Research Article

Impact of haze events on airborne bacterial consortia–a case study

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Abstract
Transboundary haze events received a noticeable attention recently, due to their frequent occurrences. They are mainly, consequences of anthropogenic activities. Sri Lanka experienced a haze event parallel to India in November 2019, the first air pollution event in Sri Lanka linked to a haze event in India. Due to the limited availability of information on haze-related microorganisms, we conducted this study in Kandy, Sri Lanka, aiming to explore the airborne bacterial consortia during a haze event. The natural sedimentation method was used for air sampling. Bacterial identification and the total bacterial load were determined using Sanger sequencing and qPCR. Notably, the total bacterial load was elevated by ~ 40% with the haze and decreased with decreasing haze intensity. The highest bacterial load was reported during the day time of the most intense hazy day (1.89×10^6 cells/µl) compared to non-hazy days (lowest; 1.12×10^5 cells/µl). Twelve bacterial species were identified and the most abundant phylum was Proteobacteria. The most common species observed during haze was *Acinetobacter modestus*. The percentage of culturable bacterial species was also high during the haze event (75% during day time of the most intense hazy day compared to 25% on the control). Two human pathogenic bacteria *Burkholderia multivorans* and *Chryseobacterium gleum* were found only during the haze event. Therefore, haze events could be hazardous to humans by means of the presence and fluctuating amounts of pathogenic bacteria. Thus, these findings are important in developing policies and guidelines to monitor and minimize the negative impact of haze events.

Keywords Haze event · Airborne bacteria · Bacterial load · Air quality monitoring

1 Introduction

Over the last decade, globally, haze events have become a topic of prime concern. They are defined as events of unexpected rise of dry particulate matter (PM) in the atmosphere obscuring its clarity [1]. Farming practices involving burning of straw stubble, open coal-fired power plants and forest fires have been identified as immediate reasons for recent haze events [2, 3]. In addition, long-term effects of other anthropogenic activities such as dump sites, factory effluent and combustion of fossil fuels could also be contributing factors [3]. Hazardous pollutants released to the air in this way may get transported through dry smoggier winds to neighboring countries resulting in haze events that cross international boundaries, as was seen in Indonesia, Malaysia and China in 1997, 2005 and 2013 [4]. There is evidence that forest fires in Sumatra had links to the September 2019 haze event in Malaysia [5]. Similarly, Saudi Arabian dust storms also are known to have associations with haze events in India [6]. Such an event occurred in 2015 and resulted in 100,000 premature deaths across Indonesia,

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Malaysia and Singapore [7]. Thus, these events pose a severe impact to public health and the absence of global agreements on monitoring and controlling may result in an increasing threat in the near future.

Sri Lanka, a country with less experience with haze events, was exposed to such an event in November 2019, parallel to the haze event in Delhi, India which was a top news at the time [8]. This was the first time that an air pollution incident in Sri Lanka was linked to a haze event in India. The most acceptable explanation for this is a haze event that crosses international boundaries. Air pollution in one area can affect the air quality in the adjoining areas causing damage in many ways as air cannot be kept constrained or divided between international boundaries [2]. The main reason behind the November haze event in India was stubble and paddy burning [9]. During this event, the $PM_{2.5}$ values were as high as 1000 µg/m^3 in Delhi and its suburbs, while the limit of safety is 60 µg/m^3 [10]. In Sri Lanka, this condition was first observed in Colombo (the capital city) and then all around the country in varying intensities. Haze was observed in Kandy, a district important in terms of economy, tourism and administration, and it was predicted that the effect could be intense as Kandy is one of the most air-polluted cities in Sri Lanka [11]. As per the central environmental authority of Sri Lanka, in both Colombo and Kandy, the air quality index showed PM_{10} values close to 100 µg/m^3 which is usually the double of a normal day [12].

Even though the physicochemical properties associated with haze events have been studied, less is known about bioaerosols, the type of particles released from marine and terrestrial eco-systems into the atmosphere [5, 13]. They may contain both living and non-living components, wherein microorganisms may compose the living component and the types of microorganisms may depend on the source of the bioaerosols. Even in Sri Lanka, chemical and physical parameters of air pollution are monitored by the relevant institutes but changes in microbial consortia are not monitored [12]. A study done in China has identified that bacterial concentration shows a positive correlation with haze intensity [1, 14], but several other studies have identified a decrease [15, 16]. Certain microbial species were also noted as being associated with haze events such as *Halomonas* and *Shewanella* species [1], but some studies have not identified any specific microorganism associated with haze events [17]. Moreover, information on the impact of haze-associated bacteria on respiratory diseases and allergies are also less explored. With reference to the November 2019 haze event in India and Sri Lanka, no studies were reported in the context of microorganisms, and hence, we undertook a study in Kandy, Sri Lanka, to assess the change in bacterial consortia due to haze conditions using advanced molecular laboratory techniques.

2 Materials and methods

2.1 Sampling site, Sample collection and processing

Air samples were collected during the haze event (06–09 November 2019) and two non-hazy days (13 and 14 November 2019) at the premises of the National Institute of Fundamental Studies, (a research institute) located in Kandy, Sri Lanka (7° 17′ 02.4" N 80°37′ 57.1" E). The sampling site is located 1.7 km away from Kandy city and is surrounded with medium vegetation density, less traffic and minimal air pollution sources. For air sample collection, the natural sedimentation method (NSM) was used, using Luria Bertani (LB) media and Whatman No.5 filter papers. In addition, two other active methods were used for confirmation of results. Detailed descriptions of the NSM and sample processing are summarized in Fig. 1, and detailed descriptions of active methods are summarized in the supplementary Fig. 1. Filter papers used in both the active and passive sampling were cut into small pieces aseptically. These pieces were suspended in sterile Milli-Q water and were shaken in an orbital shaker (ORBITEK, Scigenics Biotech, India) for 2 h. Following extraction of bacteria into Milli-Q water, extracts were centrifuged at 12,000 rpm for 5 min. The supernatants were discarded having 1 mL of sedimented bacteria remaining for further applications [18].

2.2 Quantification of bacteria

Genomic bacterial DNA was extracted from the filter paper extracts using the modified Boom's method [20] and amplified using real-time Polymerase Chain Reaction (PCR) (RotorGeneQ, Qiagen, Germany). Total bacterial load in each sample was detected amplifying the 16S rRNA gene of bacteria using a forward primer, 5′-TCC TACGGGAGGCAGCAGT-3′, reverse primer, 5′- GGACTA CCAGGGTATCTAATCCTGTT-3′ and a fluorescent probe (6-FAM)-5′-CGTATTACCGCGGCTGCTGGCAC-3′-(TAMRA) [21]. The PCR reaction was performed in a total volume of 25 µL consisting of 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 400 nM of each primer and 175 nM of a fluorogenic probe in the presence of 1 unit of Taq polymerase enzyme. The amplification reaction conditions were 50 °C for 2 min, 95 °C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Bacterial load in each sample was quantified in comparison with a standard curve generated by *Escherichia coli* DNA.

The percentage elevation of the bacterial load was calculated by taking the average bacterial load of each sampling method. Results were subjected to normality

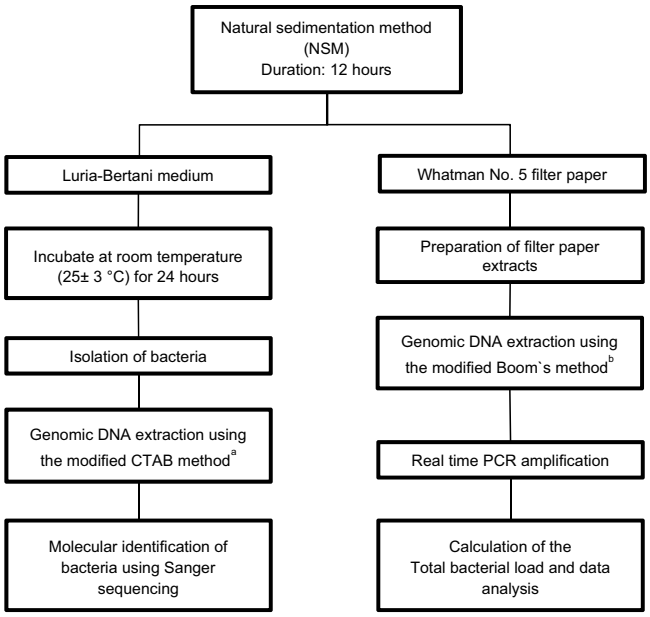

Fig. 1 Outline of air sample collection and processing methodology (CTAB; Cetyl trimethylammonium bromide, DNA; Deoxy ribonucleic acid, PCR; polymerase chain reaction). [a]Reference; [19]. [b]Reference; [20]

testing and were analyzed using ANOVA in the statistical software package Minitab (release 17.1.0, State College, PA: Minitab, Inc.) (www.minitab.com). Level of significance was set at $P < 0.05$.

2.3 Molecular identification of bacteria

Bacterial genomic DNA was extracted from the isolated pure cultures using the modified cetyltrimethylammonium bromide (CTAB) method [19]. Extracted DNA was amplified using the universal bacterial primers for the 16 s rRNA gene; 27F: 5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R: 5′-GGYTACCTTGTTACGACTT-3′. Amplified and purified DNA were sequenced at a commercial Sanger sequencing service provider (Macrogen Inc. Korea) (https://www.macrogen.com). The sequences were analyzed using MEGA v 7.0 [22] and submitted to the GenBank under the accession numbers; MT110972–MT110990. A maximum-likelihood (ML) tree was generated (MEGA v 7.0) for the sequences identified as the same species but having different morphologies. These sequences were compared with the reference sequence of the relevant species and that of a closer relative.

3 Results and discussion

This study examined the airborne bacterial consortia during the 2019 November haze event that occurred in Sri Lanka and was able to summarize the following important findings. Hazy days resulted in a ~ 40% higher total bacterial load compared to non-hazy days, displaying a decreasing in total bacterial load with the decrease of haze intensity. Proteobacteria were the dominant bacterial phylum, wherein *Burkholderia multivorans* and *Chryseobacterium gleum,* two known human pathogens**,** were recovered only during the hazy days.

Figure 2 and Supplementary Table 1 summarize the variation of the total bacterial load from each air sample collected during the study period. Accordingly, the highest bacterial load during the night time (6.00 pm–6.00 am the following day) was observed on 06 November, whereas that of the day time (6.00 am to 6.00 pm) was observed on 07 November, which had the most intense haze ($PM_{2.5}$ ~ 60 µg/m^3, PM_{10} ~ 100 µg/m^3 [12]). The lowest bacterial load during the night was observed on 13 November, which was a non-hazy day and the lowest bacterial load with respect to day time was seen on 14 November (PM2.5 ~ 15 µg/m^3, PM10 ~ 25 µg/m^3 [11]), a day without any visible haze. These two days were considered control days in the sampling process. Notably, the bacterial load decreased from 06th to 08th November, in both the night time and day time. The outcome of the two active methods used were similar to NSM as they also showed a decrease in total bacterial load with decreasing haze intensity. The significance is that the bacterial load observed during the night time as well as the day time on control days for all sampling methods were lower than that of the hazy days. The bacterial load of a NSM plate kept for 24 h on a control day, was yet lower than a plate kept for 12 h on a hazy day. The bacterial load observed during the haze event using NSM plates was 55.8% higher than that of the controls while those from a fine particulate air sampler and a laboratory-designed air sampler were 41.8 and 51% higher than a non-hazy day, respectively. However, no

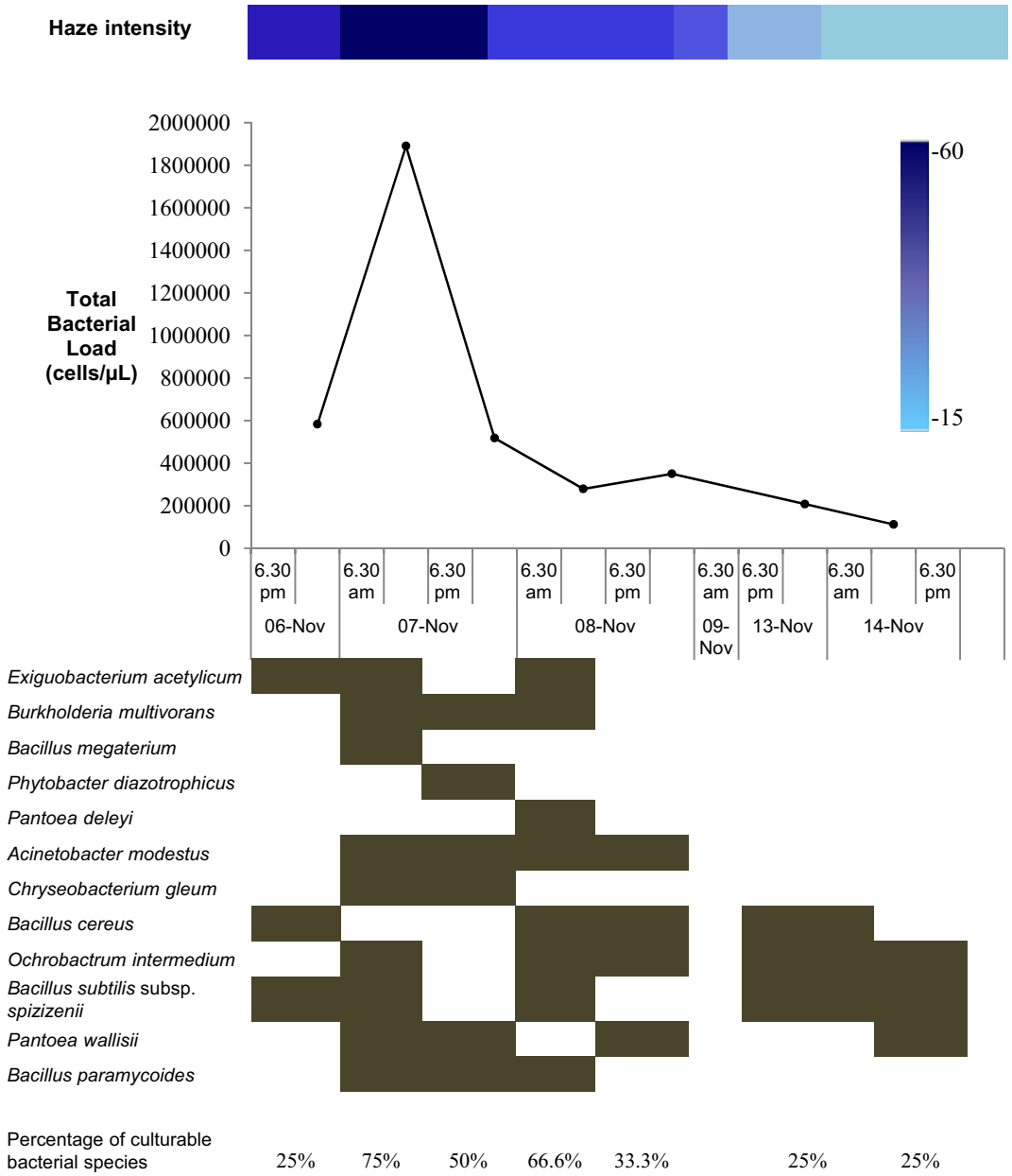

Fig. 2 A descriptive diagram of the variation of bacterial load with haze intensity and the bacterial species identified as per the date of the sample collection. The haze intensity grid was prepared as per the PM2.5 data in the central environmental authority web site [12]. All dates refer to 2019 and the scale is based on approximate PM2.5 values in µg/m^3

significant variation of the bacterial load was evident between night and day time and none of the observations were statistically significant. Consistent with some studies in China, the observations show that airborne bacterial load increases with the intensity of the haze condition and vice versa [1, 14]. As discussed before, certain studies show a decrease. Perhaps these conflicting findings might be because of the variations in the geographical setting, culture media, seasonal changes and the techniques used. Besides, certain studies have used only the culturable counts to predict the association, which is not a true representative of the whole picture [15]. A recent study shows that the bacterial cell counts increase with increasing PM; hence, polluted air consists of higher cell counts than that of non-polluted air [23]. High concentrations of air pollutants during a haze event provide nutrients like sulfur, nitrogen and ammonia for the growth and survival of certain bacteria thus, affecting their relative abundance [16]. Thus, the new conditions of increased/decreased components (nutrients,

particulate matter, etc.) in the atmosphere may have resulted fluctuations in certain microorganisms, hence effecting the bacterial load.

In this study, 12 culturable bacterial species were identified. The percentage of culturable bacterial species on hazy days was observed to be slightly higher during the day time than the night time; the highest being on 07 November 2019 (75%), the most intense hazy day (Fig. 2). It was evident throughout the study that the number of culturable bacterial species observed on hazy days was higher than that of non-hazy days (control; 25%); however, no significant association was observed which may be due to the lack of data. This shows that haze events affect airborne bacterial diversity, which encompasses both normal airborne bacteria and bacteria accompanied by haze pollutants. Most of the identified bacterial species were from the phylum Proteobacteria [6] followed by the Firmicutes [5] and Bacteroidetes [1]. This observation can be supported by the fact that Proteobacteria are the most abundant phyla during haze events [23, 24] (Supplementary Table 2). Similar to a previous study based on a haze event, the most common bacterial classes observed in this study were Bacilli [5] and Gammaproteobacteria [4] [16] (Supplementary Table 2). In our study, all identified species were observed during hazy days in different combinations while only four species were observed during non-hazy days (control) (Fig. 2). The most common species observed during the studied hazy days was *Acinetobacter modestus* and the least common were *Phytobacter diazotrophicus* and *Pantoea deleyi* (Fig. 2). Interestingly, we also encountered a *Burkholderia* sp. (*B. multivorans*) as in a study conducted in China which encountered *B. cenocepacia* as the most abundant human pathogen during haze [23]. *Bacillus subtilis* subsp. *spizizenii*, *B. cereus*, *Ochrobactrum intermedium* and *P. wallisii* were present regardless of the haze intensity. A greater proportion of the identified bacterial species during the study were inhabitants of soil, plant and water microbiota, possibly a consequence of bacterial community movements through submicron particles and aerosolization of bacteria [16]. Bioaerosols are also capable of long range transport and hence, the microbial consortia observed in this event could be associated with the bioaerosols generated from the haze origin, but, the reality and to which percentage is yet unknown. As per literature, *O. intermedium* [11], *B. megaterium*, *B. cereus* and *Exiguobacterium acetylicum* [18] have been previously identified in the Kandy atmosphere; perhaps, the haze condition might have favored their survival. Certain bacteria are capable of tolerating harsh environmental conditions through mutations in their genetic makeup. This can be seen with the discussed organisms, *E. acetylicum*, an organism extensively studied for its multi-stress radioactive tolerance [25] and *O. intermedium,* an organism studied for its heavy metal tolerance [26]. *Bacillus* spp are also capable of surviving for a long time by sporulation [27].

Among the identified bacterial strains, four bacterial species were observed to have different strains with varied colony morphologies on the LB agar medium (Supplementary Fig. 2). Colony morphology variations were observed in their color, shape, margin, texture (slimy/non-slimy) and growth rate. The highest number of strains was observed in *Burkholderia multivorans* with five different morphologies having major differences in color and growth rate. According to the maximum likelihood (ML) tree, all the five strains of *B. multivorans* clustered together with their reference sequence (NR_029358.1) and separately from their close relative *B. cepacia* (Fig. 3). The same pattern was observed for the other three species, *Bacillus cereus*, *B. subtilis* subsp. *spizizenii* and *E. acetylicum*. Hence, it can be suggested that though there were different colony morphologies and sequences, the strains belong to the same species. The five different color formats observed were yellow, transparent yellow, orangish-red, pinkish opaque and whitish opaque. Further, in comparison to the growth rate, two slow-growing, one fast-growing and two moderate growing strains were observed in *Burkholderia multivorans*. *E. acetylicum* had two colony morphologies, one with a dark orange color and the other with a yellowish opaque appearance. *Bacillus cereus* also had two colony morphologies; colonies with a distinct wavy margin and a distinct entire margin. The major differences observed between the two strains of *B. subtilis* subsp. *spizizenii* was their growth rate and the texture, which varied between extremely fast-growing with dry nature and average growth rate with slimy nature. Differences in bacterial morphology, specifically the color, are a consequence of pigmentation generated within bacteria as a response mechanism to exposure to pollutants and high radiation. The higher the pollutants in the air, the greater the variety of pigmentation [28].

As mentioned earlier bacterial consortia play a major role in haze associated health threats to humans. The samples collected during the haze event consisted of four culturable human pathogenic species namely, *Burkholderia multivorans*, *C. gleum*, *Bacillus cereus and O. intermedium*. Among them, *Burkholderia multivorans* and *C. gleum* were found only during hazy days. The species *B. multivorans*, a member of the *B. cepacia* complex is reported as a multi-drug-resistant pathogen causing chronic and debilitating lung infections in cystic fibrosis patients [29]. *C. gleum* is reported in causing infections in immunocompromised patients [30, 31]. *Bacillus cereus* mainly causes food poisoning but is also associated with potentially lethal non-gastrointestinal tract infections [32]. *O. intermedium* is an emerging opportunistic pathogen reported to be

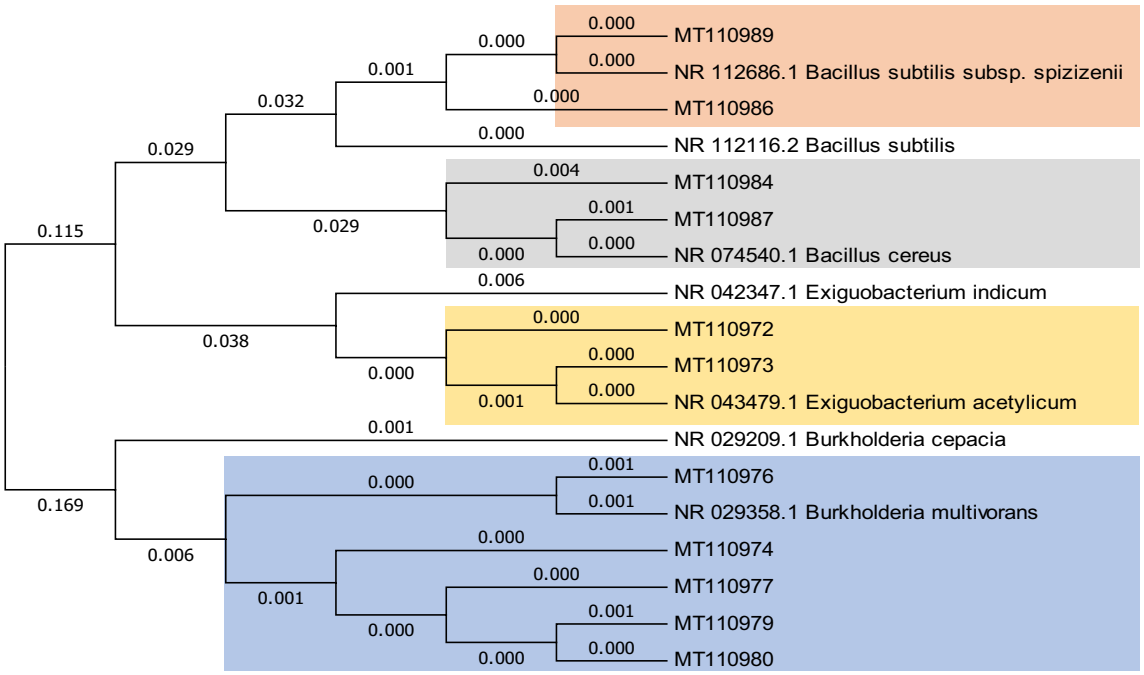

Fig. 3 Maximum likelihood (ML) tree generated for the species having morphologically (colony) varied strains (MEGA v 7.0)

associated with infective endocarditis [33]. Though not confirmed as pathogenic, *Phytobacter diazotrophicus* and *A. modestus* also have been found from clinical specimens in previous studies [34, 35]. According to the observations, it is evident that the studied haze event has had a potential impact on the health of humans in terms of encounters with pathogenic bacteria. The possibility for bacteria to acquire resistance genes due to environmental pressures is high during a haze event. Hence, it is important to study the resistance patterns and pathogenicity of these bacterial isolates to identify potential hazards.

The major limitation of the study is the low number of samples obtained, which was practically difficult as the haze event did not last long. Moreover, the study revolved only around bacteria while other microorganisms such as viruses and fungi may also show variations. However, as we conducted the analysis using molecular techniques rather than only cultures, we could obtain a complete picture on the impact of haze on airborne bacteria strengthening the study findings.

4 Conclusion

Conditions during a Sri Lankan haze event proved to have an impact on the airborne bacterial load which increases with the intensity of the haze condition. The study identified 12 culturable bacterial species. The highest number of culturable bacterial species (nine) and the highest amount of bacterial load (1.89×10^6 cells/µL) for the study were observed during the day time of the most intense hazy day, 07 November 2019. The lowest amount of bacterial load (1.12×10^5 cells/µL) was observed during the day time of 14 November 2019, a non-hazy day. The bacterial load observed during the haze event is ~ 40% higher when compared to non-hazy days. Hence, these revelations should be considered when monitoring and analyzing air quality during haze conditions. The studied haze event could have been hazardous to humans in means of pathogenic bacteria. The study emphasizes the need to implement policies to control and monitor haze events and to develop guidelines on how to prevent associated diseases during haze exposure. Further studies are needed to determine the degree of pathogenicity and resistance profiles of the identified organisms. Further study of air quality is recommended to understand the conditions favoring bacterial growth during a haze event.

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Availability of data and materials The data (sequences) that support the findings of this study are openly available in [National Center for Biotechnology Information] at https://www.ncbi.nlm.nih.gov under accession numbers [MT110972–MT110990].

Code availability MEGA V 7.0 [22] was used for the maximum likelihood tree generation and Minitab (release 17.1.0, State College, PA: Minitab, Inc.) was used for statistical analysis.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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