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AIR MICROBIOLOGY IN CHENNAI CITY: A PILOT STUDY

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Abstract: A pilot study was undertaken to assess the microbiological quality of the air in Chennai city. A total of ten representative places in Chennai city were randomly selected. Multiple air sample were collected using Air Sampler System with TSA- Agar strips and subjected to cultural isolation and colony growth ranged from 25.0 to 112.5 cfu/m^3. Pathogenic *Staphylococcus aureus* were isolated from five places of Chennai city using selective media and confirmed by suitable biochemical tests. Culture positive samples also produced a product size of 181bp by targeting Nuc gene in polymerase chain reaction. Antibiotic susceptibility pattern revealed that all the samples were resistant to cephalexin, cephalothin and penicillin. Seventy, fifty and eighty per cent of the isolates were resistant to methicillin, vancomycin and oxytetracycline, respectively. Present study indicates that microbiological quality of the air in the selected places of Chennai city is poor and appropriate measures should be taken to address this issue.
Keywords: Culture isolation, Polymerase chain reaction, *Staphylococcus aureus.*

1. Introduction

Air quality in India is deteriorating at a faster pace due to the advent of industrial era and fast growing vehicular fleet. Rapid industrialization, urbanization and transport network across India poses a new set of challenge for the country's cities. Unlike other city, Chennai represents a different pollution challenge. Although pollution levels were lower comparatively than other cities, it still varies between moderate to critical. Industrial belts and motorized vehicles added in Chennai every day makes the situation much worsen. Air pollution levels in Chennai have reached a record high with the suspended particulate matter in many pockets of the city at 45 per cent above the permissible limit. Chennai have hot and humid weather for most of the year. Meteorological factors such as wind velocity, wind direction, temperature and relative humidity together with earth surface roughness are effective agents for mixture of air pollutants. Air pollution in Chennai is always thought to be due to pollutants and other various chemicals but air microbiology is always neglected. Hence, there is an urgency to study air pollution as a public health issue and to understand the scientific health risks that arises from air pollution in Chennai in spite of this there is a

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paucity of scientific studies on the health effects caused by airborne pathogens. In this view the present study was designed to assess the bacterial load present in the air at public places with large gathering of humans and to elucidate the true status of microbial load in such environments.

2. Materials and methods

Study area

In this study, ten representative places were randomly selected from identified overcrowded places of Chennai City and designated as S1 to S10 namely; T. Nagar, Guindy, Chennai Beach, Chennai Park, Chennai Central, Chennai Egmore, Saidapet, Tambaram, Koiambedu and Parrys Corner, respectively (Fig. 1).

Collection of air samples

Particle capture mechanism of the air sampler uses centrifugal impaction. Impactor air samplers are the most widely used for the quantification of contamination [1, 2]. In this study, representative air samples were collected from each selected places of Chennai city using Air Sampler System with TSA- Agar strips (Hi-media, Mumbai) as per the manufacturer's protocol.

Culture isolation and identification

Generally airborne microbiological contaminants are less than 2.5μ despite the air sampler precipitates particles having cut off size of 1.2 to 2.1 μ. The agar media namely *viz.* Baird Parker Agar (BPA), Bismuth Sulphite Agar (BSA), Eosin Methylene Blue (EMB) agar were used for isolation of zoonotic bacterial pathogens like *Staphylococcus* spp., *Salmonella* spp. and *E. coli* organisms, respectively. Different species of *Staphylococcus* were identified and differentiated by using Histaph Kit (Hi-media, Mumbai). Mannitol salt agar was used as a selective media for the isolation of pathogenic *Staphylococcus aureus* which ferments mannitol and produce yellow colonies surrounded by yellow zones. Presence of *Staphylococcus aureus* was further confirmed by detecting the thermostable deoxyribonuclease activity in Toludine blue DNA agar. Presumptive colonies were subjected to Gram's staining to visualize the morphological characteristics of the organisms. Simultaneously, colonies were subjected to catalase and oxidase tests for further confirmation.

Calculation of Colony Forming unit (CFU)

Total count in TSA strips was calculated as follows

$$CFU/l = \frac{\text{Colonies on agar strip}}{40 \text{ X Sampling time in min}}$$

One min corresponds to 40 liters of air separation volume.

$$CFU/m^3 = \frac{\text{Colonies on agar strip X 5}}{\text{Sampling time in min}}$$

Antibiotic Sensitivity Test (ABST)

Antibiotic susceptibility pattern was studied by performing Antibiotic Sensitivity Test by following Kirby-Bauer (K-B) disk diffusion method and the diameter of zone of inhibition was measured by using antibiotic zone scale [3].

Polymerase Chain reaction

Culture positive *Staphylococcus aureus* samples were subjected to Polymerase Chain Reaction (PCR) by targeting Nuc gene at 181 bp as per the protocol performed by Hedge [4].

Sequence of primers used in this study

Target	**Primer Sequence 5' – 3'**	**Amplicon size (bp)**	**Reference**
nuc-F	GTGCTGGCATATGTATCGCAATTGT	181	Hedge,2013
nuc-R	TACGCCCTTATCTGTTTGTGATGC		

3. Results

Samples collected from selected places of Chennai city were shown colony growth on TSA with range from 25.0 to 112.5 cfu/m^3. Number of colony forming units for each sampling places are shown in Table 1. In BSA and EMB agar, specific colonies for the organisms were not noticed, whereas in BPA, grey black colonies with white rim and a clear zone were noticed. All these colonies were shown catalase positive and oxidase negative. Samples collected from five places (Koyambedu, Chennai Central, Egmore, Gundy and Park Station) were fermented the mannitol and produced yellow colonies surrounded by yellow zones in Mannitol Salt Agar (Fig. 2). Same samples produced a pink halo established around 1mm beyond the well cut in Toludine blue DNA agar and indicates that air samples containing pathogenic *Staphylococcus aureus* (Fig. 3). These samples also produced a product size of 181bp by targeting Nuc gene in PCR (Fig. 4).

Antibiotic susceptibility pattern of the air samples collected from 10 different places is shown in Table-2 and Figure-4. All the samples were resistant to cephalexin, cephalothin and

penicillin. About 70, 50 and 80 per cent of the isolates were resistant to methicillin, vancomycin and oxytetracycline, respectively.

4. Discussion

Air micro flora has significant effect in human health: Human being inhales air every moment. Even most of the micro organisms present in air are harmless but still less than 1% of the airborne bacteria is pathogens. Significance of microbes in public health: micro organisms produce a lot of disease, especially methicillin resistant *Staphylococcus aureus* and gentamicin resistant gram negative bacteria are found to be serious in nowadays.

In this study, representative air samples were collected from ten different places and subjected to culture isolation and identification by morphological, biochemical and molecular methods. The result indicated, Koyambedu is highly polluted with microbes followed by Chennai Central and other places also had significant level of microbial contamination. Koyambedu has a major hub of activity in Chennai city after the inauguration of the Koyambedu market and the Chennai Mofussil Bus Terminus. Overcrowding [5] and dumping of waste [6, 7, 8] in this area are the important determinants associated with microbial contamination of air. Chennai Central also has significant level of overcrowding with high level of floating population.

Among ten different places, *Staphylococcus aureus* was isolated from Koyambedu, Chennai Central, Egmore, Gundy and Park Station. *S. aureus* usually acts as a commensal bacterium, asymptomatically colonizing about 30% of the human population, it can sometimes causes bacteraemia, infective endocarditis, skin and soft tissue infections [9]. *Staphylococcus aureus* can survive for several months in dust particles [10] in-spite of adverse environmental conditions [11].

Antibiotic sensitivity indicated that isolates from ten different places were resistant to cephalexin, cephalothin and penicillin. Seventy per cent of *S. aureus* isolates were resistant to methicillin. Methicillin resistant *S. aureus* (MRSA) is now endemic in India [12] and incidence of MRSA varies from 25% in western part of India [13] to 50% in South India [14].

Conclusion

Present study indicates that microbiological quality of air in the Chennai city is poor. Moreover, more than 50% of the places under this study are highly contaminated with pathogenic methicillin resistant *Staphylococcus aureus*. This shows that appropriate stringent measures to be taken for monitoring and curtailing the microbiological contamination of air.

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Table 1 Colony forming units for each sampling places

Sample sites	**CFU/L**	**CFU/m^3**
T Nagar (S1)	1.1	68.75
Guindy (S2)	0.7	43.75
Chennai Beach (S3)	0.4	25
Chennai Park (S4)	0.6	37.5
Chennai Central (S5)	1.7	106.25
Chennai Egmore (S6)	0.9	56.25
Saidapet (S7)	1.5	93.75
Tambaram (S8)	0.6	37.5
koiambedu (S9)	1.8	112.5
Parrys Corner (S10)	1.2	75

Table 2 Antibiotic sensitivity pattern

Places of Chennai City	Antibiotic discs (Zone of inhibition in mm)						
	Gentamicin (10 mcg/disc)	Cephalexin (30 mcg/disc)	Methicillin (5 mcg/disc)	Oxytetracyclie (30mcg/disc)	Cephalothin (30 mcg/disc)	Vancomycin (30mcg/disc)	Penicillin (2 units)
1	20	13	-	-	-	18	-
2	25	-	-	-	-	19	-
3	18	11	10	-	10	16	-
4	21	-	10	-	-	16	10
5	24	10	-	-	-	19	-
6	21	-	-	22	-	19	-
7	18	-	12	-	-	13	-
8	25	9	-	24	-	14	-
9	24	10	-	-	11	21	-
10	21	-	-	-	10	16	-

Fig. 1 Study areas: Selected places of Chennai city

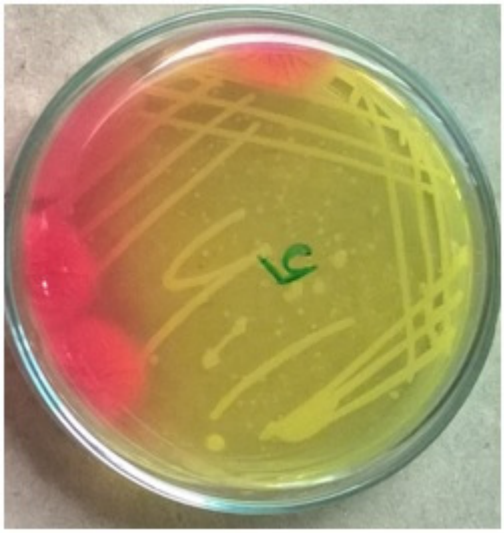

Fig. 2 *Staphylococcus aureus:* Yellow colonies surrounded by yellow zones in mannitol salt agar

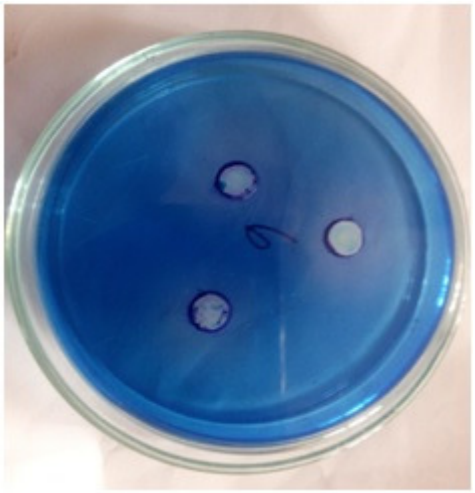

Fig. 3 *Staphylococcus aureus*: Pink halo established around 1mm beyond the well cut in Toludine blue DNA agar

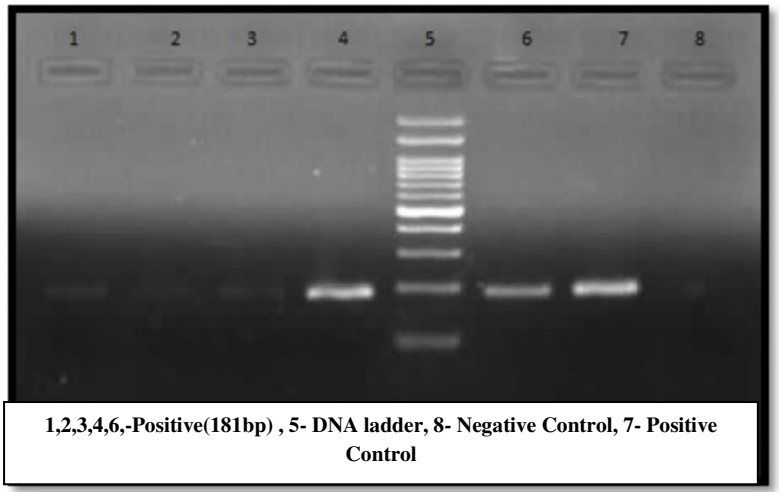

Fig. 4 *Staphylococcus aureus*: Nuc gene amplification by PCR