3.2 \times 10^{7} \pm 8.0 \times 10^{6}$	$1.0 \times 10^{7} \pm 3.4 \times 10^{6}$	$1.2 \times 10^{6} \pm 2.5 \times 10^{5}$	0.80 ± 0.03	1.69 ± 0.02
08:00 - 08:05	$9.9 \times 10^{7} \pm 2.9 \times 10^{6}$	$3.9 \times 10^{7} \pm 2.4 \times 10^{6}$	$1.7 \times 10^{6} \pm 2.1 \times 10^{5}$	0.89 ± 0.01	1.57 ± 0.01	$3.0 \times 10^{7} \pm 5.3 \times 10^{6}$	$7.4 \times 10^{6} \pm 1.8 \times 10^{6}$	$8.7 \times 10^{5} \pm 1.4 \times 10^{5}$	0.76 ± 0.02	1.63 ± 0.05
09:00 - 09:05	$8.3 \times 10^{7} \pm 2.6 \times 10^{6}$	$3.4 \times 10^{7} \pm 2.1 \times 10^{6}$	$1.6 \times 10^{6} \pm 3.5 \times 10^{5}$	0.90 ± 0.02	1.61 ± 0.02	$3.7 \times 10^{7} \pm 5.7 \times 10^{6}$	$1.1 \times 10^{7} \pm 4.4 \times 10^{6}$	$1.3 \times 10^{6} \pm 5.4 \times 10^{5}$	0.80 ± 0.07	1.63 ± 0.06
10:00 - 10:05	$5.5 \times 10^{7} \pm 2.3 \times 10^{6}$	$2.1 \times 10^{7} \pm 1.4 \times 10^{6}$	$1.2 \times 10^{5} \pm 1.7 \times 10^{5}$	0.87 ± 0.01	1.61 ± 0.01	$3.5 \times 10^{7} \pm 5.7 \times 10^{6}$	$9.6 \times 10^{6} \pm 3.6 \times 10^{6}$	$1.1 \times 10^{6} \pm 3.1 \times 10^{5}$	0.78 ± 0.05	1.63 ± 0.03
11:00 - 11:05	$4.2 \times 10^{7} \pm 2.4 \times 10^{6}$	$1.5 \times 10^{7} \pm 1.5 \times 10^{6}$	$9.0 \times 10^{5} \pm 1.4 \times 10^{5}$	0.82 ± 0.02	1.63 ± 0.02	$3.8 \times 10^{7} \pm 5.5 \times 10^{6}$	$1.0 \times 10^{7} \pm 2.8 \times 10^{6}$	$1.2 \times 10^{6} \pm 3.3 \times 10^{5}$	0.77 ± 0.03	1.63 ± 0.04
12:00 - 12:05	$7.6 \times 10^{7} \pm 4.1 \times 10^{6}$	$1.4 \times 10^{7} \pm 1.5 \times 10^{6}$	$8.8 \times 10^{5} \pm 1.3 \times 10^{5}$	0.73 ± 0.02	1.50 ± 0.02	$4.0 \times 10^{7} \pm 7.1 \times 10^{6}$	$1.3 \times 10^{7} \pm 4.5 \times 10^{6}$	$1.6 \times 10^{6} \pm 6.3 \times 10^{5}$	0.81 ± 0.06	1.67 ± 0.05
13:00 - 13:05	$6.3 \times 10^{7} \pm 1.3 \times 10^{7}$	$1.7 \times 10^{7} \pm 1.5 \times 10^{6}$	$1.1 \times 10^{6} \pm 1.6 \times 10^{5}$	0.77 ± 0.02	1.59 ± 0.06	$4.5 \times 10^{7} \pm 7.3 \times 10^{6}$	$1.5 \times 10^{7} \pm 4.7 \times 10^{6}$	$1.7 \times 10^{6} \pm 4.3 \times 10^{5}$	0.84 ± 0.06	1.68 ± 0.02
14:00 - 14:05	$6.2 \times 10^{7} \pm 5.8 \times 10^{6}$	$1.8 \times 10^{7} \pm 1.1 \times 10^{6}$	$1.2 \times 10^{6} \pm 2.3 \times 10^{5}$	0.78 ± 0.02	1.59 ± 0.01	$4.2 \times 10^{7} \pm 6.7 \times 10^{6}$	$1.2 \times 10^{7} \pm 3.9 \times 10^{6}$	$1.3 \times 10^{6} \pm 4.7 \times 10^{5}$	0.78 ± 0.03	1.63 ± 0.04
15:00 - 15:05	$6.5 \times 10^{7} \pm 7.2 \times 10^{6}$	$1.9 \times 10^{7} \pm 2.6 \times 10^{6}$	$1.2 \times 10^{6} \pm 3.1 \times 10^{5}$	0.78 ± 0.01	1.59 ± 0.03	$3.9 \times 10^{7} \pm 8.8 \times 10^{6}$	$1.1 \times 10^{7} \pm 6.1 \times 10^{6}$	$1.3 \times 10^{6} \pm 7.8 \times 10^{5}$	0.78 ± 0.06	1.61 ± 0.08
16:00 - 16:05	$6.1 \times 10^{7} \pm 1.4 \times 10^{7}$	$2.3 \times 10^{7} \pm 4.7 \times 10^{6}$	$1.4 \times 10^{6} \pm 4.6 \times 10^{5}$	0.83 ± 0.02	1.64 ± 0.02	$4.2 \times 10^{7} \pm 6.3 \times 10^{6}$	$9.9 \times 10^{6} \pm 4.5 \times 10^{6}$	$1.1 \times 10^{6} \pm 5.9 \times 10^{5}$	0.75 ± 0.04	1.58 ± 0.07
17:00 - 17:05	$7.0 \times 10^{7} \pm 8.4 \times 10^{6}$	$2.4 \times 10^{7} \pm 3.8 \times 10^{6}$	$1.5 \times 10^{6} \pm 3.8 \times 10^{5}$	0.81 ± 0.02	1.62 ± 0.03	$4.6 \times 10^{7} \pm 1.2 \times 10^{7}$	$1.1 \times 10^{7} \pm 7.0 \times 10^{6}$	$1.2 \times 10^{6} \pm 6.9 \times 10^{5}$	0.76 ± 0.06	1.57 ± 0.08
18:00 - 18:05	$7.7 \times 10^{7} \pm 4.0 \times 10^{6}$	$2.4 \times 10^{7} \pm 6.0 \times 10^{6}$	$1.4 \times 10^{6} \pm 5.6 \times 10^{5}$	0.80 ± 0.03	1.58 ± 0.07	$4.6 \times 10^{7} \pm 1.2 \times 10^{6}$	$1.4 \times 10^{7} \pm 5.5 \times 10^{6}$	$1.4 \times 10^{6} \pm 5.2 \times 10^{5}$	0.80 ± 0.06	1.65 ± 0.04
19:00 - 19:05	$7.5 \times 10^{7} \pm 3.0 \times 10^{6}$	$2.0 \times 10^{7} \pm 4.6 \times 10^{6}$	$1.3 \times 10^{6} \pm 5.0 \times 10^{5}$	0.78 ± 0.02	1.56 ± 0.08	$4.5 \times 10^{7} \pm 8.0 \times 10^{6}$	$1.1 \times 10^{7} \pm 5.6 \times 10^{6}$	$1.1 \times 10^{6} \pm 6.0 \times 10^{5}$	0.76 ± 0.06	1.58 ± 0.09
20:00 - 20:05	$6.3 \times 10^{7} \pm 2.6 \times 10^{6}$	$1.6 \times 10^{7} \pm 3.0 \times 10^{6}$	$1.0 \times 10^{6} \pm 2.9 \times 10^{5}$	0.77 ± 0.01	1.55 ± 0.05	$4.0 \times 10^{7} \pm 6.2 \times 10^{6}$	$1.1 \times 10^{7} \pm 4.6 \times 10^{6}$	$1.2 \times 10^{6} \pm 5.6 \times 10^{5}$	0.78 ± 0.07	1.62 ± 0.08
21:00 - 21:05	$6.3 \times 10^{7} \pm 1.1 \times 10^{7}$	$1.3 \times 10^{7} \pm 1.9 \times 10^{6}$	$8.1 \times 10^{5} \pm 1.7 \times 10^{5}$	0.76 ± 0.01	1.51 ± 0.06	$4.6 \times 10^{7} \pm 1.0 \times 10^{7}$	$1.3 \times 10^{7} \pm 8.8 \times 10^{6}$	$1.3 \times 10^{6} \pm 8.8 \times 10^{5}$	0.81 ± 0.13	1.59 ± 0.11
22:00 - 22:05	$4.5 \times 10^{7} \pm 1.2 \times 10^{6}$	$6.8 \times 10^{6} \pm 1.1 \times 10^{6}$	$4.4 \times 10^{5} \pm 9.0 \times 10^{4}$	0.73 ± 0.01	1.44 ± 0.03	$3.6 \times 10^{7} \pm 5.4 \times 10^{6}$	$9.0 \times 10^{6} \pm 4.9 \times 10^{6}$	$1.1 \times 10^{6} \pm 6.4 \times 10^{5}$	0.78 ± 0.08	1.60 ± 0.11
23:00 - 23:05	$3.2 \times 10^{7} \pm 2.5 \times 10^{6}$	$5.4 \times 10^{6} \pm 1.4 \times 10^{6}$	$3.6 \times 10^{5} \pm 1.3 \times 10^{5}$	0.72 ± 0.01	1.46 ± 0.05	$3.2 \times 10^{7} \pm 7.8 \times 10^{6}$	$7.2 \times 10^{6} \pm 3.3 \times 10^{6}$	$9.5 \times 10^{5} \pm 4.6 \times 10^{5}$	0.74 ± 0.04	1.61 ± 0.09
00:00 - 00:05	$3.2 \times 10^{7} \pm 1.6 \times 10^{6}$	$8.7 \times 10^{6} \pm 1.0 \times 10^{6}$	$6.4 \times 10^{5} \pm 1.1 \times 10^{5}$	0.76 ± 0.01	1.60 ± 0.02	$2.3 \times 10^{7} \pm 4.5 \times 10^{6}$	$5.6 \times 10^{6} \pm 2.4 \times 10^{6}$	$7.6 \times 10^{5} \pm 3.2 \times 10^{5}$	0.75 ± 0.04	1.62 ± 0.08

CMAD; count median aerodynamic diameter, GSD; geometric standard deviation

[1] CMAD and GSD for total aerosols between 0.5-20 µm

[2] Dybwad, M., G. Skogan, and J. M. Blatny. 2014. Temporal Variability of the Bioaerosol Background at a Subway Station: Concentration Level, Size Distribution, and Diversity of Airborne Bacteria. Applied and environmental microbiology 80:257-270.

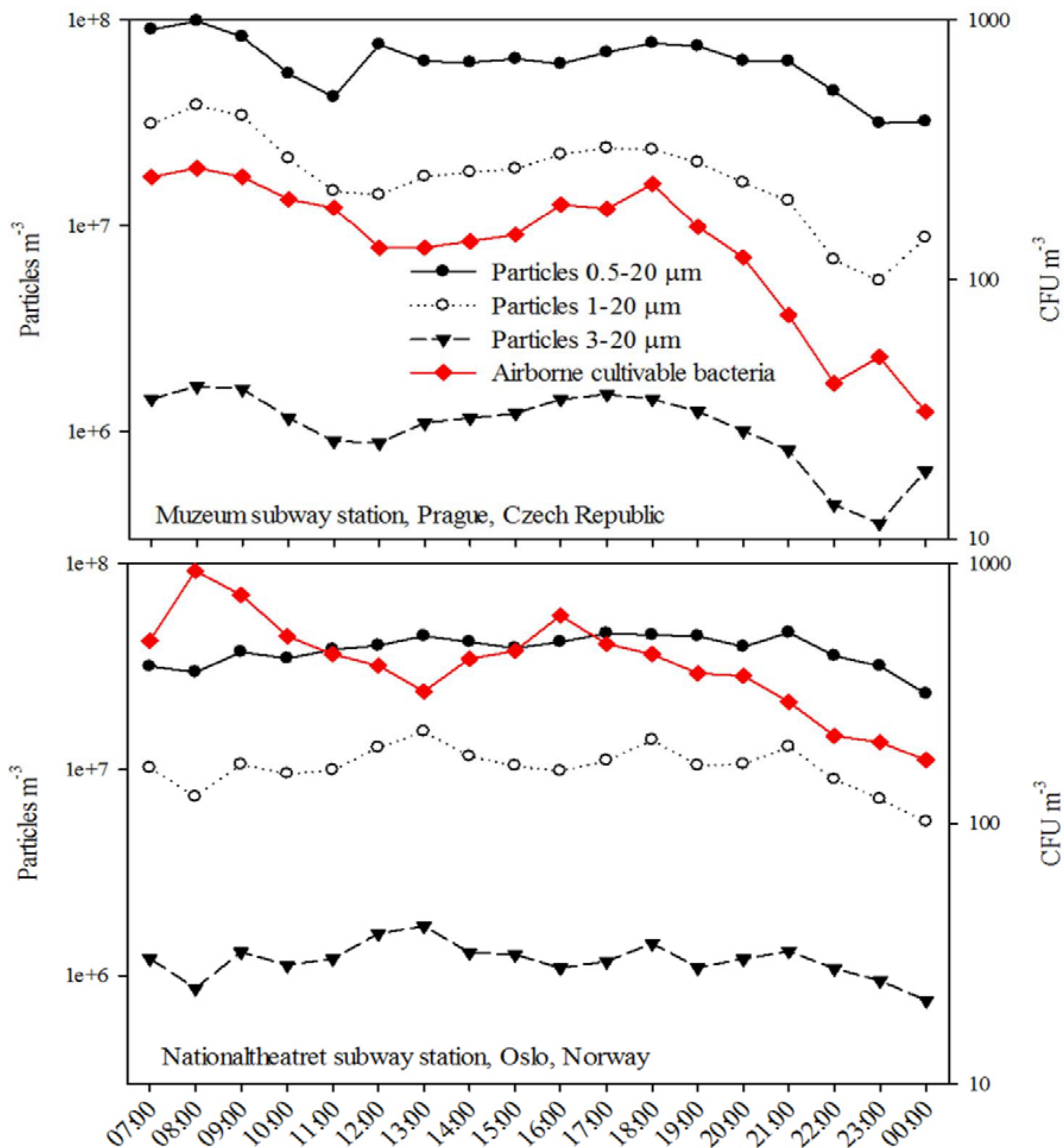

Figure 3.3 Concentration level of total aerosols in different size ranges (0.5-20, 1-20 and 3-20 µm) and airborne bacteria at the Muzeum subway station (Prague, Czech Republic, upper panel, current study) and the Nationaltheatret subway station (Oslo, Norway, lower panel, published data[9]). X-axis: Time of day (hh:mm). Left y-axis: Total aerosol level expressed as particles per cubic meter of air (particles m^{-3}). Right y-axis: Airborne bacterial level expressed as colony forming units per cubic meter of air (CFU m^{-3}).

3.4 Meteorological conditions

The temperature and relative humidity was monitored at the Muzeum station (Table 3.1). The average temperature and relative humidity at the station was 13.9±0.9 °C and 57.1±3.8 %, respectively (Table 3.1). The meteorological conditions at the station did not show substantial variation during the sampling campaign and no attempt was therefore made to investigate temporal correlation between the meteorological conditions and the airborne bacterial or total aerosol level. Compared to the Nationaltheatret station (7.5±0.4 °C and 63±5.4 %), the temperature and relative humidity at the Muzeum station was found to be significantly higher and lower, respectively (Table 1). During the sampling campaign at the Muzeum station (28 February and 13 March 2012), the average outdoor temperature and relative humidity was ~5 °C and ~82%, respectively (data not shown), and which was found to be significantly higher and not significantly different, respectively, from the outdoor temperature (~ -7 °C) and outdoor relative humidity (~80%) observed at the Nationaltheatret station (14-17 February 2011) [9].

4 Discussion

The current study represents the first published account to describe the airborne bacterial background at a subway station in the Czech Republic. While the primary objective of this work was to characterize the airborne bacterial background at the Czech station, it also served as a follow-up investigation to confirm and extrapolate results from similar investigations performed at a Norwegian subway station [7-9]. Therefore, in addition to obtain information about the airborne bacterial background at a Czech station, the study also contributed to knowledge generation concerning the variability of the airborne bacterial background between subway stations located in different European countries. The results from the Czech station have throughout this account been described in the direct context of, and related to, those from the Norwegian station. The findings from the Czech station that which could be considered to already have been sufficiently discussed in the publications from the Norwegian station [7-9] will therefore not be recapped here, and the discussion will instead be focused on similarities and differences observed between the two stations.

The concentration level of airborne bacteria at the Czech station was well within the range observed at the Norwegian station. The average level at the Czech station was however lower than in Norway. Nevertheless, the temporal concentration profile from morning to midnight was shown to be consistent between the two stations and found to be characterized by increased levels during rush hours compared to non-rush hours and decaying evening levels. The estimated airborne bacterial level at the Czech station increased when the length of the plate incubation period was extended from 24 to 48 h. While these results were expected, they may still serve as a reminder about the potential impact the choice of cultivation conditions may have on the results obtained in cultivation-based investigations of airborne bacteria. It should however be noted that the offset between the 24- and 48-h results remained stable throughout the study, suggesting that cultivation bias originating from differences in incubation time at least will be constant within the same subway environment. An in-depth investigation of the intra-station spatial and day-to-day temporal variability was outside the scope of this study due to the limited number of sampling locations (one site at the A platform and two sites at the C platform) and sampling events (28 February and 13 March 2012) at the Czech station. The consistency observed between the airborne bacterial level on different days as well as different locations inside the station may however still be taken to suggest that the airborne bacterial background at the Czech station will not be subject to substantial intra-station spatial or day-to-day temporal variability. The limited day-to-day temporal variability observed at the Czech station was in agreement with previous observations from Norway.

The airborne bacterial diversity at the Czech station was dominated by the genera *Micrococcus*, *Bacillus* and *Staphylococcus,* and which was consistent with previous results from the Norwegian station. Some differences were however observed between the two stations, with the most significant difference being the absence of certain bacterial genera at the Czech station which occasionally had been abundantly observed at the Norwegian station (e.g. *Rhodococcus*, *Arthrobacter* and *Dermacoccus*). However, a previous comparison of growth media formulations (Reasoner's 2A agar [R2A] and Trypticase soy agar [TSA]) at the Norwegian station showed that *Rhodococcus* and *Dermacoccus* could only be recovered on R2A agar [8]. In further support of this explanation, the Colombia agar-derived bacterial diversity observed at the Czech station showed a closer agreement with the TSA-derived diversity observed at the Norwegian station which did not include *Rhodococcus, Arthrobacter* or *Dermacoccus*, than the R2A-derived diversity which did. However, since the work performed at the Czech station did not include R2A it was not possible to make any definitive conclusions concerning whether the observed differences reflected true diversity differences between the two stations or whether they could be attributed to cultivation bias. Nevertheless, nearly all the bacterial genera that were observed at the Czech station had also been encountered at the Norwegian station. Thus, it is reason to believe that similar results are to be found at other subway stations.

A limitation of this study was its reliance on cultivation-based analysis methods. However, almost all the currently available information regarding concentration levels and diversity of airborne bacteria at subway stations are derived from cultivation-based investigations. In addition to the inherent limitations associated with cultivation-based methods, it should be noted that the use of different cultivation procedures may have impacted the results from the Czech and Norwegian station. Cultivation-dependent methods are inherently able to investigate only a subset of the total airborne bacterial background, which in addition to cultivable bacteria includes a complex assemblage of non-cultivable and dead bacteria [1]. It is therefore warranted to suggest that cultivation-independent analysis methods which are able to investigate the concentration level and diversity of total airborne bacteria should be used to characterize the airborne bacterial background at subway stations in future studies. Molecular analysis methods including quantitative polymerase chain reaction (qPCR) and high-throughput sequencing (HTS) have become an integral part of environmental microbiology studies, and the use of HTS-based metagenomic approaches to investigate the airborne bacterial background at subway stations have recently been demonstrated [13, 15]. Nevertheless, since many questions still remain unanswered regarding the cultivable airborne bacterial background at subway stations, in combination with the limited availability of information concerning the total airborne bacterial background, it

may be proposed that future investigations should strive to employ cultivation-based and -independent methods in parallel. Such parallel investigations may contribute to bridge the gap between past (cultivation-based) and future (cultivation-independent) airborne bacterial background results.

An important follow-up aspect of this study in light of previous findings from Norway involved the use of the MALDI Biotyper to investigate the airborne bacterial diversity at the Czech station. The results from the Czech station supported the previous conclusions from Norway concerning the applicability of the MALDI Biotyper as a rapid, cost-effective classification tool for airborne bacterial isolates. However, the results from the Czech station also supported the conclusions from Norway regarding that the performance of the MALDI Biotyper will not be optimal until the coverage of relevant environmental bacterial taxa in the reference library has been increased. Although the MALDI Biotyper has been shown to offer favorable performance in terms of speed, cost and throughput compared to other classification methods [2, 8, 10, 16], the observed issues regarding the inability of the MALDI Biotyper to cover the complete diversity of airborne bacterial isolates may reduce the benefits associated with its use. These issues have previously been attributed to an insufficient coverage of environmental bacterial taxa in the Bruker Taxonomy reference library [8] and efforts are currently underway at the Norwegian Defence Research Establishment FFI to extend the reference library to also include the part of the airborne bacterial diversity observed at the Norwegian station that could not be classified with the MALDI Biotyper [8, 9]. Such efforts can be hoped to increase even further the already existing benefits associated with the use of the MALDI Biotyper for airborne bacterial diversity investigations, at subway stations, and potentially also in other environments.

By increasing our knowledge about the airborne bacterial background this study may contribute to the development of BioDIM systems that are able to operate more reliably in subway station environments, as well as T&E paradigms that are capable of predicting more realistically the performance which can be expected in subway station environments. Although this study contributed to increase our knowledge about the spatiotemporal variability of the airborne bacterial background at subway stations, several questions still however remain unanswered. Spatiotemporal variability measures on several different dimensional scales both in terms of range and resolution could as an example provide important information about background characteristics of relevance for the performance of BioDIM systems in subway environments. It may therefore be proposed that future investigations should strive to determine, within the limitations of what is currently practically and technologically achievable, both the spatial (e.g. intra-station, inter-station, national, and international) and temporal (e.g. near-real time, diurnal, day-to-day, seasonal, and year-to-year) variability of the airborne bacterial

background at subway stations on all dimensional scales which can be considered relevant for the performance of BioDIM systems. While this study specifically addressed the airborne bacterial background, it is important to keep in mind that the bioaerosol background also will include other types of biological matter (e.g. fungi, viruses, pollen, and human and animal debris) and which could be just as relevant for the performance of BioDIM systems. Similarly, while this study focused on the airborne bacterial backgrounds at subway stations in relation to their potential impact on the performance of BioDIM systems, it should however be emphasized that the generated knowledge may also be relevant and useful within many other scientific disciplines including public health, occupational health as well as microbial ecology.

Taken together, the findings in this work concerning the temporal concentration profile of cultivable airborne bacteria from morning to midnight as well as the diversity of airborne bacteria at the Czech subway station, supported previous observations from the Norwegian station regarding that anthropogenic activities, and especially the presence of passengers in the station, appears to be a major source of airborne bacteria in subway environments. The results from the Czech and Norwegian station further suggested that the cultivable airborne bacterial background at subway stations in Norway and the Czech Republic appears to share several common features. Although these observations must be confirmed through follow-up investigations, both at the same as well as at several other stations, they may still be taken to suggest that the cultivable airborne bacterial background encountered at different European subway stations will not necessarily be very different from each other and may even share several common features.

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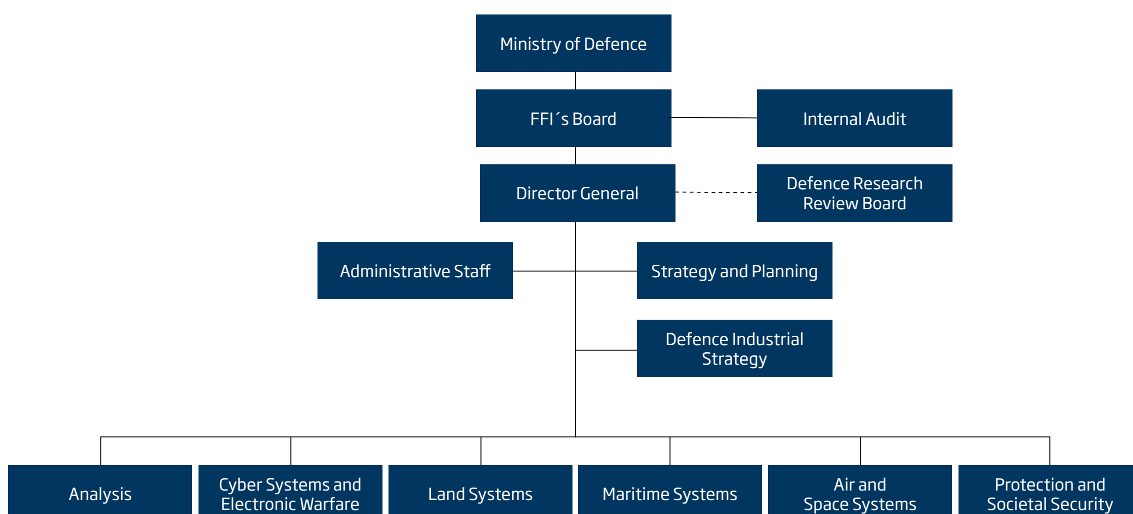

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