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Characterization of Bioaerosols in Wet-blue Leather Tanneries Environment

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Abstract. The present study was conducted for quantitative and qualitative analysis of bioaerosols in both tannery environment and control group. A total of eight air samples was collected from four wet-blue leather tanneries and the control group during September-December, 2019. Portable volumetric dust sampler was used to sample bioaerosols. Temperature, humidity and gases were monitored by portable aeroqual series 500. The respiratory micro-biota was identified through 16S rRNA gene sequencing. The bacterial count in tannery air was estimated to be 510 to 750 C.F.U/m^3, whereas the number of fungi was 600 to 900 C.F.U/m^3. The bacterial spectrum measured in the control community, on the other hand, was 400 to 492 C.F.U/m^3. However, no fungal strains was found in the control group's samples. Among four wet-blue leather tanneries, *Staphylococcus aureus*, *S. hominis*, *S. gallinarum*, *Klebsiella varricola* were the bacterial, while *Aspergillus versicolor*, *A. flavus*, *Candida parapsilosis* and *Penicillium chrysogenum* were the fungal species identified at the molecular level. However, in control group *S. hominis*, *S. warneri*, *S. epidermidis*, *Micrococcus luteus* (M) and *Bacillus subtilis* (B.) were identified without any fungal species

Keywords: air pollutants, bacteria, dust, fungi, micro-biota.

Introduction

Airborne contaminants in occupational places comprise of biotic and abiotic constituents. The biotic constituents i.e. micro-organisms are of greater significance due to their widespread exposure (Reponen, 2011). They can easily transport from one environment to another (Van Leuken *et al*., 2016). The untreated animal hides and skins bring lot of micro-organisms from slaughter houses and spread them in tannery air and surfaces (Oko and Abua, 2016). The concentration of these micro-organisms accelerates further due to the immoderate humidity and poor air circulation in the tannery, ultimately crossing the threshold level. These micro-organisms known as bioaerosols. They comprise mycelium fractions, spores, bacteria, fungi, viruses and toxins (Castellanos-Arevalo *et al*., 2015).

In recent years, these airborne micro-organisms are of greater significance due to their possible impact on human health as well as disease spread (Wang *et al*., 2019; Kim *et al*., 2018). Bioaersols have been known to involve in causing respiratory infections such as pneumonia, allergy, asthma, digestive problems and cancer (Oruko *et al*., 2019; Kim *et al*., 2018; Flannigan *et al*., 2017; Berrington and Hawn, 2013). There are many bacterial species that have been reported as normal human microflora and generally less harmful such as *S. gallinarum* (Yu *et al*., 2008), *S. epidermidis* (Levinson, 2004), *B. subtilis* (Lefevre *et al*., 2017), *M. luteus* (Greenblatt *et al*., 2004) and *S. warneri* (Nagase *et al*., 2002). On the other hand, there are some airborne bacterial genera such as *Staphylococcus* and *Klebsiella*, which may involve in respiratory infections (Kozajda *et al*., 2019; Bosch *et al*., 2013; Parker and Prince, 2012). In tanneries, the sources of gaseous emissions are majorly the chemicals used during leather processing and emit from boilers and generators. These gases may act as health hazard for tannery workers and cause skin allergy and respiratory problems (Shegani, 2014).

Data is scanty regarding bioaersols and gaseous pollutants from tannery environment in Pakistan. Therefore, this study was planned to provide baseline data regarding quantitative and qualitative analysis of

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airborne microbes and gaseous pollutants in wet-blue leather tanneries.

Materials and Methods

Meteorological and aerobiological sampling. Total eight samples of bioaerosols were collected from the air of four wet-blue leather tanneries as well as control sites in Deen Garh, Kasur city, Pakistan. Control group was taken to compare bioaerosols data between working and non-working environment. Control sites represent the residential places and samples obtained from the living area. Sampling was conducted during September to December 2019. Portable dust sampler was employed to collect bioaersols and run for 10 min with flow rate 27 L /min. Sampling was performed during working hours within breathing zones at the height of 1.5 m above the ground level. Meteorological data was obtained in terms of relative humidity (%) and temperature (ºC) by means of portable aeroqual series 500.

Monitoring of gaseous pollutants. Using proper monitoring sensors, different gases such as carbon monoxide (CO), carbon dioxide (CO_2), sulphur dioxide (SO_2), nitrogen dioxide (NO_2) and ozone (O_3) were tracked using the portable aeroqual series 500. During the exposure time, the device was run for 8 h.

Microbiological investigation. The filter paper from the dust sampler was suspended in 10 mL in sterile saline solution for each sampling site and shaked well. The filter paper suspended (FPS) saline solution was served as inoculums for the isolation and molecular identification of bioaerosols. Nutrient agar medium was prepared and autoclaved for pouring in a laminar-air flow chamber in sterile petri plates. After pouring and solidification of the medium, the petri plates were inoculated with 0.3 mL of the FPS saline solution and put in an electric incubator for 72 h at 27 ºC. The petri plates were inspected after successful incubation and the number of colonies was counted using the direct count process. The number of colony forming units per cubic meter (C.F.U/m^3) was calculated using the protocol of Sieuwerts *et al*. (2008).

After viable counting, the bacterial identification was performed through morphological features of the colonies. However, selected bacterial species, which may involve in respiratory health complications, were further identified through16S rRNA gene sequencing.

Malt extract agar (MEA) and sabouraud dextrose agar (SDA) were used to prepare petri plates for fungal examination. Following that, cotton swabs were used to scatter samples on each prepared plate. For one week, the inoculated plates were held at 30 °C. After colonies formed on plates, a single spore was taken from each plate and inoculated in the centre of freshly prepared SDA plates for pure culturing. Following that, morphological identification was performed of isolated fungal strains.

Results and Discussion

The bacterial and fungal count in tanneries air was higher than in the control group in this research. The bacterial count in tannery air was estimated to be 510 to 750 C.F.U/m^3, whereas the number of fungi was 600 to 900 C.F.U/m^3. The bacterial spectrum measured in the control community, on the other hand, was 400 to 492 C.F.U/m^3. However, no fungal strains were found in the control group's samples (Table 1). Similar results were un-covered in the tanneries of Mexico by Castellanos-Arevalo *et al*. (2015). They discovered that the experimental group's bacterial and fungal loads were 50 and 15-20 times higher than the control groups, respectively. Similarly, the microbial count in tannery air exceeded from standard guidelines of indoor bacteria and fungi concentrations maintained by some countries. When compared to the counts recommended by Sweden's guidelines, all of the tanneries tested were above the appropriate range of bacterial and fungal counts (C.F.U/m^3) in the current study (Abel *et al*., 2002). Furthermore, contrary to the level set by American Association of Industrial Hygiene (AIHA), fungal counts in all tanneries' indoor environments were above the permissible range (250 C.F.U/m^3) (AIHA, 2001).

Among identified species of bacteria, the 16S rRNA gene sequencing of Methicillin-resistant *S. aureus* (MRSA) and *K. variicola* was conducted (Fig. 1-2) among the bacteria known because of their pathogenicity and potential role in respiratory illness (Kozajda *et al*., 2019; Parker and Prince, 2012). MRSA pneumonia is found globally (Carrillo-Marquez *et al*., 2011) and accounting for 13% of all deaths (Klevens *et al*., 2007). Skora *et al*. (2014) arrived at the conclusion that in

Table 1. Average count of bacteria and fungi colony-forming units (C.F.U/ m^3) in the research sites

Bioaerosols	Exposed	Control
Bacteria	634	451
Fungi	748	00

Primer information

Seqiemcomg Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F5'(GGA TTA GAT ACC CTG GTA)3'	27F 5'(AGA GTT TGA TCM TGG CTC AG)3'
907R 5'(CCG TCA ATT CMT TTR AGT TT)3'	1492R 5'(TAC GGY TAC CTT GTT ACG ACTT)3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
CP011526.1	*Staphylococcus aureus*	2755072	503062	504548	0	2739	0.0	1486/1487	99

Kingdom	Family	Genus	Species
Bacteria	*Staphylococcaceae*	*Staphylococcus*	*Staphylococcus aureus*

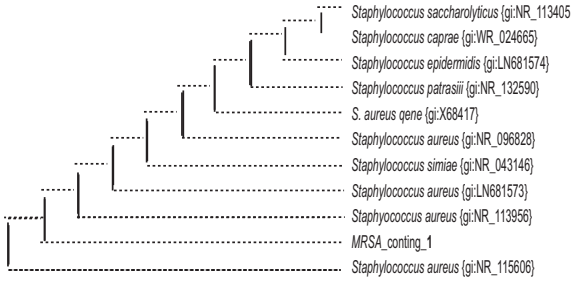

Fig. 1. 16S rRNA gene sequencing of *S. aureus*.

Primer information

Seqiemcomg Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F5'(GGA TTA GAT ACC CTG GTA)3'	27F 5'(AGA GTT TGA TCM TGG CTC AG)3'
907R 5'(CCG TCA ATT CMT TTR AGT TT)3'	1492R 5'(TAC GGY TAC CTT GTT ACG ACTT)3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
CP010523.2	*Klebsiella varicola*	5521203	4447641	4446161	0	2706	0.0	14876/1481	99

Kingdom	Family	Genus	Species
Bacteria	Enterobacteriaceae	*Klebsiella*	*Klebsiella variicola*

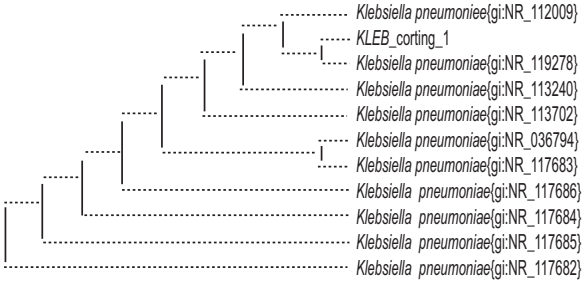

Fig. 2. 16S rRNA gene sequencing of *K. variicola.*

addition to other bacterial genera, *Micrococcus*, *Staphylococcus* and *Pseudomonas* are normally present on leather surfaces. The same bacterial species was found in tannery air of Nigeria, according to Oko and Abua (2016). They discovered *S. aureus* to be the most common bacterium in the air, with the ubiquity of 29.63%. Orlita (2004) found the same *Staphylococcus* species on the finished leather specimen. This bacterium is familiar for being present on human skin and in the nose. However, the clinical intricacies of MRSA, particularly in relation to pneumonia, have been verified in a variety of occupational settings (Kozajda *et al*., 2019). *S. aureus*, as well as other genera, migrate from the hides to the tannery air (Daliborca, 2009). It is spread to people who are exposed to the atmosphere by the air, as well as by hand and mouth (Chang and Lin, 2018; Boopathy, 2017).

K. variicola is an opportunistic pathogen that causes pulmonary disorders and renal diseases (Potter *et al*., 2018; Holt *et al*., 2015). It infects people more severely and has a higher corporality rate than *K. penumonae* (Long *et al*., 2017; Maatallah *et al*., 2014). In Dhaka, Bangladesh, *K. varricola* was also isolated and detected in tanneries (Kabir *et al*., 2018).

Similarly, *S. hominis* and *S. gallinarum* were identified in the current research from the air of tannery environment. Different researchers in different tanneries (Castellanos-Arevalo *et al*., 2015; Skora *et al*., 2014) also reported similar species of bacteria.

On the flip side *A. versicolor*, *A. flavus*, *C. parapsilosis* and *P. chrysogenum* were classified as fungal strains in all tanneries using microscopic and macroscopic recognition. Similar fungal genera were also reported in the tannery air by different researchers. In the air of Nigerian tanneries, reported Oko and Abua (2016) abundant occurrence of *A. versicolor*. Similarly, regular presence of *A. versicolor* from tannery air was outlined by Skora *et al*. (2014). Moreover, this fungal species may be involved in *Aspergillosis* in human, according to the Sabino *et al*. (2012) and Fomicheva *et al*. (2006). Different researchers have also identified *C. parapsilosis* and *P. chrysogenum* in tannery conditions, in addition to the above (Oruko *et al*., 2019; Skora *et al*., 2014). *C. parapsilosis* is present on human skin and found abundantly in the surrounding environment. It induces nosocomial infections in patients with compromised immune systems but it is not pathogenic to them. *P. chrysogenum* is a salt-resistant fungus that thrives in humid conditions (Chirila and Berechet, 2016). It is not a human pathogen in majority of cases. It can, however, cause pulmonary problems in immune compromised people when combined with *P. jiroveci* (Shokouhi *et al*., 2016), while Skora *et al*. (2014) reported oppositely and confirmed that both of these species are frequently

found in tannery conditions but do not pose a threat to tannery workers.

Comparably, three bacterial genera were isolated and classified as *Staphylococcus*, *Micrococcus* and *Bacillus* in the control group. Among these three bacterial genera, the bacterial species: *S. hominis*, *S. warneri*, *S. epidermidis*, *M. luteus* and *B. subtilis* were identified. However, no fungal strain was isolated in the control group.

Humidity and temperature have an effect on the existence of airborne microbes (Skora *et al*., 2014). The average relative humidity and temperature observed in this study were 70.32 percent and 17.72 ºC, respectively (Table 2). The results were nearly identical to those recorded by Castellanos-Arevalo *et al*. (2015) in the indoor setting of Mexican tanneries, which were 80.4 percent and 16.2 ºC. Similarly, in a wet-blue leather tannery, Chirila and Berechet (2016) reported a temperature of 24 °C and a humidity of 60%.

Tannery air was tested for five different gaseous contaminants (Table 3), while CO, CO_2 and O_3 had mean values of 6.2 mg/m^3, 4752 ppm and 69 g/m^3 after 8 h, respectively. The 10 min average value of SO_2 was 505 g/m^3 and the 1 h mean value of NO_2 was 218 g/m^3. According to World Health Organization (WHO, 2005), the safest values of SO_2, NO_2 and O_3 must be up to 500 µg/m^3 (10 min and, 200 µg/m^3 in 1 h mean, 100 µg/m^3 in 8 h) mean respectively. In the current research work, only O_3 was within the prescribed limit, while SO_2 and NO_2 were exceeding these standard values limits. However, CO and CO_2 were within the prescribed standard values (WHO, 2017; NIOSH, 2007).

Table 2. Relative humidity and temperature in tanneries

Site	Relative Humidity(%)	Temperature (ºC)
01	55.1	20.1
02	76.3	16.7
03	67.8	15.9
04	82.1	18.2
Average	**70.32**	**17.72 ºC**

Table 3. Gaseous pollutants in tannery environment

Gaseous pollutant	Unit	Time	Average mean value
CO	ppm	8 h	6.2
CO_2	mg/m^3	8 h	4752
O_3	µg/m^3	8 h	69
NO_2	µg/m^3	1 h	218
SO_2	µg/m^3	10-min	505

Conclusion

It is concluded that the airborne microbiota isolated from the tannery environment has bacterial species, which may be involved in respiratory illness and identified their presence through 16S rRNA sequencing, while in the control group, no fungal species was found except some bacterial genera, which usually have been reported as normal human skin flora. Further, research with a large number of tanneries to build complete profile of respiratory microbiota found in the working environment of tanneries is needed in future studies.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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