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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).

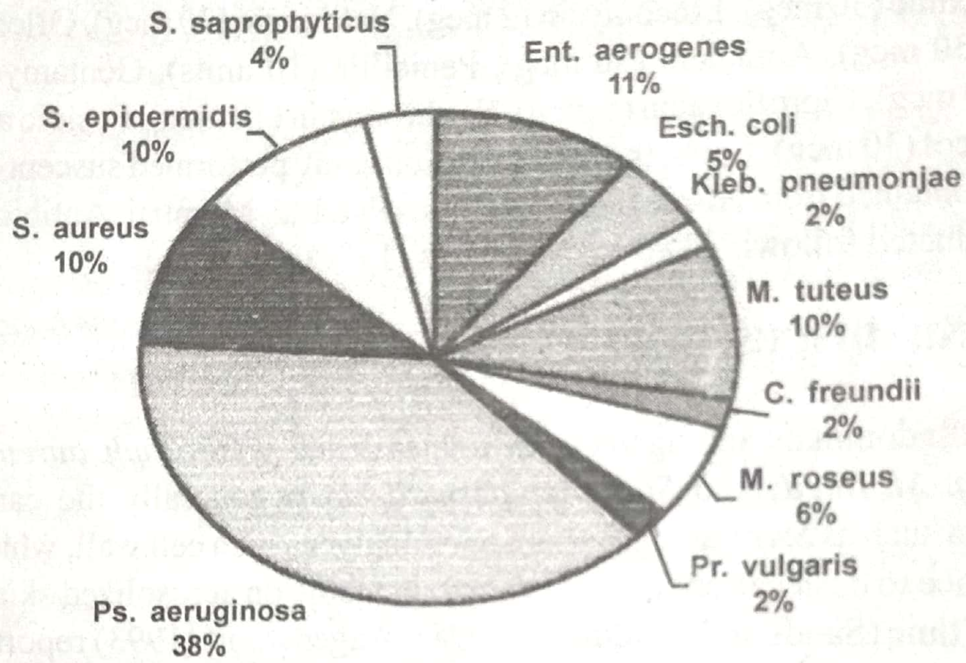

Fig. 1. Bacterial pathogens isolated from hospital environment

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*.

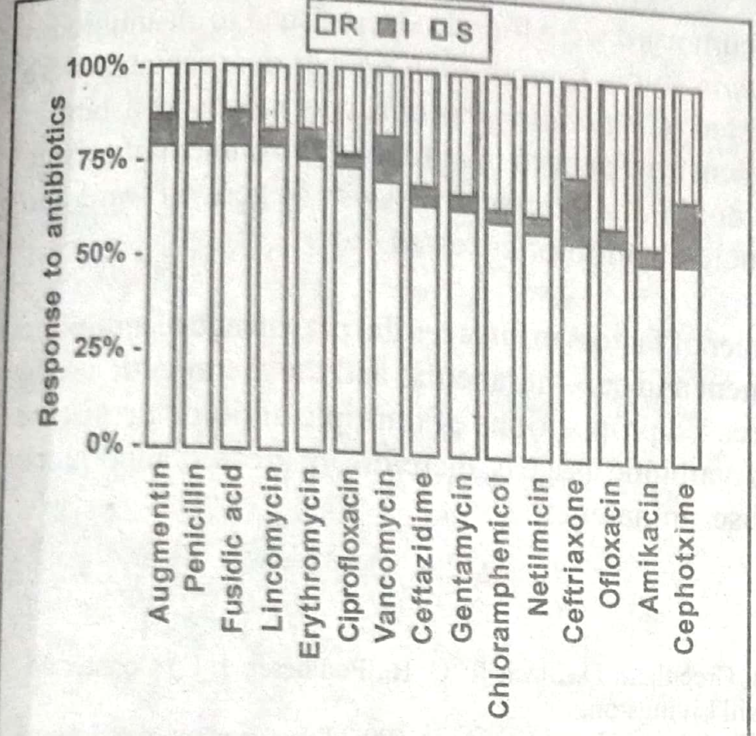

Fig. 2. Antibiotic response against bacterial isolates.

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 of'oxacin. 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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