(e) ISSN Online: 2321-9599

(p) ISSN Print: 2348-6805

ORIGINAL ARTICLE

Prevalence of Pseudomonas aeruginosa and its antimicrobial sensitivity profile among post-operative wound infections

[1]Motilal, [2]Parul Singhal

[1]Associate Professor, Department of General Surgery, Sakshi Medical College & Hospital, Guna, Madhya Pradesh, India;
[2]Assistant Professor, Department of Microbiology, Venkateshwara Institute of Medical Sciences, Gajraula, Uttar Pradesh, India

ABSTRACT:
Aim: Prevalence of Pseudomonas aeruginosa and its antimicrobial sensitivity profile among post- operative wound infections. **Materials and Methods:** During study period, the clinical specimen received in the form of post operative wound swabs were processed aerobically. All the samples were examined microscopically by performing Gram & Zeihl Nelsen staining and were cultured simultaneously on the blood agar, MacConkey agar and nutrient agar media. The media plates were aerobically incubated at 37ºC for 16-18 hours. Following incubation, the isolated bacteria were subjected to identification by standard biochemical and automated techniques. Antimicrobial Susceptibility Testing (AST) of the isolates were performed on Mueller Hinton agar by Kirby Bauer's disc diffusion method. **Results:** A total of 100 wound swabs were received from the post-operative patients admitted in the surgery. Among them 93% specimens revealed growth while 7% samples were sterile. *Pseudomonas aeruginosa* was detected among 26 % samples followed by *Escherichia coli* in 23%, *Klebsiella pneumonia* in 18%, *Staphylococcus aureus* in 17%, *Proteus mirabilis* in 4% and Acinetobacter baumannii *in* 2%. Mixed infection was detected among 3% samples. P. aeruginosa was isolated among highest number of infected wound swabs (26 %) and comparatively higher number (61.53 %) was detected among male patients. The patients in which higher number of *P. aeruginosa* isolates were detected belonged to 61-80 years of age group (46.15 %). *P. aeruginosa* revealed maximum susceptibility to colistin (96.7%) followed by meropenem (76.92%) and imipenem (73.07%). **Conclusions:** Post-operative wound infection poses an enormous burden not only on the patient but also on the health care services in terms of morbidity, mortality and the economic costs. As also observed in the present study, *Pseudomonas* infection appears to be common in healthcare settings with relaxed hygienic measures and is also found to be dependent on age, sex and even duration of stay in the hospital.

Keywords: Pseudomonas aeruginosa, antimicrobial sensitivity, post-operative wound infections

Corresponding Author: Parul Singhal, Assistant Professor, Department of Microbiology, Venkateshwara Institute of Medical Sciences, Gajraula, Uttar Pradesh, India

This article may be cited as: Motilal, Singhal P. Prevalence of Pseudomonas aeruginosa and its antimicrobial sensitivity profile among post- operative wound infections. J Adv Med Dent Scie Res 2017;5(3):176-179.

INTRODUCTION

Post-operative wound infection or surgical site infection (SSI) is a significant cause of nosocomial infection among patients who have undergone surgery. [1] Post-operative wound infection simply means a wound infection that may originate primarily i.e during the operation or may occur secondarily i.e post operation from the sources in the ward or as a result of some complications. [2] Primary infection usually appears within a week following the surgery and is related to the endogenous flora and some other environmental sources in the operation theater while the secondary infection appears within 30 days of surgery. [3] The development of wound infection depends not only on the virulence factors and invasive capability of the microorganisms but also on the physiological state of the wound tissue and immunological probity of the host. [4] SSI not only poses a financial burden on both patient and health care settings but the prolonged hospital stay continues to be a major cause of morbidity and mortality of patients undergoing the post operational care. SSI continues to be a major problem even in the health care setting with advanced facilities and standard protocols of pre-operative preparation and antibiotic prophylaxis [5]. The common pathogenic bacteria usually isolated from SSI include *Staphylococcus* spp, *Streptococcus* spp, *Enterococcus* spp, *Escherichia coli*, *Klebsiella* spp, *Enterobacter* spp, *Citrobacter* spp, *Proteus* spp, *Pseudomonas*spp, *Acinetobacter* spp, *Burkholderia* spp etc [6]. In the recent years, increasing incidence of *Pseudomonas aeruginosa (P. aeruginosa)* in SSI has been a grave problem in developing nations, as infection caused by them are often life threatening and difficult to treat because these stubborn organisms are inherently resistant to most of the drugs used to treat bacterial infections. [5] Therefore, the present study was aimed to determine the prevalence of *P. aeruginosa* among the isolates of post-operative wound infection in our hospital setting and to study their antimicrobial sensitivity profile.

MATERIALS AND METHODS

During study period, the clinical specimen received in the form of post operative wound swabs were processed aerobically. All the samples were examined microscopically by performing Gram & Zeihl Nelsen staining and were cultured simultaneously on the blood agar, MacConkey agar and nutrient agar media. The media plates were aerobically incubated at 37ºC for 16-18 hours. Following incubation, the isolated bacteria were subjected to identification by standard biochemical (HiMedia, Mumbai) [1,2,7,8] and automated techniques. Antimicrobial Susceptibility Testing (AST) of the isolates were performed on Mueller Hinton agar by Kirby Bauer's disc diffusion method.[9,10] For this test, the Mueller- Hinton agar, was uniformly and aseptically inoculated with the *Pseudomonas aeruginosa* that was isolated from the patient surgical sites. The filter paper discs, which were impregnated with the specific concentration of a particular antibiotic, were placed on the medium. Following incubation, the "zone of inhibition" were observed and measured to determine the susceptibility to an antibiotic for the isolated pathogen. The following antibiotics were tested: Amikacin (30 mcg), gentamycin (10 mcg), tobramycin (10 mcg) ampicillin (10mcg), piperacillin (100 mcg), ticarcillin (75 mcg), levofloxacin (5mcg), ciprofloxacin (5 mcg), norfloxacin (10 mcg), aztreonam (30 mcg), ceftazidime (30 mcg), cefepime (30 mcg), cefoxitin (30 mcg) piperacillin-tazobactum (100/10 mcg), colistin (e-strip), imipenem (10 mcg), doripenem (10 mcg) and meropenem (10 mcg). Epsilon-test was performed to determing minimum inhibitory concentration value for colistin. Dehydrated media and antibiotic discs/e-strips were procured from Hi-Media, Mumbai, India. The control strains used during the study was *Pseudomonas aeruginosa* ATCC 27853.

STATISTICAL ANALYSIS

The collected data was transferred to the computer and Microsoft Excel 2000 (version 9) Analysis Tool Pack was used for analysis of data. Chi-square test was performed and $p \leq 0.05$ was considered statistically significant.

RESULTS

A total of 100 wound swabs were received from the post-operative patients admitted in the surgery. Among them 93% specimens revealed growth while 7% samples were sterile. *Pseudomonas aeruginosa* was detected among 26 % samples followed by *Escherichia coli* in 23%, *Klebsiella pneumonia* in 18%, *Staphylococcus aureus* in 17%, *Proteus mirabilis* in 4% and Acinetobacter baumannii *in* 2%. Mixed infection was detected among 3% samples. (Table 1)

Table 1: Distribution of Micro-organism Associated with Post-Operative Wound Infection

Microorganism	No. of Cases (100)	Percentage (%)
P. aeruginosa	26	26
Escherichia coli	23	23
Klebsiella pneumoniae	18	18
Staphylococcus aureus	17	17
Proteus mirabilis	4	4
Acinetobacter baumannii	2	2
Escherichia coli + Proetus mirabilis	2	2
Escherichia coli + Klebsiella pneumoniae	1	1
No growth	7	7

P. aeruginosa was isolated among highest number of infected wound swabs (26 %) and comparatively higher number (61.53 %) was detected among male patients. (Table 2)

Table 2: Sex wise distribution of P.aeruginosa isolates

Gender	Number of *P.aeruginosa* isolates (26)	Percentage(%)	p-value
Male	16	61.53	0.00
Female	10	38.46	

The results were found to be highly significant (p-value=0.00). The patients in which higher number of *P. aeruginosa* isolates were detected belonged to 61-80 years of age group (46.15 %). (Table 3)

Table 3: Age wise distribution of *P. aeruginosa* isolates

Age group (years)	Number of *P.aeruginosa* isolates(26)	Percentage (%)	p-value
0 -20	03	11.53	1.54
21-40	06	23.08	
41-60	05	19.23	
61-80	12	46.15	

However, the results were not found to be significant (p value=1.54). The abscess drainage was the most common type of post operative wound (38.46%) followed by surgery of diabetic foot (30.76%) and Cesarean section (15.38%). (Table 4)

Table 4: Prevalence of *P. aeruginosa* isolated from different type of surgeries

Type of Surgery	No. of specimens (100)	No. of *P.aeruginosa* isolated (26)	Percentage(%)	p-value
Abscess drainage	29	10	38.46	1.92
Diabetic foot	25	8	30.76	
Cesarean section	21	4	15.38	
Bone excision	13	2	7.69	
Mastiodectomy	7	1	3.85	
Lipoma excision	5	1	3.84	

The results were not found to be significant (p value= 1.92). *P. aeruginosa* revealed maximum susceptibility to colistin (96.7%) followedby meropenem (76.92%) and imipenem (73.07%). (Table 5)

Table 5: Antibiotic Sensitivity Profile of *P.aeruginosa* isolated from wound swabs

Antibiotic	Sensitive (%)	Intermediate sensitive (%)	Resistant (%)
Aztreonam	38.46	19.23	42.30
Amikacin	65.38	7.69	26.92
Cefepime	23.07	11.53	65.38
Colistin	96.7	0.70	2.6
Ceftazidime	38.46	19.23	42.30
Ciprofloxacin	57.69	15.38	26.92
Doripenem	73.00	3.84	23.14
Gentamicin	57.69	11.53	30.76
Imipenem	73.07	3.84	23.07
Levofloxacin	57.69	15.38	26.92
Meropenem	76.92	7.69	15.38
Norfloxacin	57.69	15.38	26.92
Piperacillin	23.07	11.53	65.38
PiperacillinTazobactum	61.53	7.69	30.76
Ticarcillin	23.07	11.53	65.38
Tobramicin	57.69	11.53	30.76

DISCUSSION

The primary aim of this study was to determine the occurrence of *P. aeruginosa* in post-operative wound infection and its sensitivity pattern to commonly used antibiotics. In the present study, 93 % samples revealed the growth of aerobic bacteria which was comparable to the study performed by Ranjan *et al* [3] that documented 91 % culture positive wound swabs. One such study carried out by Agrawal *et al* [4] reported 77.22% of culture positive specimen. *P. aeruginosa* (26%) was the most common of all the bacterial pathogens isolated from post-operative wounds in the present study. Our results were identical to those presented by Ranjan *et al* (29.6%) [3], Kirkland KB *et al* (27.8%) [6] and Masaadeh & Jaran (27.7%) [1] in their respective studies. Oguntibegri and Nwobu [12] reported 33.3% and Anupurba & collegues [13] reported a frequency of 32 % while Agrawal *et al* [4] & Stephan et al (as referred by Oguntibegri and Nwobu in their study) [12] both reported an isolation rate of 18.8% in the separate studies. *P. aeruginosa* remarkably contributes to the wound-related morbidity and mortality throughout the globe. Its virulence factors substantially enhance the colonization and infection of host tissue denoting it as the clinically significant pathogen among non fermenters. [3] However, Lilani *et al.* [5] reported *Escherichia coli* and Livermore DM *et al.*[14] reported *Staphylococcus aureus* as the predominant organism in respective their studies. Studies have shown that postoperative wound infection is universal and it presents with the bacteriological profile that vary with geographic location, skin flora, clothing at the wound site and time lapse between development of wound and its bacteriological examination. [3] In the present study, *P. aeruginosa* was found to be more common in male patients than in female which was in accordance with the results reported by Ranjan *et al.* [3], Agrawal *et al.*[4] and Stephan et al. (as referred by Oguntibegri and Nwobu in their study) [12]. However, Oguntibegri and Nwobu [12] have reported higher prevalence of *P. aeruginosa* among female patients in their study. The highest number of *P. aeruginosa* was isolated from the patients belonging to the age group of 61 to 80 years during our study. Our results were not found to be in concordance with the authors like Ranjan & collegues [3] who reported maximum *P.aeruginosa* isolates in the age group of 20 to 40 years. However, Oguntibegri & Nwobu [12] reported higher number of isolates with *P. aeruginosa* among young (0-29 years) and elderly subjects. Results from different studies [3,4,6,12,13] clearly indicates that differences in the prevalence of *P. aeruginosa* based on age and sex may also depend upon the immunological status and underlying illnesses of the study subjects.[15]

In the present study, *P. aeruginosa* isolation rate was found to be highest from patients who underwent abscess drainage followed by diabetic foot and caesarean section which was similar to study done by Ranjan *et al.* [3] and Anupurba et al. [13]. This might be due to the prolonged stay in hospital following surgery that resulted in colonization and subsequent infection. [13] The antimicrobial susceptibility pattern revealed by *P. aeruginosa* in different studies revealed different picture in different geographical regions. Colistin was found to be sensitive in maximum 96.7% *Pseudomonas aeruginosa* isolates in our study and our results resembled those presented by Oguntibegri and Nwobu [12] who reported 100 % sensitivity to colistin. However, our study reported high meropenem (76.9%) sensitivity followed by colistin and Oguntibegri and Nwobu [12] reported Gentamicin (75%) as the second most sensitive drug followed by colistin. In contrast to this Ranjan *et al*.[3] found imipenem (76.9%) followed by meropenem (70.4%), Agrawal *et al* [4] found piperacillin-tazobactum (89.7 %) followed by imipenem (88.24%), Anupurba et al [13] found cefoperezone sulbactum (74%) followed by ciprofloxacin (58%) while Kirkland KB *et al*.[6] found amikacin (78%) as the most sensitive drug. *P. aeruginosa* has the capacity to carry plasmids containing genes that regulate antimicrobial resistance, and this feature has led to the appearance of some strains that are resistant to normally reliable antibiotics. Apart from the intrinsic resistance exhibited by them, other important causes of excessive drug resistance among *Pseudomonas aeruginosa* isolates pertains to β-lactamase production, impermeability via a loss of porin protein Opr D together with the up- regulation of multidrug efflux systems.[3]

In general, the high frequency of occurrence of *Pseudomonas aeruginosa* is related to the indiscriminate use of antibiotics without satisfactory culture and antibiotic sensitivity testing. This single factor is responsible for eliminating the normal flora and providing suitable environment for *Pseudomonas* to colonize and flourish indefinitely. The intrinsic nature of resistance to antimicrobial agents, nutritional versatility and the sub standard hygienic record of personnel involved with wound dressing and general care of patients may be the main contributing factor responsible for the high rate of *P. aeruginosa* infection. The prolonged stay in the hospital following an operation was also observed in our study that played a significant role in establishing *Pseudomonas* infections.

CONCLUSIONS

Post-operative wound infection poses an enormous burden not only on the patient but also on the health care services in terms of morbidity, mortality and the economic costs. As also observed in the present study, *Pseudomonas* infection appears to be common in healthcare settings with relaxed hygienic measures and is also found to be dependent on age, sex and even duration of stay in the hospital. Continuous monitoring of susceptibility pattern of *P. aeruginosa* through surveillance, formulation of antibiotic policy and infection control practices is essential in individual health care settings in order to prevent further emergence of resistance.

REFERENCES

1. Collee, J.G., Miles, R.S., Watt, B. 2006. Laboratory strategy in the diagnosis of infective syndrome. In: Colle JG, Fraser AG, Marimon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. Edinburg: Elsevier Churchill Livingstone; p. 84-90.
2. Forbes, B.A., Sahm, D.F., Weissfeld, A.S. 2007. Overview of bacterial identification methods and strategies. Bailey and Scott’s Diagnostic Microbiology. 12th ed. Missouri: Mosby Elsevier; p. 218-47.
3. Ranjan KP, Ranjan N, Bansal SK, Arora DR. Prevalence of Pseudomonas aeruginosa in Post-operativeWound Infection in a Referral Hospital in Haryana, India. J. Lab. Physicians, 2010; 2(2), 74-7.
4. Agarwal PK. Incidence of postoperative wound infection Aligarh. Indian Journal of Surgery. 1984; 46 (6&7), 326-33.
5. Lilani S.P, Jangale N, Chowdhary, A, Daver GB. Surgical site infections in clean and clean contaminated cases. IJMM. 2005; 23(4), 249-52.
6. Kirkland KB, Briggs JP, Trivette SL, Wilkinson WE, Sexton DJ. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. *Infect Control Hosp Epidemiol.* 1999;20:725–30.
7. Collee JG, Fraser AG, Marmian BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. Standard edition. 14th ed. London, UK: Churchill Livingstone; 2000.
8. Cheesbrough M. Microbiology. Medical Laboratory Manual for Tropical Countries. Vol.2. Cambridgeshire, England:1984, p.985.
9. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. AmJ Clin Pathol 1966;45:493-6.
10. Khan JA, Iqbal Z, Rahman SU, Farzana K, Khan A. Report: prevalence and resistance pattern of Pseudomonas aeruginosa against various antibiotics. *Pak J Pharm Sci.* 2008;21:311–5
11. Masaadeh HA, Jaran AS Incident of Pseudomonas aeruginosa in Post-Operative Wound Infection. Am J Inf Dis. 2009; 5, 1-6.
12. Oguntibeju OO, Nwobu RAU. Occurrence of Pseudomonas aeruginosa in post-operative wound infection. Pak J Med Sci. 2004; 20:187-92.
13. Anupurba S, Bhattacharjee A, Garg A, Sen MR. Antimicrobial susceptibility of Pseudomonas aeruginosa from wound infections. Indian J Dermatol. 2006;51:286-8.
14. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? *Clin Infect Dis.* 2002;34:634–40.
15. Mousa H. Aerobic, anaerobic and fungal burn wound infections. *J Hosp Infect.* 1997;37:317–23.