Isolation and Identification of Microorganisms From Air Conditioning Systems With Special Reference To Legionella Species

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Abstract- *An air conditioning system is vital in many of today's households. But there are many diseases which spread through air conditioners, like Pontiac fever, Legion fever, Aspergillosis, Mycetoma, Staphylococcal infection, pneumonia, asthma, etc. The study was carried out for the isolation and identification of bacteria, especially Legionella species, and fungi, from air conditioners in different locations. Air samples, A.C. filter samples, water from cooling tower and swabs from temperature adjustment knob were collected from hospitals, banks, laboratories, hostels and cars. They were inoculated onto appropriate media and incubated. The colonies obtained were identified by standard procedures. Three species of Legionella, namely, Legionella micdadei, Legionella pnuemophila and Legionella bozemanii were isolated from all the locations. Many bacterial and fungal isolates were identified from all the locations. Our study shows the presence of Legionella species in air conditioners. Legionella causes Legionellosis which is a condition similar to pneumonia and can appear as a mild flu-like illness or a more severe respiratory condition.*

Keywords- Air conditioning, Legionella, Indoor and Outdoor air, Air filters.

I. INTRODUCTION

An air conditioning system is vital in many of today's households. Proper use of air conditioning could help to save somewhere close to 2 billion gallons of fuel within the U.S., perhaps as much as 3 to 5 billion gallons worldwide according to estimates given by the Environmental Protection Agency (EPA)[1].

Biological contaminants of indoor air have gained considerable attention by Public authorities following outbreaks of serious diseases caused by microorganisms[2]. Indoor air contains many microorganisms capable of causing infectious disease, allergy and irritation of the respiratory system and respiratory infections many of which are transmitted inside, account for some 50-60% of all community acquired illness[3].

There are a large variety of modern appliances which provide ideal conditions for the proliferation of microorganisms in fact any uncleaned surfaces or stagnant water reservoir can act as a serious source of microorganisms. Of these, the ones with built in operation for dispersal through the air pose the greatest danger, by their very operation[4]. Contaminated water coming in contact with the rapidly moving airstream that picks up either small particulates (such as bacteria) or small droplets of water itself (containing the bacteria), spraying them into room[5]. Areas of particular susceptibility include air conditioners, humidifiers, cooling towers, vaporizers, nebulizers, cold-mist vaporizers, spas, hot tubs, showers, hot water systems, that don't maintain temperatures above 70°C[6].

The present study was done to isolate and identify the microorganisms present in air conditioning systems in different locations. To identify and characterize bacteria and fungi from the samples. To isolate and identify *Legionella* species from the air conditioning systems. To access the microbial contamination in air conditioning systems in various places like Hospitals, Banks, Laboratories, Hostels and Cars. To study the significance of the presence of various bacteria and fungi in the A.C's. To create awareness about proper cleaning and maintenance of the air conditioning systems.

II. MATERIALS AND METHODS

Samples were collected from various locations like hospitals, banks, laboratories, hostels and cars. Sterile swabs were used to collect the samples from A.C filters and adjustment knobs. The water dripping from the unit was collected in a sterile conical flask.

Air samples were collected by settle plate method[7]. Sterile media plates were opened and exposed to the air blowing out of the unit for 5 minutes. The samples were

transported to the laboratory and were inoculated on to suitable media aseptically. Air settle plates on nutrient agar (NA) and Sabouraud's dextrose agar (SDA) were incubated at 37˚C for 24 hours and at room temperature for 2-5 days, respectively. Buffered Charcoal Yeast Extract agar (BCYE) was used for isolation of *Legionella* species[8]. The plates were incubated at 35±2˚C for 3-4 days.

Water samples and swabs were inoculated on to NA, SDA and BCYE agar plates and incubated appropriately. After incubation the colonies were observed and identified by appropriate staining procedure, subculturing on to selective media (Mannitol salt agar, MacConkey agar, DNase agar) and biochemical tests.

III. RESULTS

The study was carried out for the isolation and identification of bacteria, especially *Legionella species* and fungi from air conditioners in different locations. Samples from hospitals, banks, laboratories, hostels and cars were obtained. The samples were collected from air conditioner air, filter, water and temperature adjustment knob. They were processed and the isolates obtained were identified by standard procedures. 3 species of *Legionella,* namely, *Legionella micdadei, Legionella pnuemophila and Legionella bozemanii* were isolated from all the locations. (Figure 1)

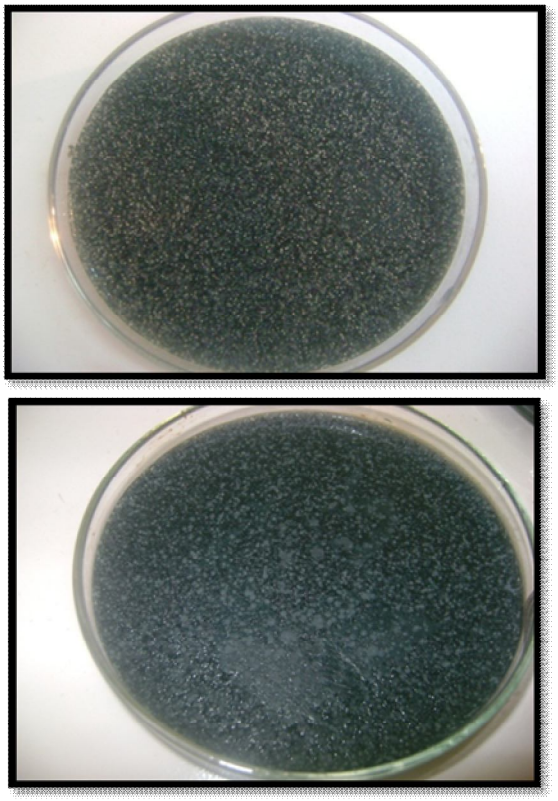


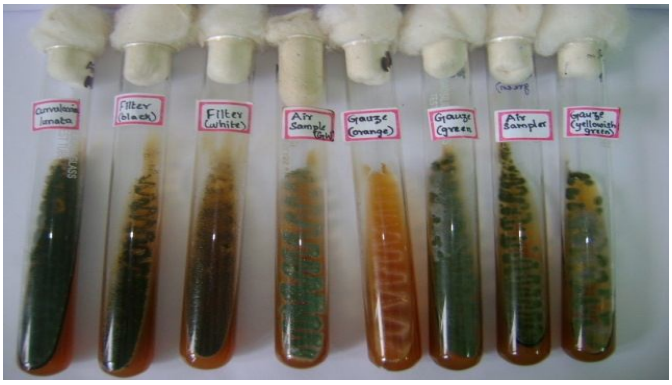
Figure 1: Growth of Legionella spp on buffered charcoal yeast extract agar

Among the bacteria isolated, hospital samples showed the presence of *Staphylococcus aureus, Staphylococcus citreus, Staphylococcus epidermidis, Escherichia coli, Bacillus species, Pseudomonas aeruginosa, Proteus mirabilis* and *Proteus vulgaris.*. Samples from banks showed the presence of *Staphylococcus aureus, Staphylococcus citreus, Staphylococcus epidermidis, Escherichia coli, Bacillus species, Pseudomonas aeruginosa,* and *Proteus vulgaris.*

Samples from laboratories showed the presence of *Staphylococcus aureus, Staphylococcus citreus, Staphylococcus epidermidis, Escherichia coli, Bacillus species, Pseudomonas aeruginosa,* and *Proteus vulgaris.* Samples from hostels showed the presence of *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Bacillus species, Proteus mirabilis and Pseudomonas aeruginosa*.

Samples from cars showed the presence of *Staphylococcus aureus, Staphylococcus citreus, Staphylococcus epidermidis, Escherichia coli, Bacillus species, Pseudomonas aeruginosa,* and *Proteus mirabilis.*

Among the fungal isolates, hospital samples showed the presence of *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor species, Penicillium species, Trichophyton species* and *Yeast.*



Samples from Banks showed the presence of *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor species, Penicillium species, Trichophyton species, Yeast* and *Curvularia lunata*. Samples from laboratories showed the presence of *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor species, Penicillium species, Yeast, Curvularia lunata* and *Rhizopus species.*

Samples from hostels showed the presence of *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor species and Penicillium species.* Samples from Car

samples showed the presence of *Aspergillus flavus, Aspergillus fumigatus, Mucor species, Penicillium species, Trichophyton species, Aspergillus niger* and *Yeast* .

IV. DISCUSSION

Air conditioners produce dew and wet conditions inside their apparatus, when in operation. Microbial contamination inside air conditioners which are used every day is much higher than those not used daily or rarely. Airborne contamination is controlled by the environmental conditions of the rooms in which air conditioners are used[12]. Our study shows the presence of *Legionella species* in air conditioners. It causes legionellosis which is a chest condition similar to pneumonia and can appear as a mild flu-like illness or a more severe respiratory condition. Legionellosis generally carries a high mortality rate (15 to 20%). These results corresponds to the work done by Sabria *et al.*, (2006) [9] who isolated Legionella species from air conditioning cooling towers.

Among the bacterial isolates *Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus citreus* were predominant. *Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa, Bacillus species* and *Proteus vulgaris* were also isolated. These finding corresponds to the results of a study conducted by Ekhaise and Omavwoya, (2008)[10].

The fungi isolated were *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium species, Mucor, Trichodermophyton species, yeast, Curvularia lunata and Rhizopus species*. This corresponds to the work done by Ekhaise *et al.* (2008)[11] who found the presence of *Aspergillus species, Penicillium, Mucor* and *Fusarium* from hospital air conditioners.

Microbial contamination inside air conditioners, which are used every day is much higher than those not used daily, example a few days per week or rarely. Cleaning is the simplest treatment for controlling microbial contamination of air conditioners, because the contaminants on the filter can be removed together with dust by vaccum cleaning or by washing with water and detergent. Fungal contamination of air conditioners is found not only in the filter but also in fan and heat exchanger[13].

Ozawa (1996)[14] reported that the number of fungi discharged from the air conditioner during the first 10 minutes of use is 10- fold greater than that during 30-40 minutes use. Opening the window for about 10 minutes is therefore very effective to prevent human ingestion of fungal spores.

V. CONCLUSION

The control of indoor microorganisms involves the elimination of sources as well as on which they are able to grow. By keeping indoor surfaces clean and dry and maintaining a relatively low humidity (ie, below 50%) microbial growth can be prevented as does regular and thorough cleaning of appliances of possible risk. Anti-microbial agents are useful in cleaning known sources but are not a long term solution unless environmental changes also occur. Disinfectants, bleaches and phenols combat most infections however, these chemicals are themselves known to have adverse effects on human health and treatment with ultraviolet (UV) lamps may be effective in controlling some bacterial contamination. Maintaining a dust free environment is necessary in keeping airborne microorganisms to a minimum.

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