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INCIDENCE AND ANTIBIOGRAM PATTERNS AMONG NOSOCOMIAL PSEUDOMONAS AERUGINOSA ISOLATES FROM MATERNITY WARDS AND LABOR ROOMS IN GULBARGA REGION, SOUTH INDIA.

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ABSTRACT

Pseudomonas aeruginosa is one of the most common nosocomial pathogen causing opportunistic infections in humans, particularly among immuno compromised patients, and because of its ubiquitous nature, ability to survive in adverse conditions and affinity for moist environment remains a common pathogen in intensive care units (ICU). In the present study, prevalence rate of *P. aeruginosa* in maternity wards and labor rooms in various hospitals of Gulbarga region, South India and their antibiotic sensitivity pattern (AST) are reported. Present study was based on the 190 samples collected from animate and inanimate objects from maternity wards and labor rooms of several hospitals in Gulbarga region, during Oct 2008 to Jan 2010. A total of 43 *P. aeruginosa* were isolated, indicating on isolation rate of 22.63% and the isolation rate was slightly higher in maternity wards than the labor rooms. There is an increased multidrug resistance among *P. aeruginosa* which may be due to the selective pressure from the use of antimicrobial agents which is a major determinant for the convergence of resistant strains, especially in hospital environments.

KEY WORDS: *P. aeruginosa,* Antibotic resistance, maternity ward

INTRODUCTION

Nosocomial infections in hospitalized persons result from adverse reaction to the invasion of an infectious agent(s) or its toxin(s) that was not present or incubating at the time of admission to the hospital [1]. In the developed countries, only 5 to 10% of patients admitted to acute care hospitals suffer from nosocomial infections [2, 3]. However, in developing countries this can exceed 25% [4]. These infections add to the mortality, morbidity and cost expected from the patients underlying disease [5, 6]. The source of nosocomial infections can be endogenous, exogenous like contaminated instruments, needles and the environment or due to cross contamination by the hospital staff [1, 7].

Infections are more severe in pregnant women and further may increase the risk of harm to the fetus or newborn. Infections during birth process are significant cause of fetal and neonatal mortality and an important contributor to early and late childhood morbidity [8]. A diverse group of microorganisms have been reported from the maternity ward which includes Gram Negative Bacteria such as, *Pseudomonas* sps*, E.coli, Proteus* sps*, klebsiella* sps*, Enterobacter* sps, *Nisseria* sps*,* and Gram positive bacteria such as *Staphylococcus aureus* and *S. epidermis.* [9-12]

Pseudomonas aeruginosa is one of the most common nosocomial pathogen causing opportunistic infections in humans, particularly among immuno compromised patients [13], and because of its ubiquitous nature, ability to survive in adverse conditions and affinity for moist environment remains a common pathogen in intensive care units (ICU) [14].
The world wide emergence of multi drug resistant bacterial strain is a growing concern, especially in Hospital Infection (HI) cases caused by *P aeruginosa.* Among the nosocomial bacterial infections, those caused by *P. aeruginosa* are associated with highest mortality rate, and are difficult to eradicate from tissues and blood because those microorganisms are highly virulent and have a limited susceptibility to antimicrobials [15]. The epidemiology of *P. aeruginosa* infections are usually studied by the analysis of phenotypic markers including biotype, serovar, pyocin production, phage type and antimicrobial susceptibility pattern [16].

In the present study, prevalence rate of *P. aeruginosa* in maternity wards and labor rooms in various hospitals of Gulbarga region, South India and their antibiotic sensitivity pattern (AST) are reported.

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MATERIALS AND METHODS

Present study was based on the 190 samples collected from animate and inanimate objects from maternity wards and labor rooms of several hospitals in Gulbarga region, during Oct 2008 to Jan 2010.

Sample collection:

Sterile cotton wool swab sticks dipped in nutrient broth solution were used for swabbing the inanimate objects that included bed sheets, towels, tables, door knob, surgical instruments, gloves, soaps, etc. Nutrient agar plates were used for air samples exposed to the environment for 10 min in the labor room and maternity ward. Samples were also collected from the anterior nares and from the skin of the forehand of mother, child and health care workers in the maternity ward by sterile swabs. The samples thus collected were transmitted to the laboratory and analyzed for bacterial pathogens using standard microbiological techniques.

Isolation and characterization:

Swab samples were inoculated on to nutrient agar and cetrimide agar plates (Hi-Media, Mumbai). The plates were incubated at 37^0C for 24hrs. Colonies were identified by morphological, cultural and conventional biochemical characteristics (Table 1). Thus obtained pure isolates of *P. aeruginosa* and were preserved in glycerol at -20^0C for further studies [17].

Antibiotic susceptibility testing:

The isolated pathogens were subjected for antibiotic susceptibility testing. The method used was disc diffusion method according to CLSI guidelines M39-A3 (18). The calibrated inoculums of the pathogenic microorganisms at 0.5 McFarland were inoculated on to Mueller Hinton Agar plates and antibiotic discs were placed on the surface of plates. Inhibition zones were measured after incubation at 37^0C for 24hrs.

Following antibiotics (Himedia, Mumbai) were used; carbenicillin (CB) 100 mcg, ofloxacin (OF) 5 mcg, amikacin (AK) 30 mcg, aztreonam (AT) 30 mcg, ciprofloxacin (CIP) 5 mcg, gentamycin (GEN) 10 mcg, piperacillin/ tazobactam (PIT) 100/10mcg, cefoxitin (CX) 30 mcg, imipenem (IPM) 10 mcg, levofloxacin (LE) 5 mcg, norfloxacin (NX) 10 mcg, cefotaxime (CTX) 30 mcg, ceftazidime (CAZ) 30 mcg, ceftazidime / clavulanic acid (CAC) 30/10 mcg..

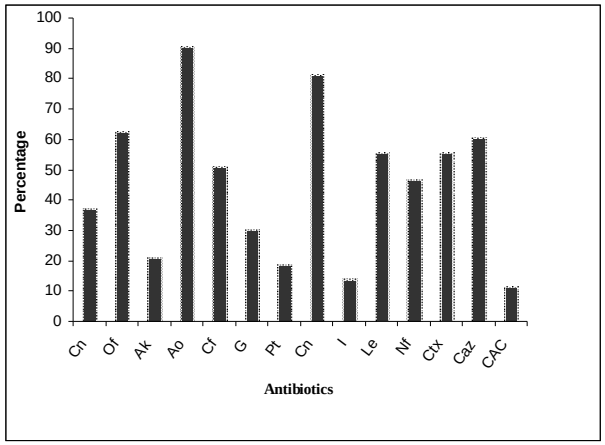
RESULTS

A total of 43 *P.aeruginosa* from 190 samples were isolated from different animate and inanimate objects as shown in table 2, indicating on isolation rate of 22.63% and the isolation rate was slightly higher in maternity wards than the labor rooms.

Maximum numbers of *P. aeruginosa* were recovered from air and inanimate objects while the nasal swabs of the babies did not show any pseudomonal colonization.

The 43 isolates of *P. aeruginosa* were subjected for Antibiotic Sensitivity Test (AST) and the results are represented in the fig. 1.

Fig.1: Antibiotic resistance pattern of *P. aeruginosa* isolates.



The fig. 1 and table 6 clearly indicate that all the isolates were resistant to one or more than one antibiotics. About 90% of the isolates were resistant to aztreonam followed by cefoxitin (81.30%), ofloxacin (62.7%) and lowest numbers of isolates were resistant to imipenem (13.90 %).

Majority of the isolates showed multi drug resistance and maximum number (9) of isolates were resistant to 5 antibiotics; however a single isolate was resistant to a maximum of 11, 12 or 13 antibiotics. On an average about 9.3% of

isolates were resistant to 3,6,7,8,9,10 numbers of antibiotics (Table 4).

Table 1: Colony Morphology and biochemical characteristics of *P aeruginosa* isolates

Sl. No.	Test	Result
1	Gram staining	Gram Negative, Single rods
2	Motility	Motile
3	Colony Morphology	
	Nutrient Agar	Bluish green coloured colonies
	MacConkey agar	Non lactose fermenting colonies
	Blood Agar	Hemolytic colonies
	Cetrimide agar	Bluish green coloured colonies
4	Oxidase	Positive
5	Catalase	Positive
6	Growth at temperature 42 ^{o}C	Negative
7	Urease	Negative
8	Indole	Negative
9	Methyl Red	Negative
10	Vogues Prosker	Negative
11	Nitrate reduction	Positive
12	Gelatin hydrolysis	Negative
13	Glucose	Positive
14	Sucrose	Negative
15	Lactose	Negative
16	Maltose	Negative
17	Mannitol	Positive
18	Xylose	Negative
19	Inositol	Negative
20	Raffinose	Negative
21	Starch hydrolysis	Negative

Table 2: Isolation rate of *P.aeruginosa* in maternity ward

Source	Type of sample	No of Swabs/plates	*P. aeruginosa* Isolated
Air	Exposed plates	4	3
Table	Swab	10	1
Door Knob	Swab	8	4
Beds	Swab	22	6
Mother			
Hand	Swab	11	3
Nasal	Swab	12	3
Baby			
Nasal	Swab	8	0
Health care Workers			
Hand	Swab	11	2
Nasal	swab	9	2
Total		95	24(25.26%)

Table 3: Isolation rate of *P. aeruginosa* in labor room

Source	Type of sample	No of swabs/Plates	*P. aeruginosa* Isolated
Air	Exposed plates	2	3
Surgical Instruments	Swabs	10	2
Gloves	Swabs	8	2
Disinfectants	Solution	20	3
Baby's tray	Swabs	8	1
Weighing machine	Swabs	10	2
Examination table	Swabs	9	1
Labor Bed	Swabs	14	2
Health care worker			
Hand	Swabs	7	2
Nasal	Swabs	7	1
Total		95	19(20.00%)
Total maternity and Labor room rate		190	43(22.63%)

Table 4: MDR pattern of *P. aeruginosa*

Resistant No. of antibiotics	*Pseudomonas aeruginosa* Isolates (%)
1	2 (4.6)
2	3 (6.9)
3	4 (9.3)
4	2 (4.6)
5	9 (20.9)
6	4 (9.3)
7	4 (9.3)
8	4 (9.3)
9	4 (9.3)
10	4 (9.3)
11	1 (2.3)
12	1 (2.3)
13	1 (2.3)
14	0

DISCUSSION

Nosocomial infections are widespread; they are important contributors to morbidity and mortality. They will become even more important as public health problem with increasing economic and human impact as a result of increasing number and crowding of people, more frequent impaired immunity (age, illness, and treatments), new micro organisms and increasing bacterial resistance to antibiotics [20]. They are the major cause of disease and death in developing countries [4].

The present study showed that the percentage of positive isolation of potential pathogenic bacteria at hospital maternity ward and labor rooms was 25.26% and 20.00% respectively

P.aeruginosa is a major cause of hospital infection. Despite advances in sanitation facilities and the introduction of wide variety of antimicrobial agents with anti pseudomonal activities, life threatening infections caused by *P.aeruginosa* continue to be hospital borne. Critical factor in the survival of *P.aeruginosa* in unfavorable environment is its ability to transform mobile "swamer" cell to a glycocalyx enclosed micro colony which serves to protect the organisms against the active phagocytes, surfactants, enzymes and high levels of specific antibodies [23]. The prevalence of new resistant strains continues in both community acquired pathogens and hospital originated infections [23]. *P aeruginosa* was also isolated as a predominant organism from the indoor air of hospitals [20]. The resistance of bacteria in hospital infections was reported by Rutala (1997), Russell (1999) and Nunez and Mortton (2007) [21-23].

Ceftriaxone and ceftazidime are the commonest antibiotics in hospital protocols. Resistance to

the cephalosporins are significant in our study (55-60%) also recorded in another study done by Holloway *et al*., by 60-70 % [24].

Reports of *P.aeruginosa* susceptibility to gentamycin have ranged from as low as 49.8% in Greece, to as high as 99.20% in the United Kingdom [22]. In our study the amino glycoside resistance was found to be low, 20.90% for amikacin and 30.20% for gentamycin. Consistent with these findings resistance to amikacin among *P aeruginosa* was still lower than that against gentamycin and this correlates with the report by Smitha *el al* [25] and Poole *et al* [26].

In various studies, increased resistance rates have been detected against carbapenems, quinolones and third generation cephalosporins for *P.aeruginosa* [27-29]. In our study resistance rates against imipenem is 13.90%. The resistance of *P.aeruginosa* to the antibiotics in the quinolone group is not consistent and variability has been reported in different centers [30-32]. In our study resistance rates against ciprofloxacin is 51.10%. Quinolone resistance in our study is high as compared to the reports of others as 31.90% in Italy and 26.80% in Latin America [33-36].

Overall we have observed that there is an increased multidrug resistance among *P.aeruginosa* which may be due to the selective pressure from the use of antimicrobial agents which is a major determinant for the convergence of resistant strains especially in hospital environments.

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