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ORIGINAL ARTICLE

Distribution of airborne microbes and antibiotic susceptibility pattern of bacteria during Gwalior trade fair, Central India

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Background/Purpose: Research into the distribution of bioaerosols during events associated with huge groups of people is lacking, especially in developing countries. The purpose of this study was to understand the distribution pattern of bioaerosols during an annual trade fair in the historical city of Gwalior, central India, a very important historical fair that was started by the King of Gwalior Maharaja Madho Rao in 1905.

Methods: Air samples were collected from six different sites at the fair ground and three different sites in a residential area before/during/after the fair using an impactor sampler on microbial content test agar and rose bengal agar for total bacteria and fungi, respectively. The representative strains of bacteria and fungi were further identified and selected bacterial strains were subjected to antibiotic susceptibility testing according to US Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: The bacterial bioaerosol count [colony-forming units (CFU)/m^3] at fair sites was found to be 9.0×10^3, 4.0×10^4, and 1.0×10^4 before the start of the fair, during the fair, and after the fair, respectively. The fungal bioaerosol count at fair sites was 2.6×10^3 CFU/m^3, 6.3×10^3 CFU/m^3, and 1.7×10^3 CFU/m^3 before the fair, during the fair, and after the fair, respectively. Bacterial/fungal bioaerosols during-fair were increased significantly from the bacterial/fungal bioaerosols of the before-fair period ($p < 0.05$); they were also significantly

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higher than the bacterial/fungal bioaerosols at non-fair sites during the event ($p < 0.0001$). The proportion of antibiotic-resistant bacteria over the fair ground was significantly increased during-fair and was still higher in the after-fair period. Methicillin-resistant staphylococci (MRS) were also reported at the fair ground.

Conclusion: The study indicates significantly higher bacterial and fungal bioaerosols during the fair event. Therefore, further research is needed to explore the health aspects and guidelines to control microbial load during such types of events.

Introduction

The load of airborne microorganisms depends on topology, geography, and environmental conditions of locations. As with any microbial habitat, the atmosphere is a heterogeneous environment, and the diversity of airborne microbes may vary spatially and temporally. Generally, microbes enter into the atmosphere from natural (vegetation and soil) and anthropogenic sources but their survival and distribution depend on the cell structure of microbes and meteorological conditions.[1–3] Many activities such as high personnel traffic, vehicle flow, constructions, and other anthropogenic activities stir up the dust from the ground resulting into high outdoor microbial load.[4,5] Airborne microbial quantity and quality can vary with time of the day, year, and location.[1,6] Their presence in air is a cumulative function of the geographical locations, anthropogenic activities, and environmental factors.[5,7] Environmental conditions such as relative humidity, temperature, and wind velocity exert a significant effect on the type of population and amount of microorganisms in the air in the long term.[2,8,9] Finally, the microbial concentration in the atmosphere relies on the abundance of sources and factors controlling their release and dispersal from the surface boundary layer.[10] However, the atmospheric bacterial bioaerosol is largely associated with human densities, like in urban areas, and found to be several fold higher when compared to surrounding rural areas.[3,5]

It is important to know the distribution pattern of live bioaerosols in the environment during the event of a huge population gathering. Many airborne microorganisms are either pathogenic or can cause sensitivities with prolonged exposure.[11] Airborne microbes attach to dust particles, condense, and enter the human body directly via inhalation or indirectly via the ingestion of contaminated foods and water resulting in the development of disease.[12,13] Airborne bacteria can also affect visibility, climate, and the quality of life.[11,14,15] Methicillin-resistant staphylococci (MRS) have become a major concern to human health because these are either pathogenic/toxigenic or cause a variety of diseases including serious skin, blood, respiratory, or soft tissue infections.[16] Antibiotic-resistant bioaerosols including MRS are commonly reported from residential homes.[17] Therefore, characterization of antibiotic-resistant bacteria and how they prevail during a human gathering is important.

The aim of the present study was to understand the distribution pattern of bioaerosols during an event associated with huge human activities. In India, there is a trend of fairs around the country; Gwalior trade fair is one of them—it is the biggest fair of Madhya Pradesh, and is also one of the most colorful fairs of the whole of north and central India. It was started by the King of Gwalior, Maharaja Madho Rao, in the year 1905. In this study, we monitored the aero-microflora over the fair ground and at nearby locations.

Methods

Air sampling site descriptions

Air samples were collected from two different locations, the fair ground and residential area at Gwalior, a historical city in Central India (longitude 78° 13′ E, latitude 26° 13′ N). Air samples were collected from six different sites over the fair ground near Madhav Institute of Technology and Science (MITS) College covering 104 acres of land at the Race Course Road and from three other sites in residential colonies surrounding the fair ground. Air sampling was done before the fair (December 7, 2010 to January 10, 2011), during the fair (January 11, 2011 to February 13, 2011), and after the fair (March 10, 2011 to March 29, 2011). Air samples were collected on a daily basis for 34 days in three replicates each from six different sites at the fair ground ($n = 34 \times 3 \times 6 = 612$ samples) and three different sites in the residential area ($n = 34 \times 3 \times 3 = 306$ samples). Air samples were collected on eight random days before the fair (fair ground $= 8 \times 3 \times 6 = 144$ samples; residential area $= 8 \times 3 \times 3 = 72$ samples) and after the fair (fair ground $= 8 \times 3 \times 6 = 144$ samples; residential area $= 8 \times 3 \times 3 = 72$ samples). During the fair period, a large number of people visited the fair every day, and it was generally more crowded on weekend days. The residential area remained generally free from the event or crowd gathering. The sampling time was between 5:00 PM and 7:00 PM, a time period when the maximum number of people came to the fair ground. To measure the diurnal distribution of airborne microorganisms, samples were collected every 3 hours each day at 0:00 AM, 3:00 AM, 6:00 AM, 9:00 AM, 12:00 PM, 15:00 PM, 18:00 PM, and 21:00 PM.

Sampling procedure

Samples for microbial analysis were collected using a Reuter centrifugal sampler (RCS; Biotest, Dreieich, Germany) from a height of 1.5 m from the surface to simulate the human breathing zone. A total of 280 L/min of air was collected on sterile air sampling strips at each sampling

time. Sterile air sampling strips were prefilled with 10 mL of microbial content test agar (Difco Laboratories, Detroit, MI, USA) containing 100 μg/mL cycloheximide (Sigma Chemicals, St Louis, MO, USA) for total bacteria and rose bengal agar (Difco) for total fungi. The sterile strips were incubated at room temperature for 48 hours for bacteria and for 2—3 days for counting and identification of fungi. The airborne bacterial and fungal bioaerosol counts were calculated and expressed as colony-forming units per cubic meter of air (CFU/m^3).

Bacterial identification

A representative number of bacterial colonies from each sampling were selected on the basis of morphology, size, color, and texture. The colonies were purified to a single clone on tryptic soy agar (TSA) plate using three-way streaks. The isolates were subjected to a series of standard biochemical assays and BD Crystal Autoreader for the identification of bacteria (Becton Dickinson, Sparks, MD, USA).

Distribution of antibiotic-resistant bacteria

A total of 300 airborne bacterial isolates (before-fair: 100 strains; during-fair: 100 strains; after-fair: 100 strains) collected from six different sites from the fair ground during the event were subjected to antibiotic susceptibility testing by the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[18] The test was conducted in duplicate for each strain.

Characterization of MRS

The methicillin-resistant bacteria were subjected to further identification screening to confirm them as MRS. Presumptive MRS were screened for coagulase and thermonuclease production. A multiplex polymerase chain reaction (PCR) was performed for the identification of the *mecA* gene and differentiation of methicillin-resistant *Staphylococcus aureus* (MRSA) from coagulase-negative-methicillin-resistant staphylococci (CN-MRS) species.[19]

Statistical analysis

The statistical analysis was carried out using SigmaStat. Microbial concentrations were compared using a paired *t* test and analysis of variance (ANOVA). A p value ≤ 0.05 was considered significant.

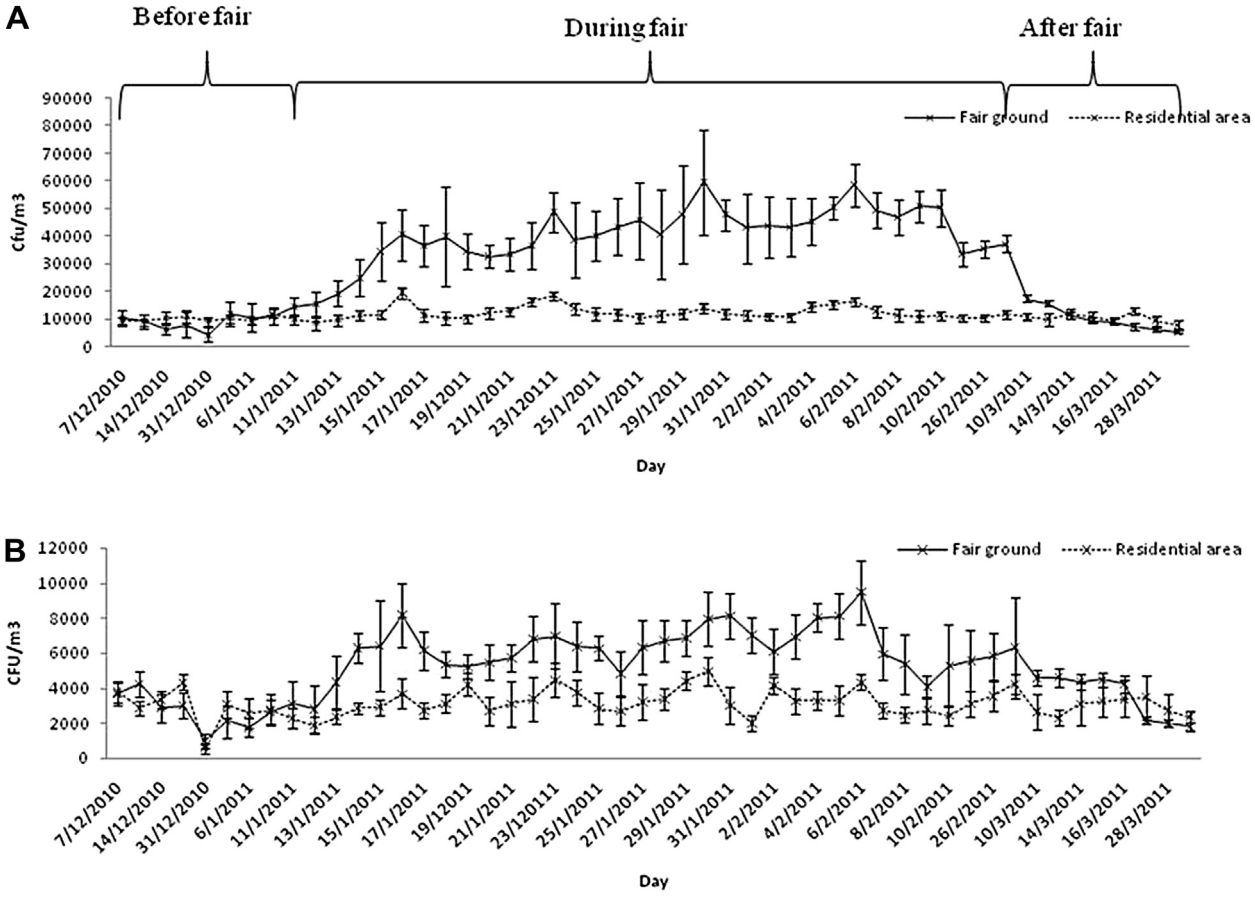

Figure 1 Daily distribution pattern of airborne microbes during Gwalior-Mela. (A) Bacterial bioaerosol in colony-forming units (CFU)/m^3; (B) fungal bioaerosol in CFU/m^3.

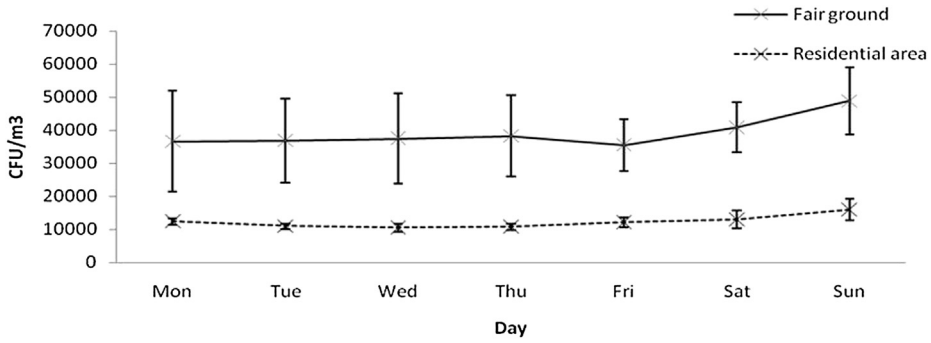

Figure 2 Weekly count of bacterial bioaerosol during Gwalior-Mela at two different localities in colony-forming units (CFU)/m^3.

Results

Daily distribution pattern of bacteria

The total bacterial bioaerosol counts at the fair ground before-, during-, and after-fair were 9035.069 CFU/m^3 (4.3×10^3 to 1.1×10^4 CFU/m^3), 4.0×10^4 CFU/m^3 (4.4×10^3 to 5.9×10^4 CFU/m^3) and 1.0×10^4 CFU/m^3 (5.6×10^3 to 1.1×10^4 CFU/m^3), respectively. The average bacterial bioaerosol during the fair was 4.43 times higher than the average bacterial bioaerosol before the fair period (Fig. 1). Thus, the bacterial bioaerosol during the fair was significantly higher than the counts before- and after-fair ($p < 0.05$). The residential housing area, which was unaffected by the event, was selected as the control site (non-fair). The bacterial bioaerosol over the residential area during the fair period was significantly lower than the bacterial bioaerosol at the fair ground ($p < 0.0001$). The bacterial bioaerosols while before- and after fair periods at the residential area were observed little bit higher than the bacterial bioaerosols over the fair ground in respective periods and this difference was found statistically nonsignificant ($p > 0.05$). Thus, human activity or human gathering affects the total bacterial bioaerosol in the environment.

Distribution of airborne fungi

Airborne fungi exhibited similar distribution patterns as shown for bacteria. The total fungal bioaerosol counts at the fair ground before-, during-, and after-fair were 2.6×10^3 CFU/m^3, 6.1×10^3 CFU/m^3, and 1.7×10^3 CFU/m^3, respectively. The fungal bioaerosol during the fair was 2.38-fold higher than the fungal bioaerosol before the fair period (Fig. 1). The fungal bioaerosol over the residential area during the fair period was significantly lower than that at the fair ground ($p < 0.0001$). However, the fungal bioaerosol counts during the before- and after-fair periods at the residential area were not significantly different from the fungal bioaerosol count over the fair ground ($p > 0.05$).

Weekday distribution of airborne microbes

The weekday distribution pattern of airborne microbes was observed at the fair ground and at the residential area during the fair. The bacterial bioaerosol at the fair ground varied from $3.5 \times 10^4 \pm 7.8 \times 10^3$ CFU/m^3 to $4.8 \times 10^4 \pm 1.0 \times 10^3$ CFU/m^3 (Fig. 2). The maximum count was observed on weekend days (Saturday and Sunday). However, the residential sites had an almost similar pattern of airborne bacterial bioaerosol throughout the week (Fig. 2).

Airborne mycoflora also exhibited almost similar distribution patterns throughout the week. The fungal bioaerosol over the fair ground varied from $4.6 \times 10^3 \pm 1.2 \times 10^3$ CFU/m^3 to $7.7 \times 10^3 \pm 1.2 \times 10^3$ CFU/m^3. Airborne fungi also showed the same weekly distribution pattern as bacteria in both of the studied sites. Higher fungal bioaerosol was observed on Saturday and Sunday as compared to other

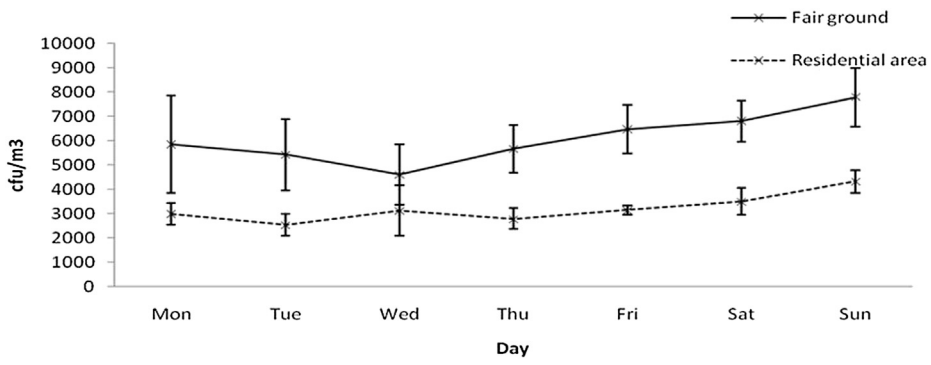

Figure 3 Weekly count of fungal bioaerosol during Gwalior-Mela at two different localities in colony-forming units (CFU)/m^3.

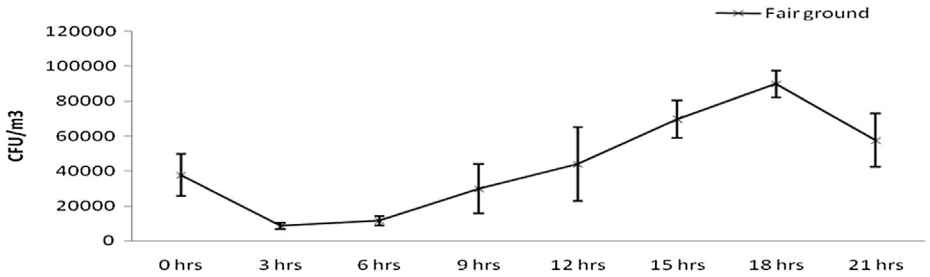

Figure 4 Diurnal count of bacterial bioaerosol during Gwalior-Mela in colony-forming units (CFU)/m^3.

weekdays (Fig. 3). Almost the same pattern of fungal bioaerosol was observed at the residential site.

Diurnal variation in microbial concentrations

Bacterial bioaerosol showed a cyclic pattern of distribution: it started increasing at sunrise, there was a gradual increment at noon, it was maximal at sunset (6:00 PM) (Fig. 4), and then it reduced at night (lowest at ~3:00 AM). Fungal bioaerosol also varied diurnally in almost the same pattern as bacteria, increasing at sunrise, a gradual increase until sunset, and a reduction into the evening with lowest concentrations between 3:00 AM and 6:00 AM (Fig. 5).

Antimicrobial resistance

A total of 300 airborne bacterial strains having clinical importance including *S. aureus*, coagulase-negative staphylococci, *Enterococcus* species, *Bacillus* species, *Escherichia coli*, and *Pseudomonas* species collected from the studied area were subjected to an antibiotic susceptibility test. Antibacterial resistance was significantly increased during-fair as compared to before-fair ($p < 0.01$) (Table 1). The antimicrobial resistance load in the atmosphere was decreased after-fair but the difference from during-fair was statistically nonsignificant ($p > 0.05$) (Fig. 6). The load after-fair was still higher when compared to the load before-fair ($p < 0.05$).

MRS

A total of 52 strains were found resistant to oxacillin on disk diffusion. Eight strains (15%) were found positive for *mecA* and staphylococci-specific 16S ribosomal DNA sequences and termed as MRS (Fig. 7, Table 2). Only one isolate was positive for *clf*A (clumping factor A) and confirmed as MRSA. The remaining seven strains were categorized as CN-MRS. Two MRS strains showed inducible macrolide-lincosamide-streptogramin B (iMLS) phenotype, one showed constitutive macrolide-lincosamide-streptogramin B (cMLS), and three were of the MS phenotype. One isolate was observed as intermediately susceptible to vancomycin.

Discussion

Daily automobile traffic and other anthropogenic activities affect bacterial and fungal bioaerosols in the air.[4,5,9] This was confirmed by higher concentrations of both bacteria and fungi during the fair. Outdoor exposures to airborne microbes are associated with allergic respiratory symptoms, infection, and asthma.[20,21] Many of the fungal species identified in the atmosphere such as *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum*, *Fusarium*, and *Rhizopus* spp. are known to cause allergic reactions.[22] In a study from India, more than half of the viable fungal bioaerosol was found to be allergenic in a skin prick test.[23] Thus, an allergic or asthmatic patient should take care when visiting the fair.

A large number of people visited the fair site at the weekend due to holiday. Human activities including movement, rafting, desquamated skin scales, sneezing, and coughing are the main contributors of elevated viable microbial concentrations in air.[24,25] Therefore, lower fungal bioaerosol was observed during working days and there were higher counts on weekends over the fair ground. Lower microbial concentrations at the residential housing

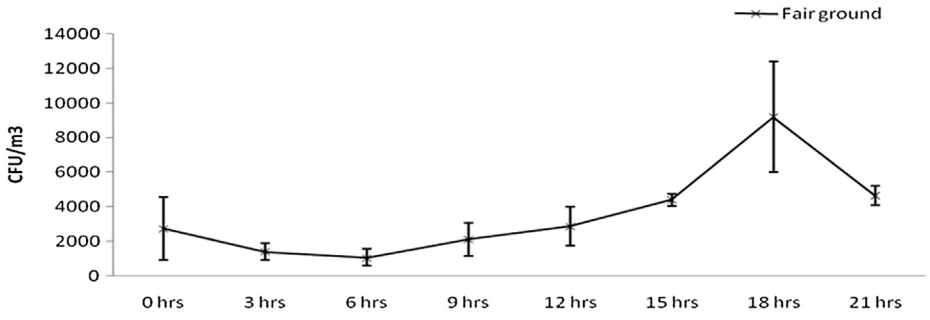

Figure 5 Diurnal count of fungal bioaerosol during Gwalior-Mela in colony-forming units (CFU)/m^3.

Table 1 Percent distribution of antimicrobial resistance of airborne bacteria towards different antibiotics with respect to three different time periods.

Antibiotics	Before-fair (%)	During-fair (%	After-fair (%)	Average (%)
Muperocin	16	33	23	23.83
Rifampicin	13	25	28	21.67
Oxacillin	9	27	16	17.33
Erythromycin	14	26	21	20.33
Clindamycin	11	23	19	17.67
Pristinamycin	9	20	17	15.33
Sulfamethoxazol/trimethoprim	7	25	19	17
Chloramphenicol	9	19	15	14.33
Tobramycin	10	19	8	12.33
Ciprofloxacin	5	17	12	11.33
Vancomycin	0	2	3	1.67
Tetracycline	0	2	0	0.67
Linezolid	0	1	0	0.33

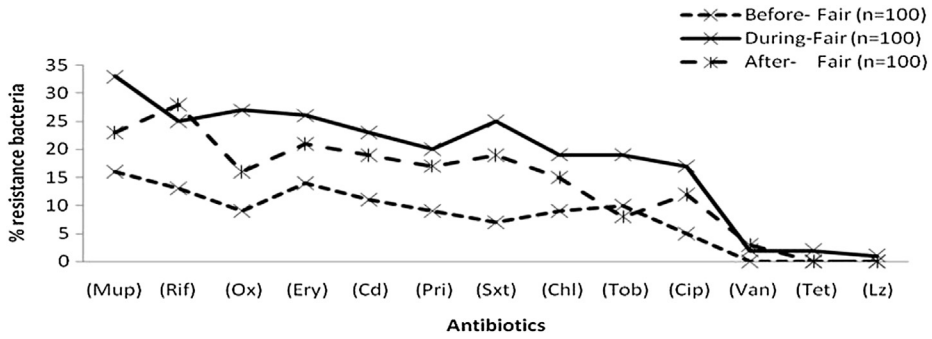

Figure 6 Percent distribution of antibiotic resistance in airborne bacteria. Cd = clindamycin; Chl = chloramphenicol; Cip = ciprofloxacin; Ery = erythromycin; Lz = linezolid; Mup = mupirocin; Ox = oxacillin; Pri = pristinamycin; Rif = rifampicin; Sxt = sulfamethoxazole/trimethoprim; Tet = tetracycline; Tob = tobramycin; Van = vancomycin.

site was not surprising because there were very little human activities during working days. With regard to diurnal distribution, gradually increasing bioaerosol counts were due to the increase in crowd numbers as the day progressed. During the night, people went to their respective places leaving the fair area silent, therefore, microbial particles settled down in the atmosphere, leading to the lowest count during early morning (3:00 AM).

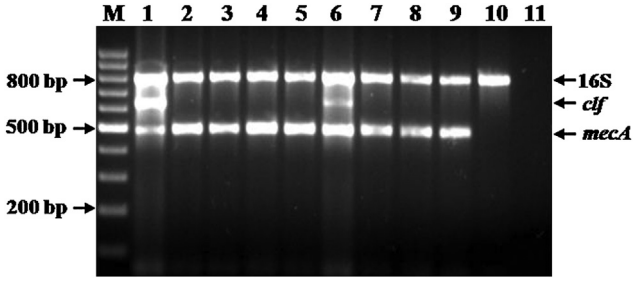

Figure 7 Multiplex polymerase chain reaction (PCR) for the detection of *mecA* gene in *Staphylococcus aureus* and coagulase-negative staphylococci. Lane 1: 100 bp ladder; lane 2: positive control of *S. aureus*; lane 3: MRB05; lane 4: MRB07; lane 5: MRB11; lane 6: MRB12; lane 7: MRB24; lane 8: MRB29; lane 9: MRB34; lane 10: MRB43.

Airborne multidrug-resistant *S. aureus*, *Enterococci*, and *Streptococci* have been isolated from areas of swine confinement, concentrated animal feeding operations, and outside nearby residential houses.[17,26,27] The most common source of airborne bacteria is soil, but antibiotic-resistant pathogens may be discharged into the air mainly from human beings. It is confirmed that flora from desquamated skin scales, sneeze, and cough contribute to elevated bacterial bioaerosols.[25,28] Staphylococci are frequent nasal colonizers in healthy people, and there is a significant increase in nasal colonization of virulent MRSA (USA300), which could be an important source of bacteremia.[29] MRSA is an important feature of modern day health care across the world that can cause superficial and serious life-threatening diseases, even in healthy individuals.[30] Airborne transmission was implicated in a number of outbreaks of MRSA.[31,32] Primary blood stream infections due to MRSA caused an approximate threefold increase in direct costs and prolonged hospital stay when compared with infections due to methicillin-sensitive *S. aureus*.[33] Infection due to multidrug-resistant bacteria and changing patterns of antimicrobial resistance make treatment very difficult because the drug of choice for treating an infection does

Table 2 Molecular and phenotypic characteristics of MRS.

Isolate code	Genotyping (PCR)			Sub-culture on MSA		Sub-culture on BPA		Antibiotic resistance profile								
	mecA	staph 16s	*clfA*	F	G	Zet black	Zone	Ery	Cln	PM	SXT	Va	Cip	Tet	Chl	Tob
MRB05	+	+	−	+	+	+	−	—	—	—	—	—	—	—	—	—
MRB07	+	+	−	+	+	+	−	R	R	R	R	—	—	—	—	—
MRB11	+	+	−	+	+	+	−	R	R^i	R	R	—	—	—	—	—
MRB12	+	+	−	+	+	+	−	—	—	—	—	—	IR	—	R	—
MRB24	+	+	+	+	+	+	+	R	—	R	—	—	—	—	—	—
MRB29	+	+	−	+	+	+	−	R	—	R	—	IR	—	—	—	IR
MRB34	+	+	−	+	+	+	−	R	—	R	R	—	—	—	—	—
MRB43	+	+	−	+	+	+	−	R	R^i	R	R	—	—	IR	—	—

BPA = Baird Parker agar; Chl = chloramphenicol; Cip = ciprofloxacin; Cln = clindamycin; Ery = erythromycin; F = fermentation; G = growth; IR = intermediate resistant; MRS = methicillin-resistant staphylococci; MSA = mannitol salt agar; PM = pristinamycin; R^i = inducible resistance; R = resistant; SXT = sulfamethoxazol/trimethoprim; Tet = tetracycline; Tob = tobramycin; Va = vancomycin.

not work and treatment requires second- or third-line medicines that may be less effective, more toxic, and more expensive.

The study concluded that airborne bacterial and fungal bioaerosols over the fair ground were much higher in the during-fair period when compared to the non-fair period and non-fair sites. The proportion of antibiotic-resistant bacteria was also higher in the during-fair period and remained high in the after-fair period. Therefore, further research is needed in the future during such types of event.

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