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PSEUDOMONAS AERUGINOSA: PREVALENCE, ANTIMICROBIAL RESISTANCE PATTERN AND RISK FACTORS IN A TERTIARY CARE HOSPITAL IN WESTERN RAJASTHAN

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ABSTRACT **Introduction-** *Pseudomonas aeruginosa* is an important nosocomial and opportunistic pathogen. Inappropriate empirical therapy has been associated with increased mortality in resistant *P.* aeruginosa infections, and may contribute to increased length of hospital stay and persistence of infection.

Aims and Objective- The main aim and objectives of this study are to detect the prevalence, antimicrobial resistance pattern and risk factors of *P. aeruginosa* isolated from different clinical specimens.

Material and Methods - This is a hospital based prospective study. A total of 920 clinical specimens were included from March 2015 to December 2015. Samples were processed in the Microbiology laboratory by standard bacteriological methods and antimicrobial susceptibility were performed by Kirby-Bauer disc diffusion method and result interpreted according to CLSI guidelines.

Results- *P.aeruginosa* were isolated in 132 out of 920 various clinical samples, The prevalence rate of *P. aeruginosa* was 14.34%. *P. aeruginosa* was maximally resistant to Piperacillin (74.24%) followed by Aztreonam (68.18%), Ceftazidime (64.39%), Amikacin (53.78%), Ciprofloxacin (50.75%), Cefepime (49.24%), Piperacillin/Tazobactam (40.90%), Imipenem (31.06%) , and least resistant to Colistin (15.15%). Duration of stay >2days was the most common risk factor (49.24%) associated with *P. aeruginosa* infection followed by wound infection (38.63%), urinary catheterization (36.36%) , Immunocompromised state of patient (34.09%), endotracheal intubation (22.72%), severe sepsis (21.96%), previous antibiotic use (18.18%), mechanical ventilation (17.42%), and post operative state (16.66%) were the other significant risk factors for the *P. aeruginosa* infection.

Conclusion- *P. aeruginosa* is becoming resistant to antibiotics due to its excessive consumption exerting selected pressure on bacteria, frequently used invasive devices and severe under lying diseases. Treatment should be carried out using antibiotic susceptibility test and efforts should be made to prevent spread of resistant bacteria. The risk factors for mortality in *P. aeruginosa* infections are similar to those of other severe infections, i.e. duration of stay, age, presence of septic shock.

KEYWORDS : ventilator associated Pneumonia, (VAP) , Clinical & Laboratory Standard Institute (CLSI), extended spectrum beta-lactamase (ESBL).

INTRODUCTION-

P. aeruginosa is rarely part of the microbial flora of healthy individuals, but it is an important cause of nosocomial respiratory tract infections including ventilator associated Pneumonia (VAP) and wound infection in burn patients[1]. *Pseudomonas aeruginosa* produces a variety of virulence factors, which aid in colonising a host[2]. Isolates of *P. aeruginosa* from healthy carriers or environmental sites are significant only when there is a risk of transfer to compromised patients[3]. *P. aeruginosa* is the most frequently isolated troublesome pathogen causing life threatening respiratory tract infection (ventilator associated pneumonia), Surgical site and Urinary tract infections in patients from intensive care units.[4]Endocarditis and septicemia carry a high mortality rate in patients compromised by severe burns, cancer or drug addiction[5]. Nosocomial infections due to multidrug resistant bacterial pathogens have been associated with increased hospital expenditures and poorer clinical outcomes[4]. Mechanisms that cause antimicrobial drug resistance and multi-drug resistance in *P. aeruginosa* due to acquisition of resistance genes are the target of the fluoroquinolones particularly ciprofloxacin[6].

AIMS AND OBJECTIVES:-

The main aim and objectives of this study is to detect the prevalence, antimicrobial resistance pattern and risk factors of *Pseudomonas aeruginosa* which isolated from different clinical specimens.

MATERIALS AND METHODS:-

A prospective study was performed during 8 months of period from March 2015 to December 2015, in a tertiary care institute in western Rajasthan. A total of various 920 clinical specimens (pus, wound swabs, body fluids, sputum, stool, urine, throat swab, etc) were investigated for bacterial culture and identification were included in our study from OPD and IPD patients of Medicine, Surgery, 2 ICU (4 Medical & 2 surgical ward), CCU, Burn, Orthopedic and Post operative wards in a tertiary care hospital, Jodhpur, Rajasthan, for 8 months. The clinical data was obtained from the respective units and wards of the patients.

The accurate isolate and identification of *Pseudomonas aeruginosa* were detected according to the standard microbiological techniques[7]. The antimicrobial sensitivity tests were performed for all isolates by Kirby Bauer disc diffusion method recommended by Clinical & Laboratory Standard Institute (CLSI)[8].

RESULTS:-

Out of total 920 samples,132 (14.34%) strains of *P. aeruginosa* were isolated, identified and tested for antibiotic sensitivity by standard microbiological procedures[7]. *P.aeruginosa* exhibited characteristic pigmentation i.e. Pyocyanin 90(68.18 %), Pyoverdin 30(22.72%), while no pigmentation seen in 12 (09.09%) samples. Maximum No. of bacterial species isolated other than *P. aeruginosa* was *Staphylococcus* species (13.04%) and least was Proteus species (04.34%).

Table no.1-Age and sex wise distribution of the patients

Age (in years)	Male	Female	Total	Percentage (%)
Below 20	13	11	24	18.18%
20-30	17	10	27	20.45%
30-40	17	11	28	21.21%
40-50	06	09	15	11.36%
50-60	14	01	15	11.36%
60-70	11	01	12	9.09%
>70	10	01	11	8.33%
Total	88 (66.66%)	44 (33.34%)	132	100%

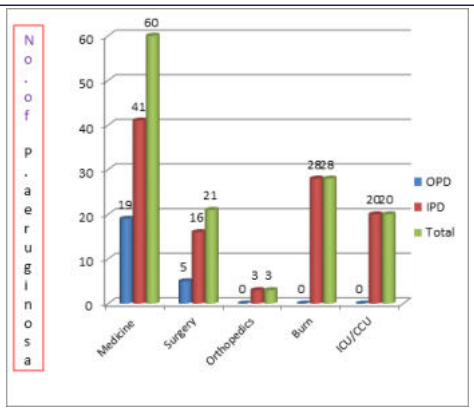

Fig. No. 1 *Pseudomonas aeruginosa* isolated in reference to admission in various wards (O.P.D./I.P.D.)

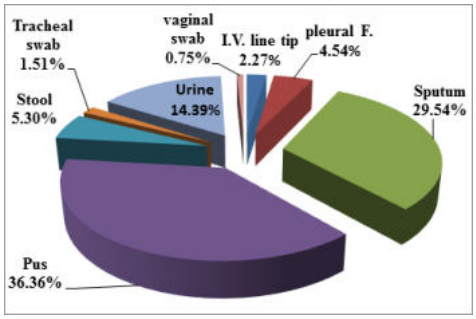

Fig. No. 2 *Pseudomonas aeruginosa* among clinical specimen

Table 2: Risk factors associated to *P. aeruginosa*

S. No.	Risk factors	OPD (%)	IPD (%)	Total
1	DURATION OF STAY > 2 DAYS	00	65	65(49.24%)
2	MECHANICAL VENTILATION	04	19	23(17.42%)
A	Ventilation associated pneumonia	00	14	14(10.60%)
B	Acute respiratory distress	04	5	09
3	CATHETERIZATION	02	52	58(43.93%)
A	Central venous catheter	00	4	4
B	Urinary catheter	02	46	48(36.36%)
C	Central line catheterization	00	2	02
4	IMMUNOCOMPROMISED PATIENT	11	34	45(34.09%)
A	Diabetic	11	14	25
B	Care in ICU	00	20	20
5	SEVERE SEPSIS	00	29	29(21.96%)
6	PREVIOUS ANTIBIOTIC USE/ inadequate treatment	02	22	24(18.18%)
7	ENDOTRACHEAL TUBE	00	30	30(22.72%)
8	POST OPERATIVE STATE/SURGICAL INTERPRETATION	02	20	22(16.66%)
09	BRONCHOSCOPY	00	18	18(13.63%)
10	LACERATED WOUND & CONTAMINATED WOUND	09	42	51(38.63%)
11	OTHERS	01	24	25(18.93%)

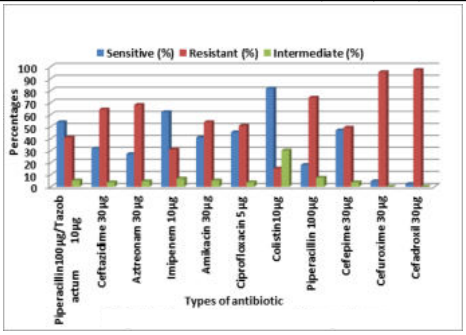

Fig. No. 3. Antibiotics sensitive patterns

DISCUSSION: -

In this present study the prevalence rate of *Pseudomonas aeruginosa* from the hospitalized and outdoor patients and their antimicrobial susceptibility patterns were determined and compared with other studies.

Our result shows that prevalence rate of *P. aeruginosa* was 14.34% of 920 pathogens isolated from all samples. The prevalence rate is similar with the work done by **Rajat RM** et. al.[9], (15.87%) as well as a previous study by **Chander** et. al. (17.05%).[10]

In this study the age wise prevalence of *P. aeruginosa* (132) was found to be more distributed among age groups between 30-40 years (21.21%) followed by 20-30 years (20.45%) and 10-20 years (18.18%) of age group. It was found least common in patients more than 70 years (18.33%) of age. This may be due to maximum occupational exposure of organism to the young adults. Mean age for male was 44.18 ± 20.87 & for female was 33.31 ± 14.61. **(Table no. 1)**

This was found similar to study done by **Chander** et. al. where most of *P.aeruginosa* belonged to the age group 21-40 years (41.40%), followed by patients of > 60 years (31%) of age.[10]

An another study done by **Rajat RM** et. al. (2012) showed 29% of patients with *P. aeruginosa* were found in age between 31-45 years.[9] While, In study by **Mohanasoundaram KM** (2011) a high prevalence of *P. aeruginosa* infection was also found in the 35-50 years age groUP.[11]

The study reveals that sex-wise, there was male patient predominance (66.66%) which constituted a larger group of patients than females (33.34%) **(Table no. 1)**. A recent study done by **Pal Ramprasad Balikaran** et. al. (2010) also reported highest incidence of *P. aeruginosa* among males than females.[12] in another study by **Jamshaid** et. al. the gender wise prevalence of clinical isolates showed that infections caused by of *P. aeruginosa* are very common in male (61.78%) as compared to female (38.22%).[13]

Out of total 132 patients 79 (59.84%) were of urban geographical area and 53 (40.16%) of rural area **.**This may be due to the more visits of hospital by urban population as compared to rural population.

In O.P.D., Out of 132 patients maximum number of *P. aeruginosa* were isolated from Medicine department O.P.D. (79.16%) as well as I.P.D. (37.96%). The distribution of *P. aeruginosa* from other I.P.D. wards were burn ward (25.92%), I.C.U. (18.51%), Surgical ward (14.81%) and least from orthopedic ward (2.77%). However on comparison p value (0.250) was statistically not significant. **(Fig. no. 1)**The distribution of *P. aeruginosa* prevalence may vary with each hospital as every hospital facility has a different environment associated with it. In contrast a study by **Rajat RM** et. al.,(2012) the highest percentage (48%) of *P. aeruginosa* infections were observed in the surgical ward, followed by paediatric ward (23%) and medical ward (17%).[9]

In our study Maximum number of *P. aeruginosa* were isolated from pus (36.36%) followed by sputum (29.54%) , urine (14.39%) and least from vaginal swab culture (0.75%) **(Fig no. 2) .** Our finding are similar to Chander **anil et**. al. study, reported that *P. aeruginosa* isolates were obtained from wound / pus (27.60%), sputum (24.10%), urine (20.70%) and tracheal aspirates (10.35%).[10]

In our study Maximum number of *P. aeruginosa* exhibited characteristic pigmentation i.e. Pyocyanin (bluish green) (68.18%), Pyoverdin (greenish yellow) (22.72%) and no pigmentation seen in 09.09%.This was found similar to B. Srinivas et. al. study in which *P. aeruginosa* exhibited characteristic pigmentation i.e. Pyocyanin (bluish green) (53.35%), Pyoverdin (greenish yellow) (20.23%) and no pigmentation in 09.53%.[14]

In our study we evaluated that among the bacterial isolates recovered from total 920 samples which are listed in**,** *P. aeruginosa*, *Staphylococcus sp*., *Streptococcus sp., Klebsiella sp*. and *Enterococcus sp*. were the most frequently isolated organisms, each representing 14.34%, 13.04%, 12.5%, 11.96% and 09.78 % respectively. Other organisms like *Esch. coli, Citrobactor sp., Enterobacter sp., Candida sp*., *Acinetobacter sp.* were represented 08.15%, 07.61%, 06.52%, 05.97%, 05.76%, 04.34% respectively. According to **Mehta** et. al.,(2007) in India, *Pseudomonas* species was the commonest pathogen isolated (51.5%) followed by *Acinetobacter*

species (14.28%), *S. aureus* (11.15%), *Klebsiella species* (9.23%) and *Proteus species* (2.3%).[15]

In our study duration of stay (>2days) was the most common risk factor (49.24%) associated with *P. aeruginosa* infection followed by wound infection (38.63%), urinary catheterization (36.36%) , Immunocompromised state of patient (34.09%), endotracheal intubation (22.72%), severe sepsis (21.96%), previous antibiotic use (18.18%), mechanical ventilation (17.42%), and post operative state (16.66%) were the other significant risk factors for the *P. aeruginosa* infection **(Table no. 2)**. In 2003 in a study done by Cheol-In Kang et. al. on *P. aeruginosa* in contrast to our study, prolonged hospital stay was risk factor in 27.2% patients only.[16] In (**Levine** et. al.**)** study *P. aeruginosa* frequently leads to colonization of the upper respiratory tract and once established, it is impossible to eradicate them and leads to VAP in 10 to 15% of patients.[17]

In another study by **Raquel Cavalcanti Dantas** et. al. Length of hospital stay [mean days](55.4)%, Mechanical ventilation (53.3%), Urinary catheter (63.3%), Central venous catheter (75%), Diabetes mellitus (11.7%), Central line catheter related (16.7%), Surgical drain (16.7%),severe sepsis (22.5%), Inadequate treatment (28.3%) were some of the risk fators.[18]

In this study *P. aeruginosa* was maximally resistant to Cefadroxil (97.72%), followed by Cefuroxime (95.45%), Piperacillin (74.24%), Aztreonam (68.18%), Ceftazidime (64.39%), Amikacin (53.78%), Ciprofloxacin (50.75%), Cefepime (49.24%), Piperacillin/Tazobactam (40.90%), Imipenem (31.06%) , and least resistant to Colistin (15.15%) **(Fig no. 3).**

A study by **Rajat RM** et. al.*,* (2012) study shows maximum isolates of *P. aeruginosa* isolated from various samples were resistant to different drugs as follows Piperacillin (50%), Piperacillin tazobactam (4%), Aztreonam (39%), Imipenem (14%), Ciprofloxacin (49%) and Ceftazidime (43%).[9]

In our study, the prevalence of resistance to Piperacillin for *P. aeruginosa* was 74.24% as compared to Piperacillin-tazobactam combination which was 40.90%. This shows that the combination (Piperacillin tazobactam) has greater anti-bacterial activity against *P. aeruginosa* compared to its monotherapy (i.e.Piperacillin alone). In **Rajat Rakesh M** et. al.*,* study resistance to Piperacillin in *P. aeruginosa* was (50%) and resistance to in combination Piperacillin-tazobactam was (4%).[116,148] It explained as concurrent administration of beta -`lactamase inhibitor markedly expands the spectrum of activity so combination therapy is better than monotherapy[9].

Ciprofloxacin is the most potent agent available in oral form for the treatment of *P. aeruginosa* infections. Second-generation quinolones have expanded gram-negative activity[19]. Third and fourth generation are considered to anti pseudomonal drugs according to CLSI guideline.[20]

In our study in Cephalosporins , Cefadroxil and Cefuroxime were highly resistant which explained as first generation cephalosporins have relatively narrow spectrum of activity focused mainly on the gram-positive cocci. The second generation cephalosporins have a greater gram-negative spectrum while retaining some activity against gram-positive bacteria. They are also more resistant to beta-lactamase. Third generation cephalosporins have a marked activity against gram-negative bacteria due to enhanced beta-lactamase stability and the ability to penetrate the gram-negative cell wall. Fourth generation cephalosporins have the broadest spectrum of activity, with similar activity against gram-positive organisms as first generation cephalosporins. They also have a greater resistance to beta-lactamases than the third generation cephalosporins and excellent activity against *Pseudomonas aeruginosa.*

CONCLUSION-

P. aeruginosa is becoming resistant to antibiotics due to excessive consumption of antibiotics exerting selected pressure on bacteria, frequently used invasive devices and severe under lying diseases. The empirical antibiotic treatment should be avoided and treatment should be carried out using antibiotic susceptibility test and efforts should be made to prevent spread of resistant bacteria. In conclusion, the risk factors for death in *P. aeruginosa* infections are similar to those of other severe infections, i.e. duration of stay, age, presence of septic shock.

Limitation-This study has a few limitations. First, there was limited number of cases with a short duration, so further work up is required & it is essential to conduct with a large number of cases, long duration of follow up and study with newer anti-pseudomonal agents which was not done. Second, molecular typing and plasmid profile of the *P. aeruginosa* isolates would have provided the much needed details about the strains and lastly extended spectrum beta-lactamase (ESBL) producing *P. aeruginosa* which have become a major cause of nosocomial infections with MDR strains should be analyzed.

Ethical Consideration:-

Ethical clearance was sought from Institutional ethical committee. Informed consent was requested from the patients, parents/guardians for participation into the study. Information about the study was given to the participants to ensure that they have the information needed to make an informed consent.

Abbreviations:-

VAP -ventilator associated Pneumonia
CLSI - Clinical & Laboratory Standard Institute
ESBL -extended spectrum beta-lactamase
MDR –multi drug resistence
IPD –indoor patient department
OPD –outdoor patient department
ICU –intensive care unit
CCU –cardiac care unit

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