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RESEARCH ARTICLE

Aeromicrobiological Study of Different Food Outlets

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ABSTRACT

Aeromicrobiology is the study of bioaerosols. An aeromicrobiological study was conducted from three different food outlets. The different sites selected for study of air microflora includes serving table, cooking site, indoor and outdoor area. A site wise analysis has shown the presence of both bacteria and fungi and very commonly identified species at all the localities were *Staphylococcus* and *Bacillus*, *Aspergillus* sp. *Penicillium* sp. *Rhizopus* sp. *Helminthosporium* sp. *Alternaria* sp. *Epidermophyton* sp. and *Trichophyton* sp. Total nine (2 bacteria and 7 fungi) microorganisms either of human or environmental origin were detected. A maximum number of microorganisms were identified in evening hours of food outlets in comparison to morning hours. The exposed plate technique was used for trapping the aeromicroflora. *Aspergillus* sp. was dominant fungal species followed by *Penicillium* sp. *Rhizopus* sp. *Helminthosporium* sp. in all the localities.

KEYWORDS

Aeromicroflora, Food outlets, Exposed plate technique, Microorganisms, Bioaerosols

INTRODUCTION

Aeromicrobiology is the study of microorganisms living in the air and transported through the air. Microbes suspended in the air are referred to as bioaerosols. Once suspended in the air column, these microbes have opportunity to travel long distance with the help of wind. Several diseases, like influenza, colds are spread by air borne microorganisms. Air borne microbes become an important source of contamination in laboratories, hospitals, industries, exposed food materials and drinks.

The main sources of air borne microorganisms are human beings. The commensal as well as pathogenic flora of the upper respiratory tract and the mouth are constantly discharged into the air by activities like coughing, sneezing and laughing.

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The microbes are discharged out in three different forms like droplets, droplet nuclei and infectious dust. Droplets may also contain hundreds of microbes which may be pathogenic, if discharged from diseased persons. The allergy causing agents present in the air are called aeroallergens, and the allergy caused by them is called aeroallergy. Some of the prominent aeroallergens are house dust, pollen grains, microbial spores or cells, etc. In addition to gases, dust particles and water vapors, air also contains microorganisms. Microbial communities present in the air include viruses, bacteria, fungi, protozoans etc. The airborne microbes are related to both the useful and harmful aspects. The useful aspects are microorganisms present in the air are involved in various fermentation products including production of alcoholic beverages, vinegar and dairy product. Harmful aspects involved microbes causes spoilage of food stuffs, spoilage of food and fermentation product and cause disease in humans. The air borne

microorganisms are the major source of contamination in food items also causing deterioration of food. Most food contains sufficient nutrients to support microbial growth. Several factors such as water availability, pH and temperature encourage/ prevent the growth of microorganisms in foods.[1,2] Microorganisms in fast food (pizza, burger) are responsible for many human diseases.[3,4,5] *Salmonella* bacteria are a common cause of food borne illness, particularly in under cooked chicken and chicken eggs.[6,7] The objective of the present study was to investigate the occurrence of aeromicroflora both bacteria and fungi in different food outlets in Haridwar and its adjoining area.

MATERIAL AND METHODS

Description of Locations

In the present study, three different food outlets of Haridwar (U.K), India especially, Roorkee (A and B) and Bahadrabad (C) were selected for the study of aeromicroflora. The different sites were serving table, cooking site, indoor and outdoor area of food outlets were selected for the quantitative screening of microflora.

Air Sampling

The exposed plate technique was used for the study of aeromicroflora in which three different types of media were used viz., Nutrient agar media, Czapex-Dox agar media, Sabouraud's agar media. In the exposed plate technique, petri plates containing a suitable agar medium were horizontally exposed for 10 minutes. After exposing, the plates were incubated in inverted position at 28^0C for 5-7 days (for fungi) and at 37^0C for 24-48 hours (for bacteria). After the incubation period fungal and bacterial colonies were enumerated. The study was conducted in morning (9:30-10:30 AM) and evening (6:00-7:00 PM).

Identification

The fungi were isolated and identified by Lacto phenol cotton blue staining while bacteria were isolated and identified by Gram's staining and characterized by different biochemical tests (Catalase test, Starch hydrolysis test, Carbohydrate fermentation test and Methyl red test).

Antibiotic sensitivity test[8]

The antibiotic sensitivity of isolated fungi and bacteria were studied by disc diffusion method. For bacteria multidisc was used, having 12 different antibiotics. For fungi single disc of fluconazole was used.

RESULTS AND DISCUSSION

Two bacteria and 7 genera of fungi were isolated and identified from all sites (Table 1). *Aspergillus* sp. was dominant fungal species followed by *Penicillium* sp. *Rhizopus* sp. *Helminthosporium* sp. in all the localities. The fungi were identified by lacto phenol cotton blue staining while bacteria were identified by Gram's staining and characterized by different biochemical test (Table 4). The antibiotic disc diffusion method was used for the isolated bacteria and fungi (Table 5, 6, 7).

In the present study the bacterial and fungal colonies were obtained as 10 CFU (Indoor), 9 CFU (Outdoor), 11 CFU (Cooking site) and 10 CFU (Serving table) (for fungi) and 205 CFU (Indoor), 186CFU (Outdoor) 280 CFU (Cooking site) and 225 CFU (Serving table) (for bacteria) respectively, which were found below the acceptable levels, when compared with many health organization standardization. The National Institute of Occupational Safety and Health (NIOSHI-USA) allows[9] up to 1000 CFU m^{-3} air while World Health Organization[10], allows 500 CFU m^{-3} air.

According to the present study number of viable counts of bacteria was exceeded over the number of viable counts of fungi. Similar results were also reported by Gorney and Dutkiewicz, (2002)[11].

The most abundant fungi isolated was *Aspergillus* (42.58%) followed by *Penicillium (36.12%)* and *Rhizopus* (12.25%) while according to Singh and Rajput, (2011)[12] the most abundant fungi was *Aspergillus (*32.39%) followed by *Microsporum* (22.29%) and *Mucor* (8.79%).While the study conducted by Singh and Parasher, (2006)[13] the most abundant fungi was

Aspergillus (36.91%) followed by *Penicillium* (32.39%) and *Alternaria* (22.99%). But according to Wanner *et al.*, (1993)[14] the most common fungi isolated from indoor air environment were *Penicillium, Aspergillus, Cladosporiuma and Alternaria*.

Antibiotic sensitivity of bacteria and fungi isolated were studied by disk diffusion method, in which *Staphylococcus* was found sensitive to Ciprofloxacin and resistant to many antibiotics such as Cefazolin, Amoxicillin, Erythromycin etc. while *Bacillus* was sensitive to Tetracyclin, Gentamicin and resistant to Ciprofloxacin, Cloxacillin. Gulhan *et al.*, (2007)[15] also reported that Methicillin resistance was seen up to 80% especially in *Staphylococcus aureus* in Turkey. Some *Staphylococcus* strains showed intrinsic heterogenous resistant to non-beta-lactum antibiotics (Erythromycin, Tetracyclin). This situation raises the difficulty in treating multi drug resistant *Staphylococci*.[16] According to Barnes, (2008)[17] prolonged exposure to variety of bacteria and fungi produce chronic health problems.

Table 1: Different types of aero microbes isolated from all sites

S.NO.	Name of different aero microbes	Different sites
1	*Bacillus* sp.	Serving table
2	*Staphylococcus* sp.	
3	*Aspergillus* sp.	Cooking site
4	*Penicillium* sp.	
5	*Rhizopus* sp.	Indoor
6	*Helminthosporium* sp.	
7	*Alternaria* sp.	Outdoor
8	*Epidermophyton* sp.	
9	*Trichophyton* sp.	

Table 2: Enumeration of isolated bacterial colonies (Average of triplicates)

A-	**Site A-Roorkee**	**Morning (Mean of CFU ± S.E)**	**Evening (Mean ± S.E)**
1	Indoor	205±5.0	245±5.0
2	Outdoor	186±3.0	242±2.0
3	Cooking site	280±14.0	TNTC
4	Serving table	225±5.0	TNTC
B-	**Site B-Roorkee**		
1	Indoor	300±20.0	235±3.0
2	Outdoor	282±2.0	280±10.0
3	Cooking site	234±4.0	196±2.0
4	Serving table	TNTC	198±3.0
C-	**Site C-Bahadrabad**		
1	Indoor	TNTC	205±2.0
2	Outdoor	281±3.0	229±2.0
3	Cooking site	TNTC	235±2.0
4	Serving table	270±2.0	320±3.0

Table 3: Enumeration of isolated fungal colonies (Average of triplicates)

A-	**Site A-Roorkee**	**Morning (Mean of CFU ± S.E)**	**Evening (Mean ± S.E)**
1	Indoor	10±2.0	11±2.0
2	Outdoor	9±1.0	10±3.0
3	Cooking site	11±1.0	12±3.0
4	Serving table	10±3.0	15±2.0
B-	**Site B-Roorkee**		
1	Indoor	12±2.0	11±4.0
2	Outdoor	11±2.0	10±4.0
3	Cooking site	20±1.0	15±1.0
4	Serving table	16±2.0	15±3.0
C-	**Site C-Bahadrabad**		
1	Indoor	12±1.0	12±1.0
2	Outdoor	15±3.0	10±1.0
3	Cooking site	16±3.0	13±1.0
4	Serving table	13±2.0	12±4.0

Table 4: Showing morphological and cultural characteristics of bacterial isolates from air

S. no	**Character-istics**	**Bacterial isolates**	
1	Gram's staining	*Bacillus* sp.	*Staphylococcus* sp.
2	Colony	Opaque growth, smooth, margin irregular	Yellowish colony, smooth, regular margin
3	Cell shape	Rods	Cocci
4	Cell arrangement	In chains	In cluster
5	Catalase test	Positive	Positive
6	Lactose	Negative	Positive
7	Sucrose	Positive	Positive
8	Dextrose	Positive	Positive
9	Starch hydrolysis	Positive	Negative
10	MR	Negative	Positive
11	VP	Negative	Negative
12	Nitrate reduction	Negative	Positive

Table 5: Showing antifungal sensitivity of fluconazole against isolated fungi (Average of triplicates)

S.no	Name of fungi	Sensitivity	Zone of inhibition
1	*Aspergillus* sp.	R	_
2	*Penicillium* sp.	R	_
3	*Rhizopus* sp.	R	_
4	*Helmithosporium* sp	S	13 mm

R= Resistant

S= Sensitive

- = No zone of inhibition

Table 6: Showing antibacterial sensitivity of *Bacillus* sp. (Average of triplicates)

S.no	Antibiotics	Symbol	Strength(mcg)	Zone of inhibition(mm)	Sensitivity
1	Amphicillin	AS	20	7	I
2	Co- Trimoxazole	BA	25	_	R
3	Cephalexin	PR	30	_	R
4	Cefotaxime	CF	30	10	I
5	Ciprofloxacin	RC	5	_	R
6	Levofloxacin	QB	5	_	R
7	Linezolid	LZ	30	_	R
8	Cloxacillin	CX	1	_	R
9	Roxythromycin	AT	15	_	R
10	Lincomycin	LM	2	_	R
11	Tetracyclin	TE	30	18	S
12	Gentamicin	GM	10	10	I

I=Intermediate R= Resistant S= Sensitive

Table 7: Showing antibacterial sensitivity of *Staphylococcus* sp. (Average of triplicates)

S.no	Antibiotics	Symbol	Strength	Zone of inhibition(mm)	Sensitivity
1	Amoxicillin	AM	10	_	R
2	Cefazolin	CF	30	_	R
3	Cephalexin	CP	30	_	R
4	Roxithromycin	TH	30	_	R
5	Cefadoxil	CD	30	_	R
6	Erythromycin	E	15	_	R
7	Ciprofloxacin	CL	5	12	I
8	Vancomycin	VN	30	_	R
9	Ofloxacin	OF	5	_	R
10	Sparfloxacin	SP	5	_	R
11	Amphicillin	I	10	_	R
12	Cloxacillin	V	5	_	R

I= Intermediate R= Resistant

CONCLUSION

From the present study it is concluded that different aeromicroflora were present in all the food outlets in which some dermatophytes and pathogenic bacteria and fungi were also found. Due to higher concentration of viable counts of bacteria and fungi in food outlets it is considered unfit for human beings both for workers and visitors there because these aeromicroflora are known to cause various allergic reactions as well as other diseases. As the fungi isolated (*Aspergillus, Penicillium*) are known to produce aflatoxins and the bacteria (*Bacillus, Staphylococcus*) isolated are also the major causative agents of various diseases such as food poisoning, nausea, vomiting and diarrhoea. So it is advisable that strict measures should be put in place to check the increasing microbial load and also it is recommended that comprehensive exposure assessment programs should be conducted in various food outlets.

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