IF : 4.547 | IC Value 80.26 Volume-6, Issue-4, April - 2017 • ISSN No 2277 - 8160

Original Research Paper Medical Science

Incidence and Antibiotic Resistance patterns of nosocomial Infections caused by *Pseudomonas aeruginosa* in a Tertiary Hospital, Nashik, India: A Epidemiological Study during 2015-2016

Jagannath D. Andhale — Assistant Professor, Department Of Microbiology, Modern Institute Of Medical Sciences, Indore, Madhya Pradesh, India, PIN:452016.

Arun P. More — Assistant Professor, Department Of Microbiology, Pacific Institute of Medical Sciences, Umarda, Udaipur, Rajastan, India, PIN: 313015

Aniket Gangurde — Dr. Gangurde's Hospital, Nashik, Maharashtra, India, PIN: 422005.

ABSTRACT

Backgrounds: *P. aeruginosa* is major causative organism of Nosocomial infections. The emergence of drug resistance and its transmission from patients to patients and from healthy persons to patients in ICU is major health concerns before the health workers in India. Moreover the accumulation of number of drug resistance genes in the this organism gives limited options for treatment of infections caused by it. Therefore, continued surveillance of drug resistance in this opportunistic pathogen is necessary to plan effective anti-microbial treatment against the infections caused by it.

Materials and Methods: From different clinical samples, *P.aeruginosa* was isolated and identified and tested for drug resistances on suitable media using standard procedures before the start and at the end of Stewardship Program. Statistical analysis was done using R statistical package in Linux system.

Results: All the drug tested for drug susceptibility testing show decreased in incidence of drug resistance after a one year time span. Gentamycin show highest decrease of 16.58% followed by ceftazidime at 13.92% and carbenacillin at 12.38%. whereas, the reduction in drug resistance to penicillin and and piperacillin is very low, i.e. only 1.19%.

Conclusions: Imipenem, with incidence of drug resistance of 4.13%, was found to be most effective drug and piperacillin and ciprofloxacin , both with incidence of drug resistance of 41.66% were least effective drug against P. aeruginosa in our study in the year 2016.

KEYWORDS : Antimicrobial Stewardship program, Nosocomial infections, Antimicrobial resistance, P. aeruginosa.

Introduction:

Pseudomonas aeruginosa, which is quintessentially ubiquitous opportunistic pathogen found in soil and water, causes number of post-operative and medical devices associated nosocomial infections in Indian hospital settings.

Spread of *Pseudomonas* infections through contaminated water is most common route of infection in hospital settings[1]. Lung infection of *Pseudomonas* is acquired by inhalation of aerosols of *Pseudomonas* contaminated water[1].

Moreover, due to high particulate pollution caused by soil dust, infections caused by Pseudomonas present in the dust is also high. Persons carry millions of dust particles and hence Pseudomonas present in them, which them easily transmit to inpatients.

The multi-drug resistance to number of routinely used anti-microbial drugs in this organism is matter of concerns among health workers in controlling and treating the infections caused by this organism, especially in hospital inpatients.

10% of the infections contracted during hospital stay of patients are caused by *Pseudomonas aeruginosa*[2].

Although the Pseudomonas infections are third leading causes of nosocomial infections that prolong the ICU stay, multi-drug resistance in this pathogen contributes to higher morbidity and mortality as compared to other pathogens in tertiary health care settings[3,4,5].

Number of studies in India have reported high prevalence of drug resistance in *Pseudomonas aeruginosa* isolated from various samples from 8.43% to 32.1%[6,7,8,9]

The higher drug resistance observed in *Pseudomonas aeruginosa* is due to presence of intrinsic drug resistance to number of drugs used against them, the selection pressure because of higher amount of antibiotics use inappropriately adds to the total drug resistance increasing incidence it many folds in this opportunistic pathogen.

To minimize the transmission of drug resistance and halt accumulation of drug resistance genes in *P. aeruginosa,* number of researchers suggested implementation of Stewardship programs[10]

One of the causes of emergence of drug resistance in bacteria, is higher selection pressure by use of higher amount of antibiotic inappropriately in hospital settings.

Stewardship program is defined as efforts of prescribing appropriate antimicrobial agents, its route, dosing and duration of its use in inpatient settings in order and reduce or prevent emergence of antimicrobial resistance, adverse consequences of its use, improve patients' health and provide cost effective therapy. Cochrane suggested one year period of the assessment of stewardship Programs on incidence of drug resistance in bacteria[10].

In the present study, researchers have studied the epidemiology of trends of drug resistance and incidence of infections caused by *Pseudomonas aeruginosa* with respect to the antimicrobial Stewardship programs for a period of one year.

Materials and Methods:

Sampling :

The number of clinical samples such as pus, urine, blood, sputum and body fluids were collected, as per standard procedures, from inpatients who developed infections after some kind of surgery or after some invasive medical procedures in order to investigate nosocomial infections, their causative organisms and incidence of drug resistance among them. The patients who have already have infections caused by *Pseudomonas aeruginosa* on admission to the hospital were excluded from this research study. Total 250 samples were processed for culture identification and drug susceptibility during each years of 2015 and 2016.

Culturing and identification of Pseudomonas isolates:

The isolates, for *Pseudomonas aeruginosa,* were cultured aerobically in Muller-Hinton broth for 16 to 24 hours at 37°C. The isolates were Gram stained and inoculated first into brain-heart infusion medium and then onto cetrimide agar. The Gram-negative bacteria from

isolated colony from cetrimide agar were confirmed as *Pseudomonas aeruginosa* by biochemical tests. The isolated were tested for oxidative fermentation and ability to grow at 42°C[11].

Implementation of Stewardship Program:

The above mentioned samples were microbiologically processed, drug susceptibility testing of isolated *Pseudomonas aeruginosa* was carried out in order to find out incidence of antibiotic resistance in this organism before the start and at the end ,after one year, of antimicrobial Stewardship program.

The antimicrobial Stewardship program was carried out between between January 2015 to December 2016 and continued thereafter also.

In the present Stewardship program, appropriate antimicrobial drug selection and its dosing was implemented. Antimicrobial therapy was started only after the culture identification and drug susceptibility testing were carried out. The antibiotic therapy was stopped as soon as a targeted infection is subsided.

Drug Susceptibility Testing:

Antibiotic susceptibility testing was carried out by Kirby-Bauer disk diffusion method as per CLSI guidelines[12,13] using antibiotics, gentamycin, piperacillin, carbenicillin, ciprofloxacin, amikacin, ceftazidime and imipenem.

Frequencies of drug resistance for each drug tested were recorded sample wise, gender wise and age group wise. Proportion and P-value was calculated using R-package of statistical analysis[14].

Results and discussion:

Results :

The total 250 various samples were selected randomly during January 2015 to December 2015, processed for drug resistance and data such as type of samples, type of infections and incidence of drug resistance was analyzed sample wise, gender wise and age-groups wise. Same procedure was followed for the 250 samples processed during January 2016 to December 2016. Sample-wise, gender-wise and age-groups wise incidences of nosocomial infections caused by *Pseudomonas aeruginosa* were recorded in **table No.1, 2** and **3** respectively.

Table No. 1 Sample-wise incidence of *Pseudomonas aeruginosa isolates* in 2015 and 2016 (sample size = 250)

Sr. No.	Specimen	Number of Samples in 2015	Number of Samples in 2016	proportions (%) in 2015	Proportions (%) in 2016
1	Wound Swab/Pus	20	14	57.14	58.33
2	Urine	10	5	28.57	20.83
3	Sputum	3	3	8.57	12.50
4	Body fluid	2	2	5.71	8.33
Total		35	24	100	100

Table No. 2 Gender-wise incidence of *Pseudomonas aeruginosa* infections in 2015 and 2016.

Sr. No.	Gender	Number in 2015	Number in 2016	Proportion(%) in 2015	Proportion(%) in 2016
1	Male	21	14	60	58.33
2	Female	14	10	40	41.66
Total		35	24	100	100

Table No. 3 Age-groups wise incidence of *Pseudomonas aeruginosa* infections in 2015 and 2016

Sr.No.	Age in years	No. of Patients in 2015	No. of patients in 2016	proportion (%) in 2015	Proportions (%) in 2016
1	0-21	3	2	8.57	8.33
2	21-40	5	8	14.28	33.33
3	41-60	17	12	48.57	50
4	61-80	10	2	28.57	8.33
Total		35	24	100	100

The data of incidence of drug resistance in *Pusedomonas aeruginosa* for year 2015 and 2016 was tabulated in **table No. 4.**

Table No. 4 Incidence of drug resistance in *Pseudomonas aeruginosa* isolates in 2015 and 2016 (Sample size = 250)

Sr. No.	Drugs (Mcg)	No. Of Sensitive Samples In 2015(%)	No. Of Sensitive Samples In 2016(%)	No. Of Resistant Samples In 2015(%)	No. Of Resistant Samples In 2016(%)	P-values
1	Gentamycin (10)	19(54.28)	17(70.83)	16(45.71)	7(29.13)	0.06391
2	Carbenicillin (100)	19(54.28)	16(66.66)	16(45.71)	8(33.33)	0.1516
3	Piperacillin (100)	20(57.14)	14(58.33)	15(42.85)	10(41.66)	0.5413
4	Amikacin (30)	20(57.14	16(66.66)	15(42.85)	8(33.33)	0.1516
5	Ciprofloxacin (5)	18(51.42)	14(58.33)	17(48.57)	10(41.66)	0.5413
6	Ceftazidime (30)	17(48.57)	15(62.5)	18(51.42)	9(37.50)	0.3075
7	Imipenem (10)	32(91.42)	23(95.83)	3(8.57)	1(4.13)	2.98e-06

Proportion of Wound and pus samples were remarkably high followed by urine samples both in years 2015 and 2016.

Male inpatients recorded higher incidence of pseudomonas infections than female patients both in the years 2015 and 2016.

The lowest resistance was observed for imipenem both for 2015 and 2016 of 8.57% and 4.13% respectively.

During the period of one year, all the drugs tested for drug susceptibility show remarkably high drug resistance.

Ceftazidime show highest resistance of 51.42%, followed by ciprofloxacin with resistance of 48.57%, in 2015 among all the drug tested whereas imipenem show lowest resistance of 8.57% in 2015.

In 2016, imipenem show lowest drug resistance of 4.13%, whereas other drugs show resistance between 29.13%(Gentamycin) to 41.66%(ciprofloxacin and piperacilin).

Discussion :

The incidence of Pseudomonas isolates and infections were 14% and 9.6% in the years, 2015 and 2016 respectively, sample-wise, gender-wise and age-group wise.

All the drugs tested for drug resistance, though show remarkably high resistance during 2015, the incidence of drug resistance has come down by 10% to 12% in 2016. During 2015, incidence of drug resistance for ceftazidime was 51.42% which observed to be 37.50% in 2016; thus incidence of drug resistance for this drug reduced by 16.58%. which is highest decrease in incidence of resistance. Penicillin, piperacillin show very negligible reduction of drug resistance, 1.19%, during a year period.

Incidence of drug resistance for Imipenem was recorded 8.57% in 2015 whereas it come down to 4.13% in 2016; a marginal reduction of 4.44%.

Thus the reduction in incidence of drug resistance is proportionate to initial incidence of the previous year, i.e. 2015; higher the incidence more is its down fall as results of various practices

followed in Stewardship program in this research work. However, the reduction in drug resistance to penicillin and and piperacillin is very low, i.e. only 1.19%, despite their remarkably high resistance in 2015; this might be due to presence of number of mutations governing a single drug resistance which take time to revert back to normal genes and this inferences can be supported by the facts that chromosomal β lactamase and MexAB-OprM MDR system are required for drug resistance to β lactam antibiotics[15]. This type of cooperative mutations show very low mutability rate in the organism[16] resulting in low decrease in incidence of drug resistance.

The high incidence of drug resistance among patients visiting this hospital might be because of accumulation of drug resistant strains of Pseudomonas aeruginosa among patients, most of who are living in dusty environment and frequently followed antibiotics regimes for acute respiratory system's illness; this inferences are drawn from oral communications by the patients since the history of such patients can not be recorded, which is the limitation of this research work.

Some significant outcomes of this research work are that that avoiding inappropriate use of anti-microbial drug, combination of more that one antibiotic use and implementation of good sanitary and aseptic practices in hospital environment help controlling spread of drug resistance in *Pseudomonas aeruginosa.*

Since the said hospital is situated in dusty environment and the dust being the major source of Pseudomonas infections, the researchers have suggested the air handling system to the administration of the hospital. The air handling system would provide microbe-free air to the modular operation theater, thus substantially reducing the microbial loan in the hospital air. The researchers, therefore, suggested to the said hospital administration to install pure air handling system in their hospital. The researchers also suggested to stop prescribing those antibiotics with high incidence of drug resistance, e.g. penicillin, piperacillin, ciprofloxacin and ceftazidime.

Conflicts of Interests:

The authors have no any kind of affiliations or financial involvement with any organization or department of government or private body with a financial interest in or conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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