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ISOLATION OF DIFFERENT MICROORGANISMS FROM HOSPITAL SITES

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ABSTRACT

Microorganisms are present abundantly on earth's atmosphere as particle or bacteria, fungi, lichen and algal cell. The concentration and composition of these particles are generally related to man's activities and atmosphere. Atmospheric pollution is one of the most common problems of our era. This pollution has now reached an advance level that possesses threat to the health and wellbeing of the people. The increase pollution is becoming threat to human population. The presence of micro-organisms in environment may be beneficial and sometimes can be harmful, especially hospital microflora may cause harm to human beings and may leads to number of diseases and in immunocompromised patients leading to nosocomial infections. In the present study nine different common bacterial species and 2 fungal species were isolated from Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre.

KEYWORDS: *Staphylococcus aureus*, bacteria, microflora and fungus.

INTRODUCTION

Bacterial infection plays a considerable role in causing diseases. Air is not a suitable medium for growth of the pathogenic bacteria, any pathogen, that airborne must have originated from a source such as humans, animals, plants, soil, food or water. There are number of people die every year from hospital infections. There are number of pathogens thrive in hospital environment which may get transmitted to other patients having long stay in hospital or may develop resistant to drugs. *Streptococcus pyogenes* was perhaps the most important cause of hospital infection, now strains have been isolated which are resistant. Another strain is *Staphylococcus aureus* strains, found resistant to number of drugs and are responsible to nosocomial infection.[1]

Human body contains normal flora which is helpful in body systems. But some microbes invade human body and cause harm and diseases. These disease causing organisms can be transmitted from one to other individuals leading to nosocomial and hospital borne infection. The collection of different microorganisms, mainly bacteria, in a host body which does not cause any harm to human body is known as flora**.** Microflora responsible for harmful diseases is often Allochthonous flora. Microbes are distributed everywhere in the environment surroundings, they are found in the air, water, soil, plant, animal, food product, in the human body and on the surface of the human body. Microorganisms are classified into Archaea, bacteria, fungi, algae, virus, protozoa and multi-cellular organisms.[2]

Nosocomial infections also known as hospital acquired infection are infections acquired from healthcare services (hospitals) during treatment, which are secondary to the patients original condition. The term Nosocomial infection is used for Hospital-Acquired Infection and Health care associated infection (HAI or HCAI). The source and spread of organisms inside the hospital are important issues, human related organisms or the body flora, also found in clothing are spread through shedding during human activities.[3] Organisms frequently involved in hospital infections include, *Staphylococcus aureus*, *Micrococcus* spp., *Pseudomonas* spp., alpha - hemolytic *Streptococci*, *Cladosprium* spp., *Aspergillus* spp. and viruses.[3] An infection can be acquired in hospital, nursing home rehabilitation facility, outpatient clinic, diagnostic laboratory or other clinical settings. Infection spreads to the susceptible patient in the clinical setting by various means. The hospital borne infection depends upon the hygienic conditions of the hospital environment and number of visitors[4] Other factors include the quality of the hospital systems and mechanical movement within the enclosed space.[5,6] Health care staff also spread infection, in addition to contaminated equipment, bed linens, or air droplets. The infection can originate from infected patients,

environment & unhygienic staff or in some cases the source of the infection cannot be determined, whereas, sometimes infection spreads from patients own skin microflora this may be due to weak immunity & patients after surgery.[7]

Hospital-acquired infections are caused by viral, bacterial, and fungal pathogens the most common type are Bloodstream infection (BSI), pneumonia (e.g., ventilator associated pneumonia (VAP), Urinary tract infection (UTI), and Surgical site infection (SSI). Hospital-acquired infection (HAI) is defined as an infection acquired by patients during a short or prolonged hospital stay.[8]

The HAIs are responsible for not only significant morbidity and mortality, but socioeconomic burden to the affected families as well. The development and frequency of HAIs are influenced by several factors which can be categorized under three major factors:- microbial agents, Susceptibility of patient, and environmental factors.

The hospital environment with relatively high counts of airborne microorganisms cannot be misunderstood as pathogenic, they can be non-pathogenic also. However, most studies are done to identify microorganisms that are pathogenic and cause severe problems to patients. Thus, highlighting the need to implement stringent and frequent disinfection procedures, training of healthcare workers on best hygiene practices, well managed surveillance methodology and installing high efficiency filtration systems to minimize the airborne transmission of infectious pathogens within the hospital. Infectious disease physicians in collaboration with other departments of hospital must initiate appropriate motivation measures and formulate evidence based policies.[9]

The most sensitive zones of hospital include Operating Theatre and Intensive Care Unit, which showed considerably higher counts of airborne microbes. Identification by molecular means revealed the presence of human pathogens in the hospital air including *Bacillus* sp, *Micrococcus* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Exiguobacterium* sp, *Enterobacter* sp, *Escherichia* sp, *Sphingomonas* sp, *Massilia* sp, *Kocuria* sp, *Fusarium* sp, and *Aspergillus* sp. Therefore, the implementation of proactive policies and strategies are needed to monitor hospital air quality in sensitive zones as well as other areas of the hospitals.[10]

Sources of organisms are found in the patient's own endogenous (normal) flora, from exogenous sources in the environment and from health care personnel. Although burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms.[11] Gram-positive bacteria that survive the thermal insult, such as *Staphylococci* located deep within sweat glands and hair follicles, heavily colonize the wound surface within the first 48 h unless topical antimicrobial agents are used. Eventually (after an average of 5-7 days), these wounds are subsequently colonized with other microbes, including Gram positive bacteria, Gram negative bacteria and yeasts derived from the host's normal gastrointestinal and upper respiratory flora or from the hospital environment or that are transferred via a HCW hands.[12]

METHODS

This study was carried in Jawaharlal Nehru Cancer Hospital and Research Center, Bhopal. The laboratory examination was done at the Department of Research, Jawaharlal Nehru Cancer Hospital and Research Center, Bhopal.

Sample collection

The samples were collected from different laboratories from Department of Research. The samples were collected from Microbiology Laboratory, Cytogenetic Laboratory, Animal house, Animal Tissue Culture Laboratory and Library. Sterile swab sticks were used for collection of samples.

Immediately after collection of samples, the samples were inoculated on nutrient agar culture plates and plates were incubated at 37 º C for 24 hours. After incubation the plates were observed next day for bacterial and fungal growth.

Identification of Bacteria

The identification of bacteria was done on the basis of morphology and gram staining.

Morphological identification

The bacterial colonies were observed on the basis of texture, color and morphology of bacterial colony or fungus growth.

Gram staining

The bacterial colony or fungus growth was picked up using sterile inoculation loop or sterile forecep. The fungus was picked up using sterile forecep. The smear was prepared on clean slide and allowed to dry at room temperature for 10 -15 minutes. The smear was air dried and followed by staining. The procedure for staining is given below.

Procedure for Gram Staining

1. The smear was prepared on clean dry slide.
2. The smear was air dried at room temperature for 10 - 15 minutes.
3. After drying, the slide was flooded with primary stain crystal violet and kept in stain for 1 minute. After 1 minute gram's iodine was added and kept for 1 minute.
4. The water was drained and the slide was counter stained with secondary stain, safranine. The slide kept in stain for 2 minute.
5. The slide was washed again with water and air dried.

6. The slide was observed under microscope at 100 x oil immersion objective.

RESULTS AND DISCUSSION

In the present study different microflora collected from Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal.

Sample Collection

The samples were collected using sterile swab sticks from different Laboratory. The swab sticks were rubbed on surfaces and taken for further inoculation. The inoculated plates observed next day showed bacterial and fungal colonies.

Number and percentage of the bacteria isolated from different Laboratory of Department of Research

Out of 30 samples that collected from different Laboratory from Department of Research, 16 of them (53%) showed positive bacterial growth. The animal house revealed positive bacterial growth of percentage of 66.7.

Table: Bacteria isolated from the laboratory. Morphology and related diseases details.

S. No.	Name	Gram Positive	Gram Negative	Morphology	Any Related Disease
1.	*Escherichia coli*	✓	-	Circular, convex smooth, colonies with distinct edges.	Urinary tract, pneumonia, diarrhea, vomiting, fever
2.	*Streptococci pyogenes*	✓		Nonspore forming, paired chains	Urinary tract, infection, pneumonia, infection
3.	*Streptococcus mutans*			Facultative anaerobic coccus	Oral deseases
4.	*Staphylococci aureus*	✓		Grape like cluster or spherical	Food poisoning
5.	*Klebsiella* spp		✓	Non-motile, encapsulated, lactose-fermenting, rod-shaped bacterium.	Pneumonia, bloodstream infections, wound or surgical site infections, and meningitis.
6.	*Pseudomonas aeruginosa*		✓	Rod shaped	Urinary tract infection, respiratory system, dermis, cancer and AIDS
7.	*Clostridium perfringens*	✓		Anaerobic spore-forming and rod-shaped bacterium	Necrotic enteritis (NE), food poisoning, and gas gangrene
8.	*Bacillus cereus*	✓		Facultative anaerobic, spore-forming, motile and rod-shaped bacterium	Food poisoning, causing severe nausea, vomiting and diarrhea
9.	*Bacillus subtilis*	✓		Obligate aerobe and rod-shaped bacterium	Bacterecemia, endocarditis, pneumonia and septicemia in immunocompromised patients
10.	*Aspergillus*			Conidial fungi, aerobic	Allergic sinusitis, a fungal ball or invasive Aspergillosis
11.	*Mucor*			moulds	Sometimes cause opportunistic infections

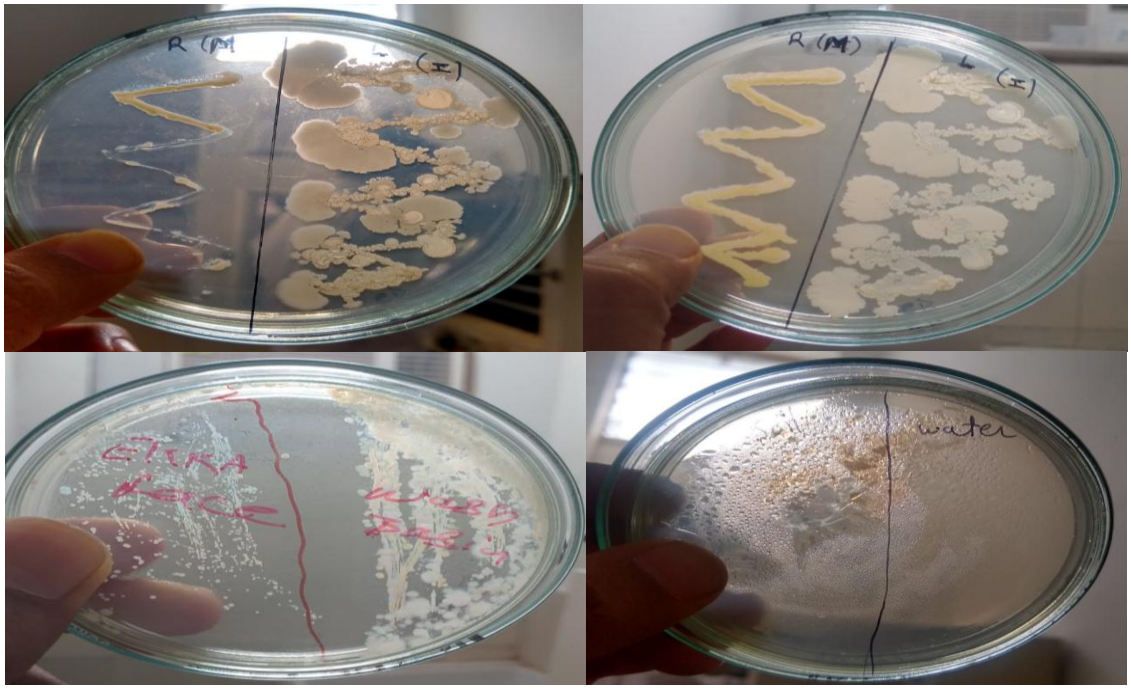

Figure 1: Nutrient Agar Culture plates with bacterial colonies.

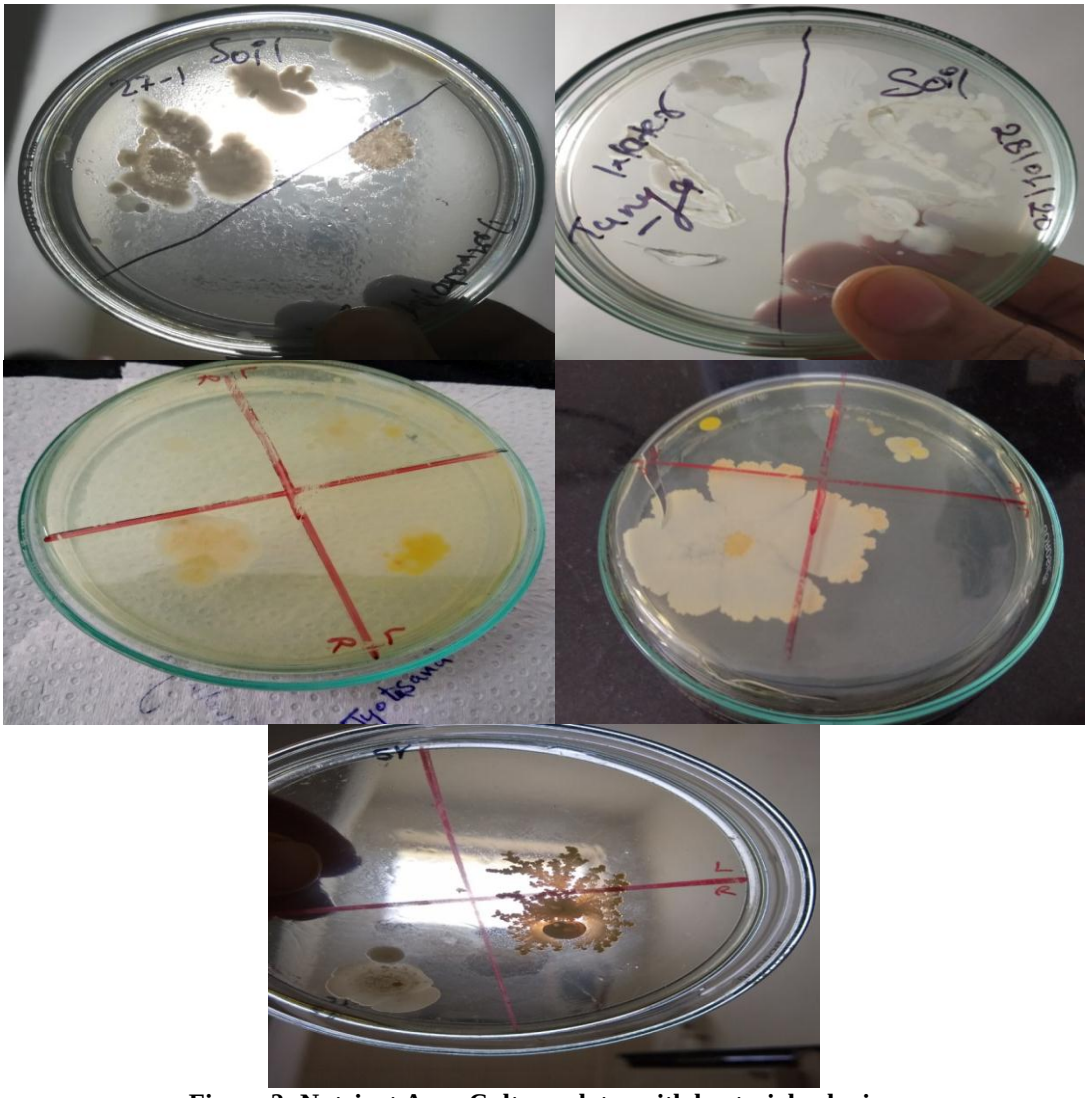

Figure 2: Nutrient Agar Culture plates with bacterial colonies.

Number and percentage of isolated bacterial species

The isolates were identified according to morphological appearance, cultural characteristic and gram's staining as gram positive bacteria, gram negative bacteria and fungus. Nine bacterial strains were isolated. The gram positive strains isolated were which further identified as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* spp. Two fungal strains were isolated namely *Aspergillus* and *Mucor*.

The most predominant isolated bacteria was *Pseudomonas aeruginosa* this was followed by *Staphylococcus aureus*. After mobbing with disinfectant the rooms showed sterile and neglible growth of *E. coli* only.

These bacterial strains are pathogenic and needs to be eliminated. In different hospitals measures are taken for pathogenic free environment. Measures include regular cleaning of floor with disinfectant. These pathogens and especially fungus is very common in hospital environment. But in Department of Research regular cleaning is done to safeguard staff and patients safety. And after cleaning the results showed no growth except *E. coli* no other strains were found. These bacterial strains were isolated by different researchers in other hospitals also and are responsible for hospital borne and nosocomial infection in immunocompromised patients.

Different researchers had reported previously about nosocomial or hospital borne infections and common microflora isolated from different wards and sites of the hospital. In one the study bacteria isolated and responsible for nosocomial infection and urinary tract infections. These includes *Staphylococcus aureus* (18.47%), *Escherichia coli* (13%), *Pseudomonas aeruginosa (9.3%)* coagulase negative *Staphylococcus* (9.12%), *Serratia marcescens* (6.58%) and *Klebsiella pneumonia* (6.36%).[13] Other study showed isolation of pathogenic bacteria isolated from the medical waste and hospital environment included *Providentia* spp. (21%), *S. aureus* (18.5%), *E. coli* (13%), *Pseudomonas* spp. (9.3%), and other coagulase negative *Staphylococci* (9.13%), *Serratia marcescens* (6.6%), *Klebsiella* spp. (6.4%).[14] Similarly, *Klebsiella*, *Pseudomonas* and *Serratia* isolated from wastes in another study.[3, 15, 16] Apart from bacteria, fungus is also very common and leads to allergic reactions namely *Fusarium* and *Penicillin* spp.[17] The prevalence rate of 16% of *E. coli* on door knobs/door handles also reported by some researchers.[18]

In the present study 9 strains were isolated from Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal. The common strains isolated were *Staphylococci aureus*, *Streptococci pyogenes*, *Streptococcus mutans*, *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* spp. Two fungal strains were isolated namely *Aspergillus* and *Mucor*.

CONCLUSION

In conclusion, our results not only confirm that the hospital environment is a dynamic microbial environment, but they also identify specific factors that may significantly influence the airborne concentration. Thus, they could provide a starting point for the recommendation of precautionary measures. With a better understanding of factors that affect the levels of airborne microorganisms in hospital services, effective control strategies can be established to reduce exposure risk of patients and healthcare workers. The efforts are urgently needed to improve hospital hygienic environment among health care workers patients and it is recommended to raise the awareness and educational status of the medical workers to reduce the hazards of air borne transmission of such potentially pathogenic microorganisms.

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REFERENCES

1. Sanaa O Yaqoub and Amani EI Agbash. Isolation of potential pathogenic bacteria from the air of hospital delivery and nursing rooms. Journal of Applied Sciences, 2010; 10(11): 1011 -1014.
2. Bhatia L and Vishwakarma R. Hospital indoor airborne microflora in private and government owned hospitals in Sagar City, India. World Journal of Medical Sciences, 2010; 5(3): 65-70. 2010.
3. Ducel G, Fabry J and Nicolle L. Prevention of hospital-acquired infections: a practical guide. A practical guide (Ed. 2), 2002.
4. Osaro EF, Ufuoma IO and Dorcas AO. Hospital indoor airborne microflora in private and government owned hospitals in Benin city, Nigeria. World Journal of Medical Sciences, 3.
5. Pearman JW. Lowbury Lecture: the Western Australian experience with vancomycin-resistant enterococci - from disaster to ongoing control. Journal of Hospital Infection. 2006; 63: 14-26.
6. Boyce JM, Potter-Bynoe G, Chenevert C, King T. Environmental contamination due to methicillin-resistant Staphylococcus aureus: possible infection control implications. Infection Control and Hospital Epidemiology. 1997; 18: 622-627.
7. Duckro AN, Blom DW, Lyle EA, Weinstein RA & Hayden MK. Transfer of vancomycin-resistant Enterococci via health care worker hands". Archives of Internal Medicine. 2005;165: 302-307

8. Akbari, Freshta, Kjellerup, Birthe. Elimination of Bloodstream Infections Associated with Candida albicans Biofilm in Intravascular Catheters Pathogens, 2010; 4(3): 457-469.
9. Borghesi A, M Stronati Journal of Hospital Infection, WHO. Prevention of Hospital-Acquired Infections: A Practical Guide, World Health Organization. Department of Communicable Disease, surveillance and Response, 2nd edition. 2008.
10. Boyce JM, Potter-Bynoe G, Chenevert C, King T. Environmental contamination due to methicillin-resistant Staphylococcus aureus: possible infection control implications". Infection Control and Hospital Epidemiology, 1997; 18: 622-627.
11. Bereket W. Update on bacterial nosocomial infection Eur Rev Med Pharmacol Sci., 2012; 16(8): 1039-1044.
12. Singh, Keerti, and K. Kishor. Bacterial contamination in operating theatres of district hospital budgam in Kashmir division. innovative journal of medical and health science, 2013; 3(2).
13. Naruka K. and Gaur J. Distribution Pattern of Airborne Bacteria and Fungi at Market Area. American-Eurasian J Sci Res, 2014; 9(6): 186-192.
14. Woodhead K. Behaviours and rituals in the operating theatre: a report from the Hospital Infection Society Working Party on Infection Control in Operating Theatres. Journal of hospital infection, 2002; 51(4): 241-255.
15. Rusin P, Maxwell S and Gerba C. Comparative surface to hand and fingertip to mouth transfer efficiency of gram positive bacteria, gram-negative bacteria, and phage. Journal of Applied Microbiology, 2002; 94: 585-592.
16. Hannigan P, Shields JW. Hand washing and use of examination gloves. Lancet, 1998; 351: 571.
17. Coia JE, Duckworth GJ, Edwards DI, Farrington M, Fry C, Humphreys H, Mallaghan C and Tucker DR. Guidelines for the control and prevention of meticillin-resistant Staphylococcus aureus (MRSA) in healthcare facilities. Journal of Hospital Infection, 2006; 63: S1-S44.
18. Givney R, Vickery A, Holliday A, Pegler M and Benn R. Evolution of an endemic methicillin-resistant Staphylococcus aureus population in an Australian hospital from 1967 to 1996. Journal of Clinical Microbiology, 1998; 36: 552-556.