Indian Journal of Clinical Practice, Vol. 31, No. 8, January 2021

Study of *Pseudomonas aeruginosa* Growth in Hospitalized Patients

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ABSTRACT

Pseudomonas aeruginosa is a leading cause of nosocomial infections, ranking second among the Gram-negative pathogens. Hence, this study was required to enhance the knowledge about this particular organism. A total of 100 isolates of *P. aeruginosa* isolated from various clinical specimens like urine, pus, blood, body fluids, sputum, collected from patients, irrespective of age and sex, were identified by standard microbiological procedures. A total of 100 culture positive samples were taken and it was found that *P. aeruginosa* was predominantly present in urine samples of males aged between 21 and 30 years.

Keywords: *Pseudomonas aeruginosa*, National Nosocomial Infections Surveillance system, intensive care unit

Pseudomonas aeruginosa is the perfect example of an opportunistic pathogen of humans. Infection due to *P. aeruginosa* is seldom encountered in healthy adults. Now the organism has become increasingly recognized as the etiological agent in a variety of serious infections in hospitalized patients with impaired immune defenses. It causes infections particularly in burns patients as the skin host defenses are destroyed, orthopedic-related infections, respiratory diseases, immunosuppressed and catheterized patients. It may be the cause of the chronic debilitating pulmonary infections, which is one major cause of death in patients with cystic fibrosis. Generally, it contributes substantially to wound-related morbidity and mortality worldwide. *P. aeruginosa* is a leading cause of nosocomial infections, ranking second among the Gram-negative pathogens reported to the National Nosocomial Infections Surveillance (NNIS) system. Hence, this study is required to observe the growth of *P. aeruginosa* in various samples according to parameters related to hospitalized patients, to enhance the knowledge about this particular organism.

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MATERIAL AND METHODS

This study was conducted in the Dept. of Microbiology, Pt. BD Sharma Post Graduate Institute of Medical Sciences, Rohtak, over a period of 1 year.

A total of 100 isolates of *P. aeruginosa* isolated from various clinical specimens like urine, pus, blood, body fluids, sputum, etc., were collected from patients, irrespective of age and sex, and were identified by standard microbiological procedures.

Collection of specimen

1. Urine: clean catch midstream urine samples were collected.
2. Pus: aspirated samples of pus or swabs were collected.
3. Blood: blood samples were collected by aseptic venipuncture.
4. Body fluids: body fluids were aspirated under aseptic conditions.
5. Sputum: expectorated sputum samples were collected.

Processing and Culture of Organism

Microscopy and culture of all the above mentioned samples were done. Cultures were performed on blood agar and MacConkey agar. Inoculated media were examined for growth after overnight incubation at 37^0C. Blood samples were cultured in glucose broth and subcultured on blood agar and MacConkey agar after incubation at 37^0C for 24 hours, 48 hours, 72 hours and

on 7th day. The evaluation of colony morphology on the plating media was done and the subsequent identification procedures were carried on the isolated bacteria, using standard procedures.

Blood agar and MacConkey agar were inoculated within half an hour of collection with the specimen. Inoculation of samples on culture medium was done using an ordinary reusable inoculating loop.

IDENTIFICATION AND SCREENING OF *P. AERUGINOSA*

Gram Staining

The smear was prepared on clean, grease free slide, air dried and heat fixed. Crystal violet was poured on the slide, allowed to remain for 1 minute and rinsed with tap water. Gram's iodine was then poured on the slide, retained for 1 minute and then rinsed with tap water. The smear was decolorized with acetone and rinsed immediately with tap water. The slide was counter stained with carbol fuchsin for 30 seconds and rinsed with tap water and air dried. The slide was finally examined under an oil immersion lens for presence of Gram-negative bacilli.

Detection of Motility Using Hanging Drop Preparation

A part of colony was passed into peptone water and incubated at 37°C for 2 hours. After 2 hours, hanging drop was prepared by taking a loopful of growth from peptone water. It was kept on a cover slip and was inverted on a slide with a plasticine ring over it. First, the edge of the drop was focused under 10X of microscope and then it was examined under 40X. Gliding type of motility was seen in maximum number of isolates.

Biochemical Reactions

The various biochemical reactions used were oxidase test, catalase test, motility, growth at 42°C, oxidative/fermentative medium (Glucose, Maltose, Lactose), nitrate reduction test, MR/VP, mannitol motility medium, triple sugar iron agar, indole production, urea hydrolysis, citrate utilization.

RESULTS

A total of 100 isolates of *P. aeruginosa* isolated from various clinical specimens like urine, pus, blood, body fluids, sputum, etc., collected from patients, irrespective of age and sex, were included in the present study. *P. aeruginosa* isolates were identified on the basis of Gram staining, motility and biochemical reactions.

Out of 48,218 clinical samples received in the laboratory during the study period, 12,854 (26.66%) showed bacterial growth, while rest 35,364 samples (73.34%) were either culture sterile, or showed the growth of bacterial contaminants or fungal isolates. The overall isolation rate of *P. aeruginosa* was 12.21%.

Table 1 shows the sex distribution of patients with *P. aeruginosa* infection among different age groups. The male-to-female ratio among patients with *P. aeruginosa* was 1.27:1. Majority of the patients from whom *P. aeruginosa* was isolated belonged to age group 21-30 years (40%), followed by age group 31-40 years (17%) and age group 41-50 years (16%).

Table 2 shows the distribution of *P. aeruginosa* isolates among a total of 100 clinical isolates. The maximum number of *P. aeruginosa* isolates were from urine samples (49%), followed by pus samples (20%), blood samples (19%), sputum (11%) and body fluids (1%).

Table 1. Age and Sex-wise Distribution of Patients from whom 100 Isolates of *P. aeruginosa* were Taken

Age groups (years)	Male		Female		Total	
	n	%	n	%	n	%
0-10	2	3.57	5	11.36	7	7.0
11-20	9	16.07	5	11.36	14	14.0
21-30	17	30.36	23	52.27	40	40.0
31-40	11	19.64	6	13.64	17	17.0
41-50	12	21.43	4	9.09	16	16.0
51-60	3	5.36	1	2.27	4	4.0
>60	2	3.57	0	0.0	2	2.0
Total	**56**	**56.0**	**44**	**44.0**	**100**	**100.0**

Table 2. Distribution of *P. aeruginosa* Isolates Among Various Clinical Samples

Name of sample	Number of *P. aeruginosa* isolates (n)	Percentage (%) of *P. aeruginosa* isolates
Urine	49	49
Pus	20	20
Blood	19	19
Sputum	11	11
Body fluids	1	1
Total	**100**	**100**

DISCUSSION

The purpose of this study was to enhance the knowledge about this organism according to various patient-related parameters.

Nosocomial infections caused by *P. aeruginosa* are frequently life-threatening and often challenging to treat. In the current study, the rate of isolation of *P. aeruginosa* isolates from culture positive samples was 12.21%, which was lower than studies by other authors who have reported an isolation rate of 19-31.71% from all culture positive samples. This discordance may be due to implementation of better infection control measures in our hospital, like barrier precautions, frequent hand washing by hospital staff, removal of catheters at frequent intervals, regular environmental sampling from ICUs, operation theaters and wards. However, Sherertz et al have reported an isolation rate of 12.5% which was similar to current study. Gales et al and Khan et al have reported an isolation rate of 9.46% and 6.67%, respectively from culture positive samples, which was low as compared to this study. This may be due to different prevalence rates of *P. aeruginosa* isolates in different geographical areas. In addition, prevalence rate may also vary from hospital to hospital.

The present study showed maximum rate of isolation of *P. aeruginosa* isolates from urine samples (49%), followed by pus samples (20%), blood samples (19%), sputum (11%) and body fluids (1%). The results of current study were in accordance with those of a study by Pitout et al who have also reported maximum rate of isolation of *P. aeruginosa* isolates from urine samples (43%), followed by pus samples (21%) and respiratory tract samples (20%) and blood samples (7%). However, Khan et al reported maximum rate of isolation of *P. aeruginosa* isolates from pus samples (57.64%), followed by urine (24.2%) samples. The difference in rates of isolation may be due to difference in type of samples received in different laboratories.

The male-to-female ratio among the patients with *P. aeruginosa* infections in the present study was 1.27:1, which was in accordance with the study done by Sherertz et al who also reported the male-to-female ratio in patients with *P. aeruginosa* infection to be 1.3:1. Khan et al reported the male-to-female ratio among patients with *P. aeruginosa* infection to be 1.6:1. Higher incidence of infection among males in the present study was in accordance with these studies.

In the present study, the majority of patients from which *P. aeruginosa* was isolated belonged to age group 21-30 years (40%), followed by age group 31-40 years (17%) and age group 41-50 years (16%). Another study by Ruhil et al revealed the occurrence of *P. aeruginosa* infection was highest in patients aged 16-40 years. However, Mahmoud et al reported more *P. aeruginosa* infections in patients in the age group >45 years (mean) and Sherertz et al reported majority of *P. aeruginosa* infections in patients in age group 50-80 years.

CONCLUSION

This study concluded that *P. aeruginosa* was grown predominantly in urine samples, especially in young adult hospitalized patients. Hence, suspicion of *P. aeruginosa* should not be avoided, especially in northern part of India.

SUGGESTED READING

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New Coronavirus Variant Raises R Number by up to 0.7

The new variant of coronavirus is more transmissible than the virus's previous version, reported a study by the Imperial College, London. It further stated that the new variant increases the Reproduction or R number by 0.4 to 0.7.

The UK's latest R number is estimated at between 1.1 and 1.3, and should be below 1.0 for the number of cases to start falling. Prof Axel Gandy of London's Imperial College has stated that the differences between the virus types were quite extreme. The study suggests that the transmission of the new variant tripled during England's November lockdown while the previous version was reduced by one-third… (*BBC*)

CRC Risk in Young Adults Not as High as Previously Estimated

The risk for colorectal cancer (CRC) in young adults appears to be lower than what has been previously estimated, as previous studies did not differentiate between colorectal adenocarcinoma and carcinoid tumors, which are incidental findings, suggest experts.

New estimates for the risk of CRC in young adults, which differentiate colorectal adenocarcinoma from other types, appear in a study published in the *Annals of Internal Medicine*. The new analysis revealed that 4-20% of the lesions previously described as CRC were not adenocarcinoma but carcinoid tumors.

Investigators determined the incidence rates of early colorectal cancer, using Surveillance, Epidemiology and End Results (SEER) data from 2000 to 2016, and stratified the data by histologic subtype (primarily adenocarcinoma and carcinoid tumors), age group (20-29, 30-39, 40-49 and 50-54 years) and subsite. The absolute incidence rate in the age groups of 20-29 and 30-39 years was very low compared to 40-49 and 50-54 years age groups. The greatest changes in adenocarcinoma 3-year average annual incident rates (2000 to 2002 vs. 2014 to 2016) were for rectal-only cases in those aged 20-29 years (+39%), as well as rectal-only cases in the 30-39 years (+39%), and colon-only cases in the age group of 30-39 years (+20%)… (*Medscape*)