Appendix A

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QUALITATIVE ASSESSMENT OF AEROMICROBIOLOGY OF HOSPITALS IN AMRAVATI CITY

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Hospital is commonly contaminated with various microbes based on patients of various diseases admitted in it. The 478 air samples were analyzed from 43 private and 07 general hospitals in Amravati city for microbial air contamination. The results showed that *Staphylococcus aureus* (32.57%) was predominant followed by *Micrococcus luteus* (19.74%), *Pseudomonas aeruginosa* (19.54%) and *Staphylococcus epidermidis* (19.30%). The *Pseudomonas mirabilis* (1.72%) *Staphylococcus saprophyticus* (1.72 %) *Escherichia coli* (0.94%), *Pseudomonas fluorsence* (0.15 %), *Serratia marcescens* (0.62%), *Citrobacter freundii* (0.31%) and *Morganella morganii* (0.31%) were occurred in less frequency in hospital indoors and outdoors environment.

INTRODUCTION

Hospitals are commonly contaminated with various microbes based on patients of various diseases admitted in it. Bioaerosol particles are usually present in indoor and outdoor air of various sections of hospitals, although their composition and concentration may vary. Human exposure to these airborne microorganisms may result in variety of infectious diseases, allergic and irritant responses, respiratory problems, and hypersensitivity reactions.[1,2]

The prominent pathogenic microorganisms found in hospital air are multidrug resistant strains of *Staphylococcus aureus*, which remained a major clinical and epidemiological problem among hospital personnel and patients.[3] Mathias, *et al.*[4] studied reservoirs of multi-resistant nosocomial pathogens in a Secondary Care Hospital, and measured the indoor and outdoor air contamination of various sections in hospital and recorded most contaminated site was labour room, followed by the dressing room and the operation theatre. Coagulate negative *Staphylococci* (30%), *Pseudomonas aeruginosa* (24.4%) were prominent and others in less frequency such as *Staphylococcus aureus*; Micrococcus, Enterococci, *Bacillus spp.*, *Pseudomonas aeruginosa* and members of Enterobacteriaceae.

Nanoty, *et al.*[5] at Akola studied hospital air flora and recorded the presence of *Staphylococcus aureus* *Pseudomonas aeruginosa*. Annadurai, *et al.*[6] (2001) at Kanchipuram studied the indoor air micro flora of government and private hospitals and reported heavy contamination of *Aspergillus, Penicillium, Alternaria spp, Pseudomonas spp., Proteus spp.* and *Bacillus spp.* Saoji and Giri[7] at Nagpur sampled the indoor air from hospital wards and recovered sixty-nine species of fungi, the prominent were *Aspergillus* spp.

However, the impact of airborne microorganisms on indoor or outdoor air quality of hospital and impact on human health remains poorly understood. Therefore, the present study was conducted in 50 hospitals of Amravati city to assess air contamination by bacterial pathogens. The main objective of this study is to make aware the people from the infections caused by hospitals indoors and outdoors environments and identification of significant pathogens, which can give information on various hospital borne infections for proper treatment.

MATERIALS AND METHODS

The study was conducted at 50 private and general hospitals in Amravati city. The 478 air samples were analyzed from 43 private and 07 general hospitals by

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exposing the agar plates in indoor and outdoor environment of each section of hospitals. Air samples were analyzed from various sections of hospital including Operation theatre (OT), Labour Room (LR), General Ward (GW), Private Room (PR), Pathology Laboratory (PL), Intensive Care Unit (ICU) and Out Patient Department (OPD). Measurement of the contamination of hospital air was carried out by sedimentation method. The three media used for sampling includes Mannitol salt agar, MacConkey agar and Cetrimide agar. Petri dish containing medium were exposed for 5 min. at height of one meter above the ground level in indoor and outdoor air of various sections in hospital. Mediums were then incubated at 37^0C for 24 to 48 hrs. and examined for colony types. The isolated colonies were identified by using standard procedure.[8]

RESULTS AND DISCUSSION

The hospital environment has patients and staff and a large amount of human traffic including visitors, shift changer staff, and support workers. Because of this, hospital environment is always laden with various microorganisms, which may cause infectious diseases[3]. The most prominent microorganisms in the hospital environment were *Staphylococcus aureus* and other *Ps. fluorescence, Sr. marcescens, Citro. freundii* and *Morg. morganii* were found in very less number because it occurs in traces of moisture on the hands[9] on patient's skin and on articles that come in direct contact with patients.[10]

A total 50 hospitals in which 07 General and 43 Private hospitals were analyzed for microbial air contamination. In the present project, various t types of hospitals such as Maternity hospitals and Children hospitals, Cardiac hospitals, orthopedic hospitals, Cancer hospital, ENT hospital and General hospitals were studied. Total 478 air samples were analysed from indoor and outdoor environment of different sections such as Out Patient Department (OPD), General Ward (GW), Private Room (PR), Labour Room (LR), Intensive Care Unit (ICU), and Pathology Laboratory (PL). Out of which 433 (90.58%) air sample contaminated with various airborne pathogenic microorganisms, which included, *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Pseudomonas aeruginosa, Pseudomonas fluorescence, Proteus mirabilis, Micrococcus luteus, Serratia marcescens, Morganella morganii, Citrobacter freundii,* and *Escherichia coli* (Table 1).

Among this *Staph. aureus* was predominant (32.57%) followed by *Micro. luteus* (19.74%), *Ps. aeruginosa* (19.54%) and *Staph. epidermidis* (19.30%). The *Pr. mirabilis* (1.72%) *Staph. Saprophyticus* (1.72 %) and *Esch. coli* (0. 94%) found in less number. There was very less contamination of the air by *Ps. fluorsence* (0.15 %), *Sr. marcescens* (0.62%) *Citro. freundii* (0.31%) and *Morg. morganii* (0. 31%). (Fig.1)

The maximum bacterial contamination was recorded in general ward (indoor), followed by general ward (outdoor), OPD, Private rooms (indoor), private rooms (outdoor), operation theater (indoor), OT (outdoor), labour room (indoor), labour room (outdoor) and least in pathology laboratory and ICU (Fig.2).

Staph. aureus showed highest contamination in hospital air due to its carriage in the nose, throat, skin and toe-webs and patients with superficial infections and respiratory infections disseminate large number of *Staphylococci spp.* into environment. It was found that General Ward, Private Room, Operation Theatre, and Intensive Care Unit were mostly contaminated by *Staph. aureus.* In General Ward, Private room and Intensive Care Unit, the *Staph. aureus* is transmitted by sneezing coughing from nose and throat as well as through contact via hands of doctors and nurses. Even some times Doctor's stethoscope[3] or surgeons hair are also the source of *Staph. aureus* during lengthy operations and orthopedic surgery, it may get disseminate from the hair and hands and can be transmitted into the wound because wound is the susceptible site for infection.[11]

The next most common occurrence was of *Staph. epidermdis, Micro. luteus* and *Ps. aeruginosa* in which *Staph. epidermidis* and *Micro. luteus* are the skin commensals and community transmitted through contact mainly via hands. While *Ps. aeruginosa* was found to colonise in the nose, throat, skin as well as oral cavity and thus can cause infection easily through airborne droplets and by contact. *Staph. saprophyticus* is a non-pathogen, whereas *Pr. mirabilis, Esch. coli* and other gram-negative bacteria are not ordinarily colonised in oropharynx, but in the compromised host, these organisms can be found there in relatively high number. The occurrence of *Ps. fluorescence, Sr. marcescens,*

Table 1: Pathogenic bacterial load in various sections of hospitals

Name of Organism Isolated	Number of Organisms		General Ward (Indoor)	General Ward (Outdoor)	Private Room (Indoor)	Private Room (Outdoor)	Operation Theatre (Indoor)	Operation Theatre (Outdoor)	Labour Room (Indoor)	Labour Room (Outdoor)	Pathology Laboratory (Indoor)	Pathology Laboratory (Outdoor)	Intensive Care Unit (Indoor)	Intensive Care Unit (Outdoor)	Out Patient Dept. (Indoor)	Out Patient Dept. (Outdoor)
	Total	%														
Staph. aureus	227	35.59	36	30	26	22	20	13	13	7	2	2	4	4	18	30
Micro. luteus	126	19.76	17	13	16	10	4	8	7	10	2	0	2	2	21	14
Ps. aeruginosa	125	19.54	23	12	19	9	12	6	16	6	3	2	3	1	3	10
Staph. epidermidis	123	19.30	19	13	13	16	10	9	7	5	1	0	1	2	10	17
Staph. saprophyticus	11	1.74	2	2	2	1	0	1	1	1	0	0	0	1	0	0
Pr. mirabilis	11	1.74	3	1	2	0	3	0	1	0	0	0	0	0	0	1
Esch. coli	6	0.94	2	0	0	0	2	0	2	0	0	0	0	0	0	0
Sr. marcescens	5	0.62	1	1	0	0	0	0	2	0	0	0	0	0	0	1
Citro. freundii	2	0.31	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Morg. morganii	2	0.31	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Ps. fluorscence	1	0.15	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Total	639	100%	105	73	79	58	51	37	50	29	8	4	10	10	52	73
		%	16.43	11.41	12.36	9.0	7.98	5.8	7.82	4.53	1.25	0.62	1.56	1.56	8.13	11.42

253

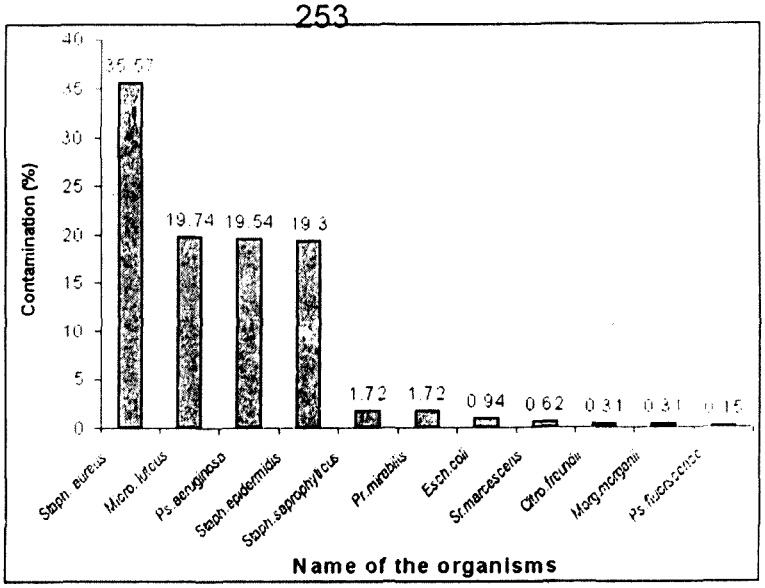

Fig 1 Presence of various organisms in Hospitals

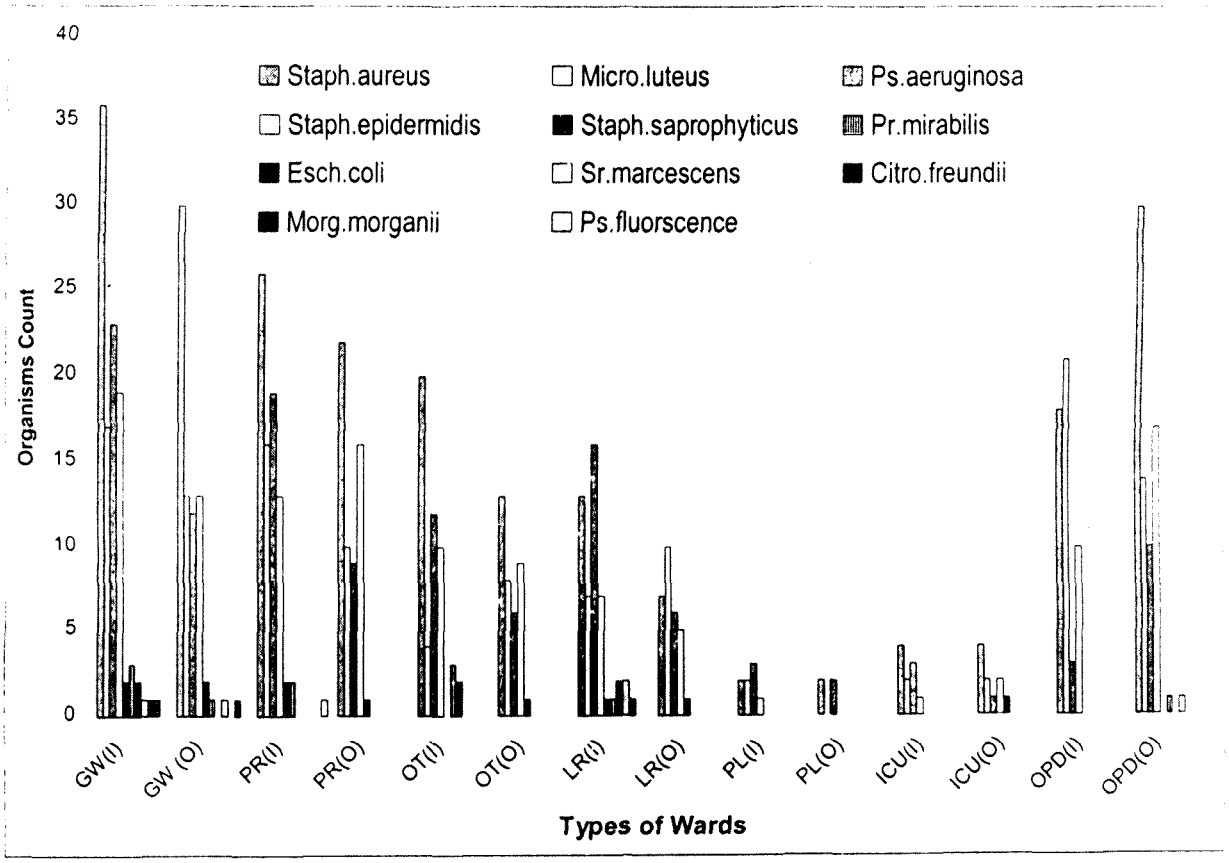

Fig 2 Occurrence of air flora in various sections of Hospital

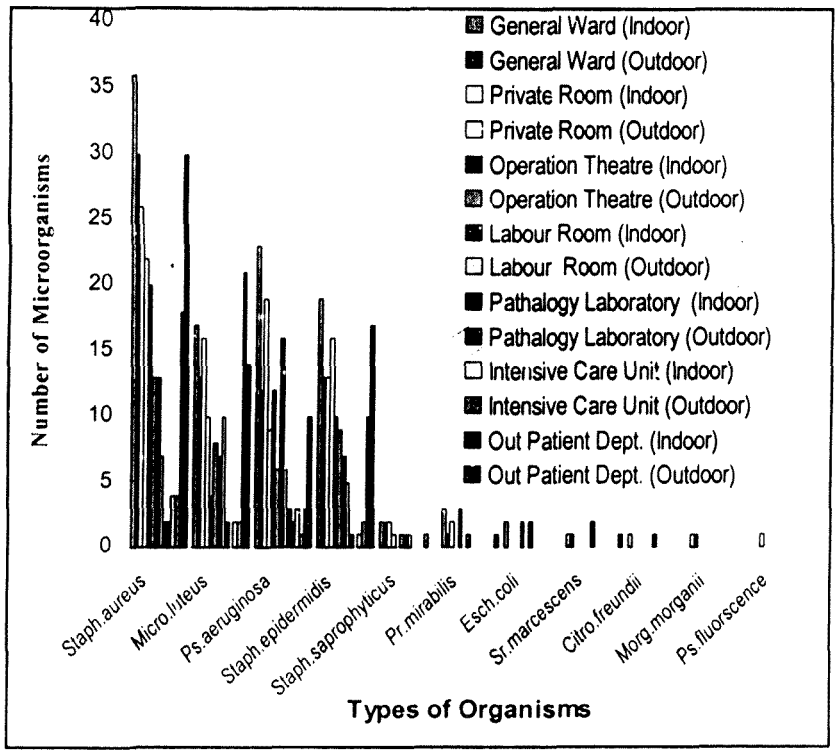

Fig 3 Bacterial load in various sections of Hospital

Citro. freundii and *Morg. morganii* was very less number in hospital environment because they are usually not colonized on human skin (Fig.3).

The study showed that the prominent bacterial pathogens in the hospital indoor and outdoor environments were *Staphyloccous aureus, Micrococcus luetus, Pseadomonas aerogenosa, and Staphylococcus epidermidis*. The other pathogens isolated in less frequency were *Pr. Mirabilis, Staph. saprophyticus, Esch. coli, Ps. fluorescens, Sr. marcescens, Citro. freundii* and *Morg. morganii*.

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Assessment of multiple antibiotics resistant airborne pathogens in hospital's environment

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ABSTRACT

The emergence of nosocomial infection and multiple antibiotic resistant bacteria has become a major challenge in the treatment of infectious diseases. Bacterial isolates from hospital air of Amravati were examined to assess multiple antibiotic resistance patterns. Isolated 100 bacterial spp. included *Staphylococcus aureus*, Staph. *saprophyticus*, Staph *epidermidis*, *Micrococcus luteus*, *Micrococcus roseus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Citrobacter freundii*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* were examined for antibiotic sensitivity against 15 different types of antibiotics such as amikacin, augmentin, ceftazidime, ceftriaxone, cephotaxime, ciprofloxacin, chloramphenicol, erythromycin, fusidic acid, gentamycin, lincomycin, netilmicin, ofloxacin, penicillin and vancomycin. The multiple antibiotic resistant (MAR) indexes of the isolates showed the highest (0.0073) with *Enterobacter aerogenes*. The 1500 tests of antibiotics were made against 100 organisms. Out of them 1015 antibiotic tests were found to be resistant, 103 tests were found to be intermediate sensitive and 382 tests were found to be sensitive to various antibiotics tested. The 80 % isolates were resistant to augmentin and penicillin, 79 % to fusidic acid and lincomycin, 76 % to erythromycin, 74 % ciprofloxacin, 71 % vancomycin, 66 % to ceftazidime and gentamycin, 62 % to chloramphenicol, 60 % to neilmicin, 58 % to ceftriaxone and ofloxacin, 53 % to amikacin and cephotaxime.

Keywords: *Hospital air, Multi-resistant pathogens, Indoor environment, Air-borne pathogens.*

INTRODUCTION

Airborne transmission refers to infections, which are contracted from micro-organisms contained in droplet nuclei produced by coughing, sneezing or some other form of aerosolation and also apply to dust particles and skin squamae carrying pathogenic microorganisms. The contribution of airborne microorganisms to the spread of infection is likely to be greater than is currently recognized. This is because many airborne microorganisms remain viable and may not detected and some infections arising from contact transmission involve the airborne transportation of microorganisms onto inanimate surfaces (Beggs, 2003). When a person coughs or sneezes many thousands of droplets are expelled at high velocity into the atmosphere (Wells, 1995). According to Beggs (2002), droplet nuclei are so small that they settle slowly and remain suspended in air for a considerable period of time and distributed widely throughout in indoor hospital buildings.

Hospital air commonly contaminated with various microbes based on the patients of different diseases admitted in the hospital. These pathogens may contaminate the air and are able to survive in the adverse condition and can cause hospital infection and develop resistance to antibiotics. Even in the developed countries despite so many advances in the treatment of infectious diseases "cross infection" in the hospital tends to be high (Nanoty *et al.*, 2003). Bioaerosol particles are usually present in indoor and outdoor air of various sections of hospitals, although their composition and concentration may vary. Human exposure to these airborne microorganisms may resulted in variety of infectious diseases, allergic and irritant responses, respiratory problems and hypersensitivity reaction (Tambekar and Gulhane, 2003).

The prominent pathogenic microorganisms found in hospital air are multidrug resistant strains of *Stphylococcus aureus*, which remained a major clinical and epidemiological problem among hospital personnel and patients (Cynthia *et al.*, 1998). Mathias *et al.* (2000) studied reservoirs of multi-resistant nosocomial pathogens in a Secondary Care Hospital, Ramnagar and measured the indoor and outdoor air contamination of various sections in hospital and recorded most contaminated site was labour room followed by dressing room and operation theatre. Tambekar (2004) conducted a study at 50 private and general hospitals in Amravati city (India) and reported the maximum bacterial contamination was recorded in general ward (indoor), followed by general ward (outdoor), OPD, private room (indoor) private room (outdoor), operation theatre (indoor), operation theatre (outdoor), labour room (indoor), labour room (outdoor) and least in pathology laboratory and ICU.

Furthermore, changing patterns of susceptibility and the availability of new antimicrobial agents require continuous updating of knowledge concerning treatment of diseases caused by such pathogens. The impact of airborne microorganisms on indoor and outdoor air quality of hospital and impact on human health remains poorly understood. Therefore the present study was conducted in 76 hospitals of Amravati city to assess air contamination by bacterial pathogens and to make aware the people from the multiple antibiotic resistant airborne pathogens, which can give information on various hospital borne infections for proper treatment.

MATERIALS AND METHODS

Sample collection: The aero-biological survey was carried out in indoor and outdoor environment at 76 hospitals in Amravati which includes 6 general, 37 maternity and children, 5 multi-specially, 9 cardiac, 6 each of orthopedic and ENT, 1 each of cancer, dental and mental hospitals and 4 clinics.

Microbial analysis: The total 953 organisms are isolated from 670 air samples by performing sedimentation method. The air samples were analyzed from indoor and outdoor environment of hospitals by exposing the mannitol salt agar, MacConkey agar and Cetrimide agar plates.

Qualitative estimation of *Escherichia coli, Cirobacter freundii, Serratia marcescens, Enterobacter aerogenes, Klebsiella pneumoniae* and species of *Staphylococcus, Micrococcus, Pseudomonas* and *Proteus* were identified by applying various cultural, morphological and biochemical tests.

Antibiotic sensitivity test: The total hundred clinical isolates were tested for antimicrobial susceptibility tests against the following antibiotics: Ceftriaxone (30 mcg), Ceftazidime (30 mcg), Cephotaxime (30 mcg), Lincomycin (2 mcg), Netilmicin (30 mcg), Ofloxacin (2 mcg), Vancomycin (30 mcg), Amikacin (30 mcg), Penicillin (10 units), Gentamycin (10 mcg), Augmentin (30 mcg), Ciprofloxacin (5 mcg), Erythromycin (10 mcg), Fusidic acid (10 mcg), Chloramphenicol (30 mcg). Using the agar diffusion assay performed susceptibility test and the disks were obtained from Hi-media Laboratories Pvt. Ltd, Mumbai. Antibiotic sensitivity tests were conducted following Davis and Stout's (1971) procedure.

RESULTS AND DISCUSSION

The most dominant among the aerial contaminants ware *Staph. aureus* followed by *Ps. aeruginosa, M. luteus* and *Staph. epidermidis*. It is generally the case that gram-positive bacteria such as *S. aureus*, possess a peptidoglycan-rich cell wall, which gives them relative resistance to desiccation. It can also remain viable on aerosolized skin squamae for long periods of time (Sands and Goldmann, 1998). Wagenwoort (1993) reported MRSA on ventilation grilles in an orthopedic ward and Cotterill (1996) identified colonies of MRSA in the exhaust air from an isolation room as the source of an outbreak in an intensive care unit. *S. aureus* grows on the nasal mucosa; hands then touch the nose and *S. aureus* are transferred to the skin; they colonize the skin and are ultimately disseminated back into the air on skin squamae. Even sometimes Doctors stethoscope or surgeons hair are also the source of *Staph aureus*. During lenghty operations and orthopedic surgery, it may get disseminated from the hair and hands and can be transmitted into the wound because wound is the susceptible site for infection (Tambekar, 2004).

The dominance of *Ps. aeruginosa* denoted its minimal growth requirements and its survival and replication within the hospital environment. The disperse of *Ps. aeruginosa* from colonized patients and personnel of the hospital might have resulted in further contamination of the environment of the hospital as well as the hands of the medical staff (Mathias *et al.*, 2003). According to Tambekar *et al.* (2005) *Pr. mirabilis, Esch coli* and other gram-negative bacteria were found in less number in hospital environment. Some species of Enterobacteriaeceae such as *Pr. vulgaris, Morganella morganii, Citrobacter freundii, Serratia marcescens* and *Klebsiella pneumoniae* showed least air contamination, as the main source is the contaminated water droplets.

The various bacterial strains were isolated in hospital environment on a variety of media belong to the species of 11 different types of microorganisms. The present distribution of different organisms resulted into 10 % each of *Staphylococcus aureus, Staph epidermidis* and *Micrococcus luteus*, 4 % *Staph. saprophyticus*, 6 % *M. roseus*, 38 % *Pseudomonas aeruginosa*, 2 % *Proteus vulgaris*, 5 % *Escherichia coli*, 11 % *Enterobacter aerogenes*, 2 % each *Citrobacter freundii* and *Klebsiella pneumoniae* (Fig. 1).

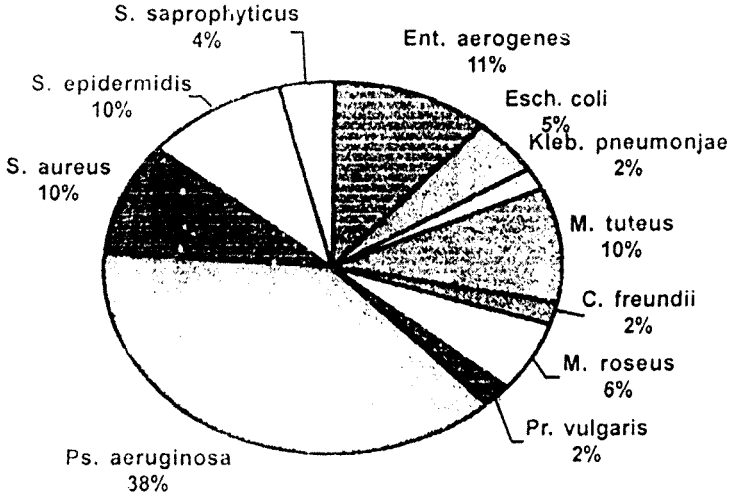

Fig. 1. Bacterial pathogens isolated from hospital environment

A total of hundred isolates were tested for antimicrobial susceptibility tests against 15 antibiotics. Out of which near about 80 % isolates resistant to commonly used antibiotics such as penicillin, augmentin, ciprofloxacin, erythromycin, fusidic acid, ceftriaxone and netilmicin. The 25 % isolates were resistant to all 15 antibiotics, which included *Ps. aeruginosa, Micrococcus luteus, Staph. aureus*. Nearly 80 % *Staph. aureus* were resistant to penicillin. This is due to enzyme beta-lactamase or penicillinase, which destroy the drug.

Out of the isolated strains of bacteria 80 % strains were resistant to augmentin and penicillin, 79 % to fusidic acid and lincomycin, 76 % to erythromycin, 74 % to ciprofloxacin, 71 % to vancomycin, 66 % to ceftazidime and gentamycin, 62 % to chloramphenicol, 60 % to netilmicin, 58 % each to cephotaxime and ofloxacin, 53 % each to amikacin and cephotaxime (Fig. 2). The study showed that *Pr. vulgaris* was found highly resistant to tested antibiotics. Next to them were *M. luteus, E. coli, M. roseus, Ps. auruginosa, Staph. aureus, Ent. aerogenes, S. epidermidis, Kleb. pneumoniae, Staph. saprophyticus* and finally *C. freundii.*

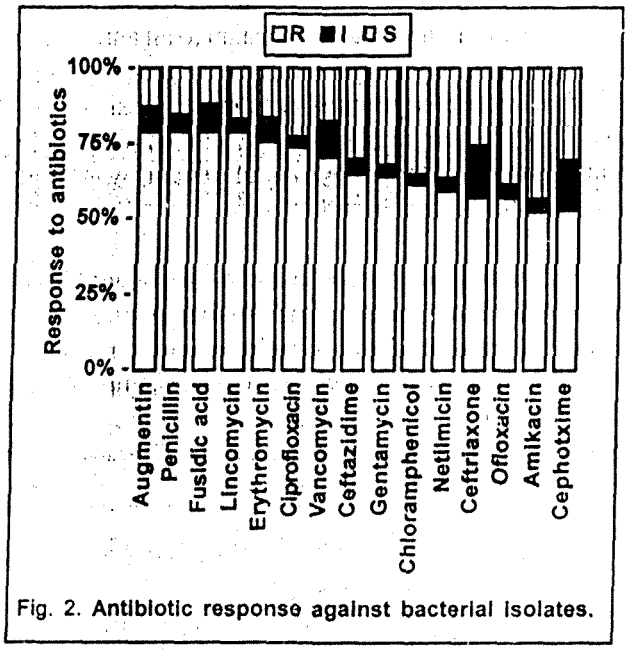

Fig. 2. Antibiotic response against bacterial isolates.

The study showed the highest MAR index (0.0073) with *Enterobacter aerogenes* against augmentin followed by *Micrococcus luteus*, which was 0.0066 against ofloxacin and chloramphenicol. *Staph. aureus* was highly resistant of fusidic acid (MAR 0.006) and least against ofloxacin and Vancomycin. Staph. *epidermidis* was highly resistance (MAR index 0.0053) to penicillin while *Micrococcus roseus* had highest MAR 0.004 against ceftazidime. *Escherichia coli* had MAR index 0.0033 against fusidic acid, penicillin, vancomycin, netilmicin and chloramphenicol. *Staph. saprophyticus* had highest MAR index 0.002 against ceftazidime, erythromycin, fusidic acid, lincomycin and penicillin while *Pseudomonas aeruginosa* had 0.022 MAR index against erythromycin. *Proteus vulgaris, Citrobacter freundii* and *Klebsiella pneumoniae* had highest MAR index 0.0013 against lincomycin. *Proteus vulgaris* was highly resistance against commonly used antibiotics such as lincomycin, fusidic acid, penicillin, augmentin, vancomycin, ciprofloxacin, gentamycin, ceftriaxone, cetrazidime, netilmicin, cephotaxime and ofloxacin. While *Citrobacter freundii* had the same MAR index against vancomycin and lincomycin. *Klebsiella pneumoniae* also had same MAR index (0.0013) against lincomycin, augmentin, ciprofloxacin, erythromycin, ofloxacin and netilmicin (Table 1).

Ps. auruginosa were maximally resistance due to the ability to produce a large number of extra cellular protective and toxic substances, and found resistance to commonly used antibiotics such as penicillin, augmentin, erythromycin, fusidic acid and lincomycin. The 80 % species of *Micrococcus luteus* were resistant to all 15 antibiotics tested. Thus, it deserves special attention among recently existing

Table 1. Bcterial isolates and antibiotic sensitivity.

Organisms	R	I	S
C. freundii	11	3	16
Ent. aerogenes	104	14	47
Esch. coli	62	0	13
Kleb. pneumoniae	19	04	07
M. luteus	130	08	12
M. roseus	62	08	20
Pr. vulgaris	27	0	03
Ps. aeruginosa	377	39	154
S. aureus	98	05	47
S. epidermidis	93	14	43
S. saprophyticus	32	08	20
Total	**1015**	**103**	**382**

hospital resistant organisms. *Staphylococcus* spp. of general ward was sensitive while that private room, out patient department and burn ward were found to be resistant to all antibiotics tested. Out of 60 isolates of *Pseudomonas* studied, more than 64 per cent isolates were resistant to more than six antibiotics tested. *Citrobacter freundii* was mostly resistant in indoor of private room whereas it shows sensitive pattern in outdoor environment of private room. *Escherichia coli* of surgery ward and *Enterobacter aerogenes* of general ward and X-ray, sonography sections were resistant to all antibiotics tested.

Thus, study suggested that a number of factors influences the prevalence of antibiotic resistant in bacteria in hospital environment and sub-therapeutic and the therapeutic usage of antimicrobial drugs will result in increased propositions of multiple antibiotic resistant hospital pathogens. The periodic review of antibiotic usage is, therefore, of greatest importance to ensure that antibiotics are not used indiscriminately.

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Research Paper

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Studies on Environmental Monitoring of Microbial Air Flora in the Hospitals

D.H. Tambekar, P.B. Gulhane and D.D. Bhokare

Indoor air quality is an important determinant of human health and comfort. Airborne bacteria can also contributes to indoor air pollution. The aerobiological survey was carried out in indoor and outdoor environment at 76 hospitals in Amravati. The total 670 air samples were analyzed from indoor and outdoor environment of hospitals by using sedimentation method. The most prominent bacteria isolated were *Staphylococcus aureus* (29.59%), *Pseudomonas aeruginosa* (19.72%). The *Staphylococcus saprophyticus*, *Proteus mirabilis*, *Escherichia coli and Enterobacter aerogenes* were in the range of 2-6%. The rest of bacterial pathogens *Pseudomonas fluorescence*, *Proteus vulgaris*, *Morganella morganii*, *Citrobacter freundii*, *Serratia marcescens* and *Klebsiella pneumoniae* were below 1%. Out of all the hospital examined, maternity and children hospitals showed highest (50.68%) bacterial isolates, which were the highest among all types of hospitals.

Key words: Airborne pathogens, hospital environment, hospital air flora

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INTRODUCTION

Indoor air quality is an important determinant of human health and comfort. There are large evidences on the hazardous nature of indoor air pollutants, on their sources or conditions leading to human exposure. The indoor air quality of hospitals has become an important issue now days. The airborne route of transmission is important for a number of pathogenic microorganisms in hospital buildings (Beggs, 2003). As it is, 5% of all patients who go to hospitals for treatment will develop an infection while they are there. This is because the density of pathogens is greater in hospitals than in most other environments. Indeed, it has been estimated that the airborne route of transmission accounts for between 10 and 20% of endemic nosocomial infections (Brachman, 1970). Unfortunately, hospitals tend to be places where harmful organisms are concentrated.

Airborne transmission is known to be the route of infection for diseases. It has also been implicated in nosocomial outbreaks of methicillin resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Greene *et al.* (1962) reported 42.6% gram-positive cocci and 14% gram-negative rods in hospitals air. Human exposure to these airborne microorganisms may results in adverse health effects, infectious diseases (Sattar and Ijaz, 1987), allergic and irritant responses (Croft *et al.*, 1986), respiratory problems (Jacobs, 1989) and hypersensitivity reactions (Woodward *et al.*, 1988; Tambekar and Gulhane, 2003).

The contribution made by airborne pathogens towards nosocomial infection and the role played by aerosolized microorganisms is unclear. The fact that many airborne microorganisms are viable even though they are non-culturable (Heidelberg *et al.*, 1997) is of importance. Indeed, it might explain why Greene *et al.* (1962) found relatively few gram-negative bacilli when they sampled the air in hospitals. It therefore follows that airborne transmission of infectious agents in hospital buildings is likely to be greater than is currently recognized.

Up till the work on indoor air quality has been conducted in farms, caves, industries, dwelling houses, library buildings, poultry sheds, green houses (Tilak *et al.*, 1985), museums and libraries (Manoharachary *et al.*, 1997), school building (Razek *et al.*, 2000), college field, market area, saw mill area (Basumatary *et al.*, 2002). In all the above-mentioned projects, the study has been focused on studies on fungal flora. In comparison, relatively little work has been undertaken on the bacteriological aspects of indoor air quality. The impact of airborne microorganisms on indoor and outdoor air quality of hospital and impact on human health remains poorly understood. Thus, the relative lack of research into the airborne transmission of bacteria tends to conduct the present study for assessment of air contamination by bacterial pathogens in the 76 hospitals of Amravati city.

MATERIALS AND METHODS

Sample collection site: The aero biological survey was carried out in indoor and outdoor environment at 76 hospitals in Amravati which includes 6 general hospitals, 37 maternity and children hospitals, 5 multi-specialty hospitals, 9 cardiac hospitals, 6 each of orthopedic hospitals and eye, nose and throat (ENT) hospitals, 1 each of cancer hospital, dental hospital and mental hospital and 4 clinics.

Aerobacterial flora analysis: The total 670 air samples were analyzed from indoor and outdoor environment of hospitals by using sedimentation method (Mathias *et al.*, 2000) and air sampler (Hi-media, Mumbai). The petridishes containing mannitol salt agar, MacConkey agar and cetrimide agar were exposed for 5 min in air to sample particles at 1 cubic foot height. The plates were incubated at 37°C for 48 h and examined for types of bacteria. The bacterial isolates were identified using standard procedure (Bergey's Manual of Determinative Bacteriology, 1974).

RESULTS AND DISCUSSION

The total 670 air samples were analyzed from indoor and outdoor environment of hospitals, out of these, 953 strains of 15 bacteria were isolated. Out of them 457 were from indoor and 496 from outdoor hospital environment. The most prominent bacteria isolated were *Staphylococcus aureus* (29.59%), *Pseudomonas aeruginosa* (19.72%), *Micrococcus luteus* (16.05%) and *Staphylococcus epidermidis* (15.84%). The *Staphylococcus saprophyticus*, *Proteus mirabilis*, *Escherichia coli and Enterobacter aerogenes* were in the range of 2-6%. The rest of bacterial pathogens *Pseudomonas fluorescence*, *Proteus vulgaris*, *Morganella morganii*, *Citrobacter freundii*, *Serratia marcescens* and *Klebsiella pneumoniae* were below 1% (Fig. 1).

The indoor environment refers to inside of general wards, private rooms, Operation Theater (OT), labour rooms, Intensive Care Unit (ICU), pathology laboratories, X- ray rooms, Trade Meal Test-Pulmonary Function Test (TMT-PFT) rooms, Electro Cardio Graphy-Electro Encephalo Graphy (ECG-EEG) rooms, sonography rooms, lithotripsy rooms, pschychologist's rooms, dressing rooms, gynecology wards, medicine wards, pediatric

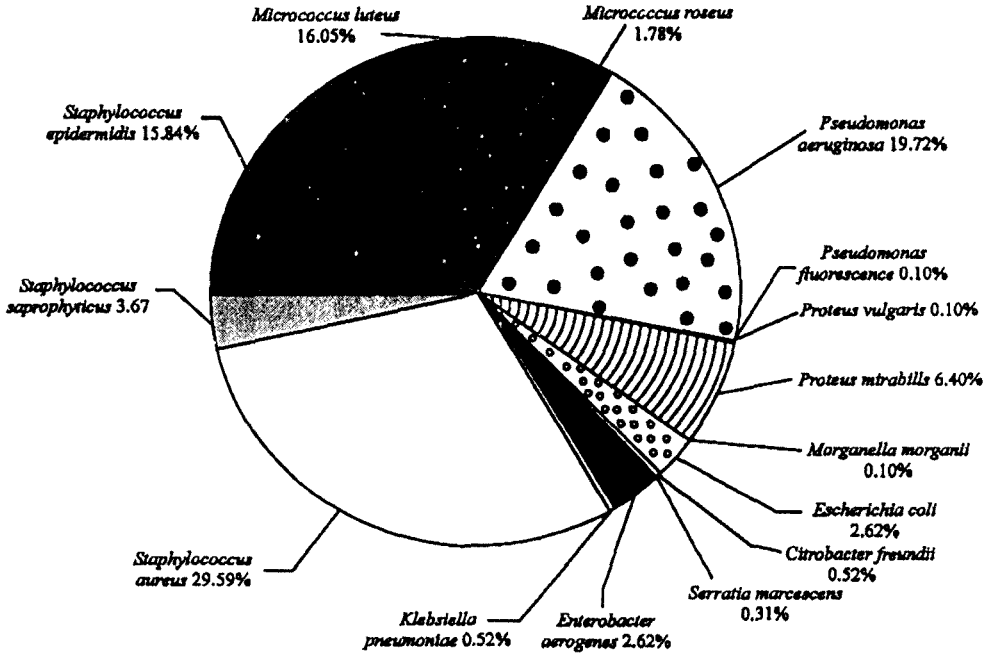

Fig. 1: Total types of airborne pathogens isolated in hospital environment of Amravati

wards, surgery wards, ENT wards and burn wards. The outdoor environment refers to corridors of these wards and sections including OPD's, which are the out patient department or the examination areas.

The occurrence of *Staphylococcus aureus* (13.22%) and *Micrococcus luteus* (6.71%) was less in indoor than in outdoors i.e., 16.36 and 9.33% respectively. At any one time approximately 30% of healthy people are carriers of *Staphylococcus aureus*. It is an opportunistic pathogen, which causes infection at sites of lowered host resistance, such as damaged skin or mucous membranes (Arbuthnott, 1992). The micrococci are parasitic on mammalian skin (Ananthanarayan and Paniker, 2000).

The occurrence of more percentage in the outdoor air of these organisms suggested that the source was the shedders. Shedders can disperse large numbers of cocci into the environment, resulting in high concentrations of airborne staphylococci, which may remain viable for long periods of time. If the visitors, auto rickshaw drivers, healthcare personnel and other people are heavy shedders then, the outdoor air becomes occupied with *Staphylococcus aureus* and *Micrococcus luteus*. It is generally the case that gram-positive microorganisms survive much longer in the aerosolized state than gram-negative bacteria (Sands and Goldmann, 1998). Thus their presence was more in outdoor air.

Coagulase negative staphylococci (CNSs) (19.51%) were found to be very less as compare to *Staphylococcus aureus*. They are commonly found on the skin of healthy persons and rarely cause infections, except in immuno-compromised patients (Arbuthnott, 1992). The transmission route for coagulase negative staphylococci is airborne, which has been observed from staff in an operating room during implant surgery (Lidwell *et al.*, 1982).

Pseudomonas aeruginosa concentration was high in indoor air (10.38%) than outdoor air (9.33%) while other members of enterobacteriaceae were found less in number in the outdoor air of the hospitals. *Pseudomonas aeruginosa* is difficult to eradicate from hospital wards as it is resistant to and may multiply in, many of the disinfectants and antiseptics commonly used in hospitals. This is the main reason why *Pseudomonas aeruginosa* is more in indoors than outdoor. The few studies suggested that airborne transmission played an important role in *Pseudomonas* sp. infection as it was isolated in burns units via the airborne route (Govan, 1992). Blessing-Moore *et al.* (1979) recovered *Pseudomonas aeruginosa* from settle plates near patients with cystic fibrosis. *Pseudomonas* sp. along with other gram-negative bacilli can be recovered from hospital air. However, the few studies indicated that *Pseudomonas* sp. play an important part in airborne transmission (Zimakoff *et al.*, 1983).

The concentration of gram-negative bacteria (18.99%) was more in indoors than outdoors. Although it is generally true that gram-positive bacteria survive longer in the aerosolized state than gram-negative bacteria, there

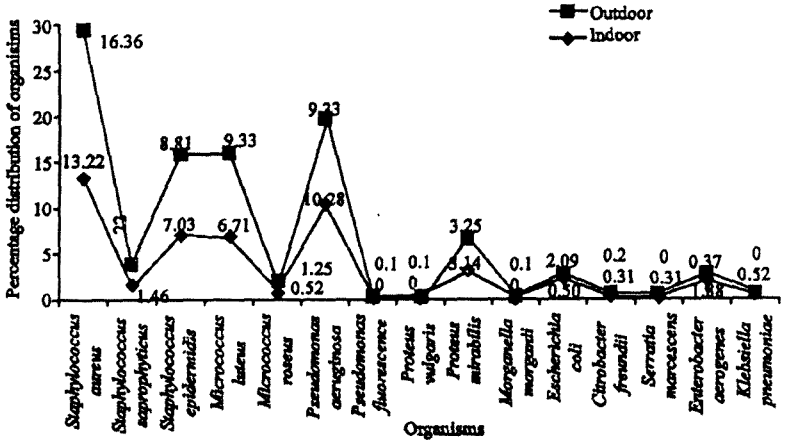

Fig. 2: Bacterial pathogens isolated from indoor and outdoor hospital environment in amravati

is growing evidence that gram-negatives can survive in the aerosolized state (Jawad *et al.*, 1996). According to Beggs (2003) Tambekar *et al.* (2005) the enterobacteriaceae members such as *Proteus vulgaris, Morganella morganii, Citrobacter freundii, Serratia marcescens* and *Klebsiella pneumoniae* were least air contaminated, as the source of contamination may be water droplets and they may not survive for long period in the aerosolized state (Fig. 2).

Maternity and children hospitals showed 50.68% bacterial isolates which were the highest among all types of hospitals (Fig. 3). This may be due to unhygienic state of children patient with their parents and more crowds as well as nearby and open defecation. The airborne contamination of fomites, i.e. curtains and furnishings and of floors plays a role in the spread of airborne bacteria (Beggs, 2003). Thus the dust, skin squamae on the surfaces may get airborne and contributes in the highest microbial flora of maternity hospital. Intestinal organisms, through dried particles of feces, from napkins of infants, also get disseminated (Ananthanarayan and Paniker, 2000).

General hospitals had 17.62% pathogenic bacterial flora. The most common contaminants were *Staphylococcus aureus* (4.61%), *Staphylococcus epidermidis* (3.67%), *Micrococcus luteus* (2.51%) and *Pseudomonas aeruginosa* (3.88%). As patients attending these hospitals have lower socio-economic status. The dirty clothes and the skin of those people may contribute in the airborne organisms in the hospitals. The ultimate source of common pathogenic organisms is dust derived from human beings. The more occurrence of *Pseudomonas aeruginosa* suggested that it has minimal growth requirements and has ability to produce a large number of extracellular protective and toxic substances (Whitby and Rampling, 1972). It can survive and replicate within the hospital environment, where it colonizes sinks, hospital distilled water systems (Zimakoff *et al.*, 1983), mattresses (Fujita *et al.*, 1981), hand wash basins, humidifiers, floor mops, plastic washing bowels, soap dishes, nail brushes, bedrails (Lowbury *et al.*, 1970) and even disinfectants (Burden and Whitby, 1967).

The cardiac hospitals indoor and outdoor had 12.69% bacterial pathogens. The patients in the cardiac hospital were usually immunocompromised. Frequent visit of doctors and nurses, visitors and relatives add to the contamination of airborne pathogens. According to Cairns *et al.* (2000) most airborne microorganisms found in hospitals are generated within the building by the staff, patients and visitors. Respiratory droplets produced by patients coughing or sneezing can impact upon the conjunctivae or oro-nasal mucosae of susceptible patients and healthcare personnel resulting in subsequent infection. If these healthcare personnel go to treat patients, the organisms on their uniforms might be expelled into the air in the form of cloth dust (Boyce *et al.*, 1997).

The orthopedic hospitals showed 6.71% of airborne pathogens contamination and reported the presence of *Staphylococcus aureus* (1.88%), *Staphylococcus epidermidis* (0.83%) and *Micrococcus luteus* (1.57%) (Table 1). The microbiological studies confirmed that gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* are the primary pathogens responsible for wound infection in prosthetic joint surgery (Fig. 3).

All the examined air flora of the hospitals was contaminated with airborne pathogens. The most dominant pathogens within all examined hospitals were *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Table 1: Percent bacterial aerial flora of various hospitals in amravati

Types of Hospitals	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total % isolates
Clinic	0.52	0	0.41	0.52	0.10	0.83	0	0	0.10	0	0.10	0	0	0	0	2.62
Cancer hospital	0.62	0	0	0.10	0.10	0.20	0.10	0	0.10	0	0	0	0	0	0	1.15
Cardiac hospital	4.82	0.73	0.94	2.09	0.20	1.88	0	0	1.46	0	0	0.10	0	0.41	0	12.69
Dental hospital	0.52	0	0.20	0.20	0	0	0	0	0	0	0	0	0	0	0	0.94
ENT hospital	0.31	0	0.52	0.10	0	0.83	0	0	0.10	0	0	0	0	0	0	1.88
General hospital	4.61	0.52	3.67	2.51	0.31	3.88	0	0	0.83	0	0.52	0.10	0.10	0.31	0.20	17.62
Maternity and Children hospital	15.32	1.67	8.28	8.28	0.83	9.86	0	0.10	3.46	0.10	1.57	0.20	0.20	0.62	0.10	50.68
Mental hospital	0.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.10
Multispeciality hospital	0.83	0.10	0.94	0.62	0.20	1.46	0	0	0.10	0	0.31	0	0	0.73	0.20	5.56
Orthopedic	1.88	0.62	0.83	1.57	0.10	0.73	0	0	0.20	0	0.10	0.10	0	0.52	0	6.71
Total bacterial Pathogens	282	35	151	153	17	188	1	1	61	1	25	5	3	25	5	953

1. *Staphylococcus aureus*, 2. *Staphylococcus saprophyticus*, 3. *Staphylococcus epidermidis*, 4. *Micrococcus luteus*, 5. *Micrococcus roseus*, 6. *Pseudomonas aeruginosa*, 7. *Pseudomonas fluorescence*, 8. *Proteus vulgaris*, 9. *Proteus mirabilis*, 10. *Morganella morganii*, 11. *Escherichia coli*, 12. *Citrobacter freundii*, 13. *Serratia marcescens*, 14. *Enterobacter aerogenes*, 15. *Klebsiella pneumoniae*

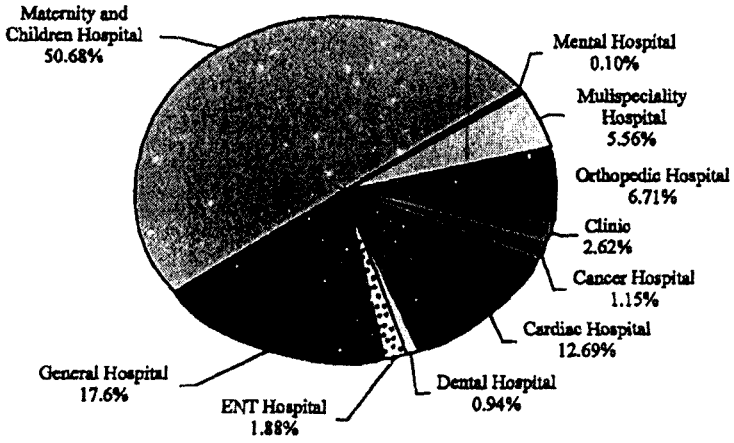

Fig. 3: Total pathogenic bacterial air flora isolated from various hospitals in amravati

Out of all of the hospitals examined, maternity and children hospitals showed highest bacterial contamination, which may be due to unhygienic state of children patient, parents and more crowds as well as nearby and open defecation of children.

The observations strongly recommend periodical recording of such data to keep the sudden outbreak of airborne infections in hospital patients at minimum. For the pathogens that can spread through the air, there must be proper ventilation and exhaust fans in the hospital wards. Spitting and gargling etc. should be at proper place and not at anywhere which may spread infection in the air. Infectious person should try to avoid sneezing, coughing; talking in the open air or in crowded area and the handkerchief should be used. The bed sheets of the earlier patients should not be reused for the next patients. In OPD, crowd should be avoided or minimised. The signboards should be used indicating the use of napkins etc. and coughing, sneezing and talking by open patients. Maternity and children hospitals should be hygienically clean so that there should be proper disposal of the children pads. During entrance in the hospital, shoes should be kept out side so that the dust cannot enter inside. Highly infectious diseased patients should be hospitalized in quarantine or in isolation. The floor of the wards and hospitals should be swabbed with disinfectants daily. Visitors and relative's visits to the patients should be as low as possible. Limit the movement

and transport of the patient from the room to essential purposes only. If transport or movement is necessary, minimize patient dispersal of droplet nuclei by placing a surgical mask on the patient, if possible. Strategies should be such that prevention is better than cure.

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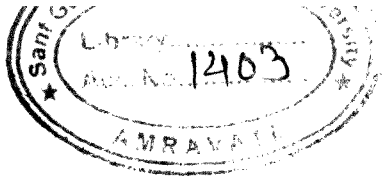
Appendix B

Papers Presented in Conferences/Symposium/Seminar



STUDIES ON PATHOGENIC MICROBIAL AIR FLORA OF HOSPITALS IN AMRAVATI

Gulhane P.B. and D.H. Tambekar

44th Annual Conference of Association of Microbiologists of India, Dharwad, Nov. 12-14' 2003

The investivation was carried out to assess the air microbial contamination in the hospital ward and its surroundings in the Amravati City. Out of the 13 departments, 3 of local civil hospitals investigated, 12 departments and ward rooms were found to be contaminated by members of enterobacteriaceae such as *Escherichia coli. Pseudomonas aeruginosa* and *Staphylococcus aureus.* The general ward was the most contaminated while the operation theatre was free from pathogenic microorganisms. The contamination of air was due to the talking, coughing and sneezing activities of patients, which released droplets containing several thousands of pathogenic microorganisms in air.)

STUDIES ON AIRBORNE BACTERIAL PATHOGENS IN HOSPITALS OF AMRAVATI CITY

Gulhane P.B. and D.H. Tambekar

45th Annual Conference of Association of Microbiologists of India, Karnal, Nov. 23-25' 2004

The emergence of nosocomial infections and multiple antibiotic resistant bacteria has become a major challenge in the treatment of infectious diseases. The continuous updating of knowledge regarding hospital associated pathogens and their antibiotic sensitivity pattern is the need of time. In our present study we examined 953 samples of air quality of 76 hospitals of Amravati by the sedimentation technique on Mannitol Salt Agar, MacConkey agar and Cetrimide agar plates. The different sections of hospitals were studied for the presence of airborne pathogens in indoor and outdoor environment. The aggregate percentage distribution of different bacterial pathogens was recorded as 26.54% in general ward, 22.77% in private room and 15.32% in OPD, 1.88% in ICU, 1.99% in pathology laboratory and in dressing room 0.41%. The comparative percent distribution of individual pathogen in different hospitals was found to be 29.59% *Staphylococcus aureus*, 19.72% *Pseudomonas aeruginosa*, 15.84% *Staphylococcus epidermidis* and 16.05% *Micrococcus luteus.* The dominance of *Staphylococcus aureus* (29.59%) and *Pseudomonas aeruginosa* (19.72%) was commonly observed in all the hospital environment of Amravati city.

ASSESSMENT OF MULTI-RESISTANT AIRBORNE PATHOGENS IN HOSPITALS

Gulhane P. B. and D. H. Tambekar

46th Annual Conference of Association of Microbiologists of India, Hyderabad, Dec. 8-10' 2005

Emergence of nosocomial infection and multiple antibiotic resistant bacteria has become a major challenge in the treatment of infectious diseases. Bacterial isolates from hospital air of Amravati were examined to assess multiple antibiotic resistance patterns. Isolated 100 bacterial spp. included *Staphylococcus aureus, Staph. saprophyticus, Staph. epidermidis, Micrococcus luteus, Micrococcus roseus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Citrobacter freundii, Enterobacter aerogenes* and *Klebsiella pneumoniae* were lined for antimicrobial sensitivity against 15 different types of antibiotics such as amikacin, augmentin, cetazidime, ceftriaxone, cephotaxime, ciprofloxacin, chloramphenicol, erythromycin, fusidic acid, gentamycin, lincomycin, netilmicin, ofloxacin, penicillin and vancomycin. The multiple antibiotic resistant (MAR) indexes of the isolates showed the highest (0.0073) with *Enterobacter aerogenes.* The 1500 tests of antibiotics were made against 100 organisms. Out of them 1015 antibiotic tests were found to be resistant, 103 tests were found intermediate sensitive and 382 tests were found to be sensitive to various antibiotics tested. The 80% isolates were resistant to augmentin and penicillin, 79% to fusidic acid and lincomycin, 76% to erythromycin, 74% to ciprofloxacin, 71% to vancomycin, 66% to ceftazidime and gentamycin, 62% to chloramphenicol, 60% to netilmicin, 58% to ceftriaxone and ofloxacin, 53% to amikacin and cephotaxime.