# MALDI Biotyper Characterization of Microorganisms Colonizing Heating Ventilation Air-Conditioning Systems at a South African Hospital

N. J. Malebo *and K. Shale

Central University of Technology, Free State, Department of Life Sciences, Private Bag X2053 Bloemfontein, 9300, South Africa; E-mail: nmalebo@cut.ac.za

**Abstract:** Airborne microbial contaminants can spread from heating ventilation air conditioning (HVAC) systems resulting in exposure to indoor surfaces and building occupants. Rapid identification of airborne contaminants is a necessity especially in healthcare settings. In the current study, samples were collected from a 5X5 surface area of ventilation grills at a public hospital using swabs. In general bacterial counts were above 12cfu/cm$^{-2}$ while fungal counts were below 5cfu/cm$^{-2}$, ranging from clean (<5) to very contaminated (>10). Microbial contaminants were identified and characterized using the MALDI-TOF MS and Scanning Electron Microscopy. Identified genera included *Bacillus*, *Arthrobacter*, *Rhodotorula* and *Penicillium*. Microorganisms identified in the current study using the MALDI TOF MS have no reported clinical implications; however, some are potential pathogens due to the presence of immune-compromised patients in these settings. The probable aerosolization of these microorganisms from ventilation systems could result in microorganisms settling on hospital surfaces and possible contamination of medical equipment. The current study also demonstrates the MALDI-TOF MS as a rapid, inexpensive and effective method for microbial characterization of microorganisms isolated from HVAC systems.
[N. J. Malebo and K. Shale. **MALDI-TOF MS Characterization of Microorganisms Colonizing Heating Ventilation Air-Conditioning Systems at a South African Hospital.** *Life Sci J* 2013;10(4):13-17] (ISSN:1097-8135). http://www.lifesciencesite.com. 54

**Keywords:** Bioaerosols, Heating Ventilation Air-Conditioning systems, Hospital Acquired Infections, MALDI Biotyper, Scanning Electron Microscopy

## 1. Introduction

The vital role that heating, ventilation and air-conditioning (HVAC) systems play in healthcare settings has long being recognized (Dascalaki et al., 2008; Balloco, 2011). An efficient HVAC system controls indoor air quality by ensuring safe and suitable thermal conditions for hospital staff and patients (Balloco, 2011). In indoor environments including hospitals, factors such as inadequate ventilation, chemical use in the building, outdoor pollutants, or growth of microorganisms inside the HVAC system can compromise air quality (Dascalaki et al., 2008; Schmidt et al., 2012). In most cases the exchange between indoor and outdoor air in hospitals is restricted and the key source of contamination could be indoor pollutants or the filtration system of the ventilation system (Yu et al., 2009).

In addition to air filters, heat exchanger coils and fins, condensate drain pans and air ducts are areas where contaminants mostly accumulate within HVAC systems. Bioaerosols have been reported to spread from these sites resulting in exposure to indoor surfaces and building occupants (Schmidt et al., 2012). Since hospitals are enclosed environments, bioaerosols in this environment can possibly place patients at greater risk than outside environments as aerosols can be confined and allowed to build up to infectious levels (Ekhaise, 2008) leading to possible hospital acquired infections (HAIs).

Hospital acquired infections are on the increase in South Africa and globally, often causing morbidity and mortality in patients receiving healthcare (Brink et al., 2006; Ryan et al. 2011). In developing countries HAIs can possibly place strain on limited financial resources allocated to health delivery. HAIs spread via through three main ways, contact spread, droplet spread and aerially. Infection control measures for contact and droplet spread such as hand washing are encouraged since these transmission routes are well understood. However, recent evidence suggests an increase of infectious disease (including methicillin resistant *Staphylococcus aureus*) contracted via the aerial route from particles possibly distributed through HVAC systems (Ryan et al. 2011; Thompson et al., 2011; Schmidt et al., 2012). Unfortunately, there is a lack of research on microbes colonizing HVAC systems in South African hospitals.

As a result of the abovementioned information, sampling of ventilation systems and characterization of isolated microorganisms thus becomes essential as they affect the indoor environment air quality (Dascalaki *et al*., 2008). Microbial identification using conventional phenotypic methods is often difficult, time-consuming and may sometimes be inconclusive. While the inability to differentiate between viable and non-viable cultures when using molecular

techniques can lead to an overestimation of microbial loads (van Veen et al., 2010; Mandal and Brandl, 2011). The MALDI-TOF MS has been described as a rapid and reliable method for identification of microorganisms in clinical settings when compared to other techniques. Therefore, the current study aims to quantify and characterize microbial contaminants on ventilation grills at a South African hospital using MALDI-TOF MS.

## 2. Material and Methods

*Sample collection*

Samples from ventilation grills were collected over a period of six months in 5 wards (wards A-E). A 5X5 cm area of the outlet of the ventilation system facing each ward was swabbed using sterile swabs. Following serial dilutions, samples were transferred to plate count agar (PCA) for bacterial isolation and potato dextrose agar (PDA) for fungal isolation. Plates were incubated for 48h at 37°C and 25°C for bacteria and fungi respectively. Subsequently, plates were examined for microbial growth and colonies were counted.

**Microbial characterization**

*Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS/MALDI Biotyper).*

Identification of bacterial and fungal colonies by MALDI Biotyper was performed on a Microflex LT instrument (Bruker Daltonics GmbH, Leipzig, Germany) with flex control (version 3.0) software (Bruker Daltonics). Pure bacterial and fungal colonies were prepared and analyzed by smearing a thin film of colonies directly on a MALDI steel target according to the standard direct transfer method recommended by the manufacturer. Subsequent to drying of the microbial colony smear, colonies were overlaid with 1µl of a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile/2.5% tri-fluor-acetic-acid was prepared according to the manufacturer's instructions (HCCA matrix solution) and left to air dry at room temperature. The manufacture's recommended extraction method was used for yeast and mold colonies. Subsequently, extracts were placed on the MALDI steel target plate and allowed to dry; extracts where overlaid with matrix solution and left to air dry before analysis. The MALDI steel plate was placed in the MicroFlex MALDI-TOF-MS instrument for analysis. The Biotyper 2.0 system was run in automatic classification mode and the reference database used was the Bruker Taxonomy database.

*Light Microscopy and Scanning Electron Microscopy (SEM)*

Fungal cells were further characterized using light and scanning electron microscopy. A light microscope (Axioplan Zeiss, West Germany) coupled to a Colorview Soft Digital Imaging System (Münster, Germany) was used for morphological characterization of filamentous fungi cultures. To prepare fungal cells for SEM analysis, cells were collected from agar plates and suspended in 3% sodium phosphate buffered (0.1 M, pH 7.0) glutardialdehyde and fixed for 3 h. The suspension was rinsed once by centrifugation with the same buffer and then post-fixed for 1 h in 1% osmium tetroxide in similar buffer solution. The suspension was rinsed twice by centrifugation and dehydration commenced in an ethanol series (50%, 70%, 95% and two changes of 100%). The cell and ethanol suspension was centrifuged between each dehydration step. The cells and spore pellet was finally transferred to 5 µm critical point dryer baskets (Biorad, London, United Kingdom) for the critical point drying process. The dried pellet of cells was dispersed over a thin layer of epoxy glue (Pratley, Gauteng, South Africa) on SEM stubs for mounting. The material was coated by 200 nm gold in a sputter coater (Biorad, London, United Kingdom) and examined with the scanning electron microscope (Jeol 6400 WINSEM, Jeol Japan, London, United Kingdom branch).

## 3. Results and Discussion

In general, bacterial microbial counts from sampled HVAC systems were above 12 cfu/cm$^{-2}$ while fungal counts were below 5 cfu/cm$^{-2}$. According to the guide to monitoring surface hygiene (2010), the current results ranged from clean (<5) to very contaminated (>10). Similar results were observed at a government hospital elsewhere (Qudiesat *et al.,* 2009; Chuaybaroong *et al.,* 2008). The presence of microorganisms on HVAC systems is often worrying because dust contaminated with MRSA from ventilation grills has reportedly led to HAI outbreaks (Thompson *et al*., 2011). Results from the current and previous studies indicate the significant role played by ventilation grills as potential sources of airborne microbial contaminants in hospitals.

Identified microorganisms isolated from ventilation grills in category rooms A-E are shown in Table 1. The MALDI-TOF MS scores are reported as a logarithmic value, the value is obtained by alignment of peaks of the unknown raw spectrum and the best matching database spectrum. Acquired spectra were interpreted using the manufacturer-recommended scores (genus, ≥1.7; species, ≥2.0).

Samples collected from ventilation system grills were dominated by bacterial genera *Anaerococcus*, *Arthrobacter*, *Bacillus,* *Staphylococcus* and *Streptomyces*. The only fungal genera isolated were the yeast genus *Rhodotorula* and the filamentous fungus, *Penicillium chrysogenum*. All microbial isolates were identified up to species level except *Staphylococcus* and *Streptomyces* (results not included in Table).

**Table 1.** Identified microbial species collected using swabs from a 5X5 cm area of HAVC grills in 5 wards.

| Ward | Total Bacterial Counts | Total Fungal Counts | MALDI Biotyper Score | Organisms |
|---|---|---|---|---|
| **A** | ≥12cfu.cm$^{-2}$ | ≥4cfu.cm$^{-2}$ | 2.1<br>2.1<br>1.6 | *A. oxydans* DSM 20119T DSM<br>*B. megaterium* DSM 32T DSM<br>*P. chrysogenum* DSM 895 HED |
| **B** | ≥12cfu.cm$^{-2}$ | | 1.7<br>1.7 | *A. pigmenti* DSM 16403T DSM<br>*B. megaterium* DSM 32T DSM |
| **C** | ≥12cfu.cm$^{-2}$ | ≥4cfu.cm$^{-2}$ | 1.7<br>2.1 | *R. minuta* DSM 4043 DSM<br>*Anaerococcus* spp. HU40261 PNU |
| **D** | ≥12cfu.cm$^{-2}$ | | 2.2 | *A. polychromogenes* DSM 20136T DSM |
| **E** | ≥12cfu.cm$^{-2}$ | | 2.1<br>2.2 | *B. megaterium* DSM 32T DSM<br>*A. polychromogenes* DSM 20136T DSM |

The MALDI-TOF MS is a technique that has been used successfully for rapid identification of bacterial and yeast genera from clinical isolates (van Veen *et al*., 2010). It measures highly abundant proteins that are found in all microorganisms. The characteristic patterns of these abundant proteins are used to reliably and accurately identify a particular microorganism by matching the respective pattern with an extensive open database to determine the identity of the microorganism down to the species level. The advantages of this technique over biochemical and molecular techniques for healthcare settings isolates have been demonstrated in numerous studies (Prod'hom *et al*., 2010; van Veen *et al*., 2010; Carbonnelle *et al.,* 2011). In the current study, MALDI-TOF MS was successfully utilized to identify and characterize microorganisms colonizing ventilation systems.

Amongst the bacterial genera isolated in the current study was a species from the *Anaerococcus* genus (Table 1). Bacteria from this genus have been mostly been isolated from the human vagina, but have also occasionally been identified in the nasal cavity, on the skin, and in various opportunistic infectious processes including ovarian and cervical abscesses, bacteremias, foot ulcers, sternal wounds etc. (Hugan et al., 2012). This is the first report of the isolation of *Anaerococcus* species from ventilation grills. In addition, three *Arthrobacter* species were identified namely *Arthrobacter oxydans*, *Arthrobacter pigmenti* and *Arthrobacter polychromogenes* (Table 1). The presence of *A. oxydans* on ventilation grills was expected as this organism is found in soil and dust and its presence on ventilation systems has been reported in other studies (Gołofit-Szynczak and Górny, 2010). However, there were concerns regarding its presence as *A. oxydans* was isolated from clinical isolates elsewhere (Mages et al., 2008). Information concerning clinical implications due to other isolated *Arthrobacter* species is currently limited.

Previous studies have reported the presence of bacilli in HVAC systems (Schmidt *et* al., 2012); however, using molecular techniques the viability of these strains could not be confirmed as these bacteria are spore formers. Here, *Bacillus megaterium* was isolated from HVAC systems in rooms A, B and E (Table 1). The viability of the isolated bacterium was confirmed as colonies are grown on agar plates before identification with the MALDI TOF MS, demonstrating the advantage of this technique over molecular techniques. Molecular techniques could still be performed using plate isolates; however this could be time consuming which is not the case with the MALDI TOF MS. *Bacillus megaterium* species are generally non-pathogenic and mainly used in industrial applications because they have efficient protein secretion systems and they are also able to grow on different cheap carbon sources (Bunk et al. 2010).

Formerly deemed nonpathogenic, *Rhodotorula* a reported emerging opportunist pathogen (Wirth and Goldani 2012) was the only yeast genus isolated on ventilation grills in room C (Table 1), again this was not the first report of this yeast on ventilation systems (Gołofit-Szynczak and

Górny, 2010). The presence of microbes on ventilation grills is a worrying factor because microbes can be aerosolized leading to the spread and settling on surfaces (Ryan *et al*., 2011; Thompson *et al*., 2011) of these organisms in the wards. In addition, due to the yeast's strong affinity for plastic, its presence could lead to possible contamination of medical equipment such as dialysis equipment, bronchoscopes etc. (Wirth and Goldani 2012). The presence of *Rhodotorula* in hospital rooms could have serious health complications as such as bloodstream infections.

Since the MALDI-TOF MS score value for *Penicillium chrysogenum* was below 1.7, further characterization of this fungus was conducted using Light and Scanning Electron Microscopy for morphological characterization. Morphological characteristics of filamentous fungi remain the traditional method for identification because filamentous fungi have more distinctive morphologies than bacteria and yeast (Santos et al., 2009). Molecular techniques such as polymerase chain reaction (PCR) are also used to identify filamentous fungi. However, difficulties breaking fungal cell walls and the presence of PCR inhibitors in fungal cultures often hinder these techniques (Bandh *et al*., 2011).

A fungus isolated in the current study was identified by light and scanning electron microscopy as *Penicillium chrysogenum*. Light and Scanning Electron micrographs (Figures 1a-d) indicate *P. chrysogenum* characterized by sub-globose conidia, smooth finely roughened conidia in ornamentation, a smooth stipe, flask shaped phialide and a quarte-verticillate branching pattern, confirming the identity of this fungus (Bandh et al., 2011). The presence of *Penicillium*, although not unexpected, was a worrying factor because fungi are known to release spores, fungal fragments, mycotoxins and volatile organic compounds (Cabral 2010) which comprise the health of patients with already weak immune systems.

The HVAC system is an important building parameter that controls indoor moisture and mold growth; however, if not properly designed, maintained or operated it can lead to poor indoor air quality which could be detrimental in health-care settings. Some of the microorganisms identified in the current study using the MALDI TOF MS have no reported clinical implications; however, some are potential pathogens due to the presence of immune-compromised patients. In addition, the potential aerosolization of these microorganisms from ventilation systems cannot be ruled out and it could result in microorganisms settling on hospital surfaces and possible contamination of medical equipment leading to HAIs. Frequent change of filters and air monitoring are suggestions provided for the hospital. Future studies should be carried out in other wards at the hospital and at other public hospitals to determine if similar challenges regarding ventilation systems exist.

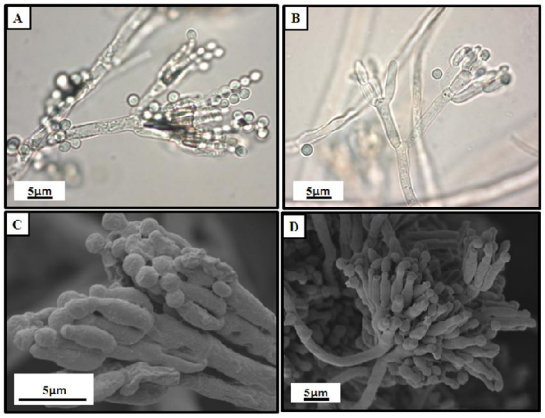


Figure 1. Light (A-B) and scanning electron (C-D) micrographs showing morphological structures of *Penicillium chrysogenum* isolated in all wards.

**Acknowledgements**
The authors would like to acknowledge the National Research Foundation of South Africa and the Central University of Technology for financial support. University of the Free State (Microbiology and Microscopy departments) is acknowledged for access to microscopes.

**Corresponding Author:**
Dr. Ntsoaki Joyce Malebo
Department of Life Science
Central University of Technology, Free State; Private Bag X2053 Bloemfontein, 9300, South Africa;
E-mail: nmalebo@cut.ac.za

**References**

1. Balloco C. Hospital ventilation simulation for the study of potential exposure to contaminants. Build Simul 2011; 4(1): 5-20.
2. Bandh SA, Kamili AN, Ganai BA. Identification of some *Penicillium* species by traditional approach and morphological observation and culture. AJMR 2011; 5(21): 3493-3496.
3. Bunk B, Biendendieck R, Jahn D, Vary PS. *Bacillus megaterium* and other bacilli: Industrial applications. Encyclopedia of Industrial, Biotechnology: Bioprocess, Bioseparation and Cell Technology; 2010.
4. Cabral JPS. Can we use indoor fungi as bioindicators of indoor air quality? Historical

perspectives and open questions. Sci Total Environ 2010; 408: 4285-4295.
5. Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, Beretti J, Ferroni A, Gutmann L, Nassif X. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. Clin Biochem 2011; 44: 104-109.
6. Chuaybamroong P, Choomseer P, Sribenjalux P. Comparison between hospital single air unit and central air unit for ventilation performances and airborne microbes. Aerosol and Air Qual. Res 2008; 8(1): 28-36.
7. Ekhaise FO, Ighosewe OU, Ajakpovi OD. Hospital Indoor Airborne Microflora in Private and Government Owned Hospitals in Benin City, Nigeria. W J Med Sci 2008; 3(1): 19-23.
8. Gołofit-Szynczak M, Górny RL. Bacterial and fungal aerosols in air-conditioned office buildings in Warsaw Poland – The winter season. JOSE 2010; 16(4): 465-476
9. Hugon P, Mishra AK, Robert C, Raoult D, Fournier P. Non-contiguous finished genome sequence and description of *Anaerococcus vaginitis*. Standards Gen Sci 2012; 6: 356-365.
10. Mages IS, Frodl R, Bernard KA, Funke G. Identities of *Arthrobacter* spp. and *Arthrobacter*-like bacteria encountered in human clinical specimens. J Clin Microbiol 2008; 46(9): 2980–2986.
11. Mandal J, Brandl H. Bioaerosols in indoor environment –A review with special reference to residential and occupational locations. Environ & Biol Monitoring 2011; 4: 83-96.
12. Prod'hom G, Bizzini A, Durussel C, Bille J, Greub G. MALDI-TOF mass spectrometry for direct bacterial identification from positive blood culture pellets. J Clin Microbiol 2010; 48: 1481–1483
13. Qudiesat K, Abu-Elteen K, Elkarmi A, Hamad M, Abussaud M. Assessment of airborne pathogens in healthcare settings. AJMR 2009; 3(2): 066-076.
14. Ryan RM, Wilding GE, Wynn RJ, Wellivers RC, Holm BA, Leach CL. Effect of enhanced ultraviolet germicidal irradiation in the heating ventilation and air conditioning system on ventilator associated pneumonia in a neonatal intensive care unit. J Perinatol 2011; 1-8.
15. Santos C, Paterson RRM, Vena A, Lima N. Filamentous fungal characterization by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Applied Microbiol 2009; 108: 375-385.
16. Schmidt MG, Attaway HH, Terzieya S, Marshall A, Steed LL, Salzberg D, Hamoodi HA, Khan JA, Feigley CE, Michels HT. Characterization and control of the microbial community affiliated with copper and aluminum heat exchangers of HVAC systems. Current Microbiol 2012; 65: 141-149.
17. van Veen SQ, Claas ECJ, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol 2010; 48(3): 900-907.
18. Wirth F, Goldani LZ. Rhodotorula: An emerging pathogen. Interdisciplinary Perspectives on Infectious Diseases 2012; 465717: 1-7.
19. Yu BF, Hu ZB, Yang HL, Kong QX, Liu YH. Review of research on air-conditioning systems and indoor air quality control for human health. Int J Refrig 2009; 32: 3-20.

7/1/2013