Section: Miscellaneous

Original Research Article

TO EVALUATE THE PREVALENCE OF PSEUDOMONAS AERUGINOSA AND ITS ANTIMICROBIAL SENSITIVITY PROFILE AMONG POST OPERATIVE WOUND INFECTIONS

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Received : 02/09/2020
Received in revised form : 15/10/2020
Accepted : 01/11/2020

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

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DOI: 10.29228/jamp.44922

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2020; 2 (3); 334-338

Abstract

Background: Pseudomonas aeruginosa is a significant contributor to infections that occur in healthcare settings. The organism has inherent resistance as well as acquired multidrug resistance to several antibiotics, resulting in heightened rates of illness and death. Consequently, this leads to challenging circumstances and escalated healthcare costs. **Aim:** Prevalence of Pseudomonas aeruginosa and its antimicrobial sensitivity profile among post- operative wound infections. **Materials and Methods:** The samples were subjected to microscopic examination using Gramme staining and were cultivated concurrently on blood agar, MacConkey agar, and nutrient agar medium. The media plates were incubated under aerobic conditions at a temperature of 37 degrees Celsius for a duration of 16-18 hours. After the incubation period, the bacteria that were separated were analysed using conventional biochemical and automated methods for identification purposes. The isolates underwent Antimicrobial Susceptibility Testing (AST) on Mueller Hinton agar using Kirby Bauer's disc diffusion technique. **Results:** Out of the total, 100 specimens (90.91%) showed growth, whereas 10 samples (9.09%) were sterile. Pseudomonas aeruginosa was found in 22.73% of the samples, followed by Escherichia coli in 20%, Klebsiella pneumonia in 18.18%, Staphylococcus aureus in 16.36%, Proteus mirabilis in 4.55%, and Acinetobacter baumannii in 3.64%. A co-infection was identified in 5.45% of the samples. P. aeruginosa revealed maximum susceptibility to colistin (92%) followed by meropenem (76%) and imipenem (72%). **Conclusions:** Post-operative wound infection not only burdens the patient, but also imposes a substantial burden on healthcare services in terms of morbidity, mortality, and financial costs. The prevalence of Pseudomonas infection seems to be extensive in healthcare facilities where cleanliness requirements are not strictly enforced, as shown in the recent study.

INTRODUCTION

The Centres for Disease Control and Prevention (CDC) provide a definition for surgical site infections (SSIs) as infections that happen at the location where the incision was made during a 30-day period after any surgical procedure. Although advanced surgical methods are available, surgical site infections (SSIs) remain a prevalent source of hospital-acquired infections, resulting in increased mortality, morbidity, and medical expenses.[1] The uncontrolled proliferation of antibiotic resistance among bacterial agents poses a greater difficulty in clinical and surgical practise for effectively managing surgical site infections. The escalation of resistance in bacteria has a direct impact on the efficacy of antimicrobial agents, leading to a global predicament. The inappropriate prescribing of antibacterial drugs exacerbates the severity of surgical site infection and wound infection in underdeveloped countries.[2] Pseudomonas aeruginosa is a prominent contributor to infections acquired in healthcare settings, ranking as the second most prevalent gram-negative bacteria according to the United States national nosocomial infection monitoring system. Pseudomonas aeruginosa significantly adds to the global burden of illness and death associated with wounds. The bacterium infiltrates the bloodstream, resulting in sepsis that might potentially disseminate to the skin and result in ecthyma gangrenosum, characterised by a black necrotic lesion.[3] It generates many chemicals believed to facilitate the colonisation and invasion of host tissue.[4] Pseudomonas aeruginosa is considered the most medically important pathogen among non-fermenting bacteria because to its combination of many virulence factors, including as lipopolysaccharides (LPSs), exotoxin A, leukocidin, extracellular slime, proteases, phospholipase, and other enzymes. Pseudomonas aeruginosa has the ability to harbour plasmids that contain genes responsible for regulating antimicrobial resistance. As a result, several strains of this bacterium have emerged that are resistant to antibiotics that are often effective.[5] Among them, there are many established and confirmed risk factors for postoperative wound infections.

A risk factor refers to any identified factor that contributes to an elevated likelihood of postoperative wound infection.[6] The pathogenicity and capacity to spread of the microorganisms have been shown to impact the likelihood of infection, but, the condition of the tissue in the wound and the immune system's integrity of the host seem to be equally significant in influencing the occurrence of infection.[7] Initial infections often have a greater level of severity, manifesting during a timeframe of 5-7 days after surgery. These infections mostly arise from the body's own microorganisms and sometimes from external sources in the operating theatre. The occurrence of sepsis that originates within 30 days after a surgical procedure and prior to the dressing of the wound indicates an infection at the surgical site. Several studies indicate that there is a clear correlation between higher education and understanding of the causes of postoperative wound infection and a decrease in its occurrence. Additionally, the implementation of infection control practises that have been thoroughly studied plays a crucial role in preventing such infections.

The occurrence of primary wound infection is directly linked to the bacteriological cleanliness of the surgical procedure. The clean procedure, with a minimal occurrence rate of less than 2%, does not need the opening of viscous substances or the cutting of mucous membranes. In operations classified as contaminated (20%), an incision is made in a viscous substance that typically contains bacteria or a membrane that is typically colonised with bacteria. In clean-contaminated procedures (<10%), an incision is made in a viscous substance or membrane that is generally sterile. Health care linked infections often manifest as superficial infections and often occur after treating wounds in the hospital ward. Likewise, the occurrence of skin infections like boils or abscesses in areas other than the surgical site suggests that the infection was acquired inside the hospital ward.[7-9] Wound infection after contaminated surgeries often occurs due to the presence of bacteria that naturally reside in the exposed viscera or on the incised mucous membrane. These bacteria either belong to the patient's own normal microbial population or have entered during the patient's hospital stay. These operations include procedures performed in a field that is already contaminated by germs, such as abscesses and colon surgeries.[10,11] Bacteriological investigations have shown that postoperative wound infection is widespread, and the kinds of bacteria found differ depending on the geographical region, bacteria present on the skin, clothes near the wound site, and the time elapsed between the wound and examination.[12] Facultative anaerobic gram-negative bacilli, Streptococci, and Staphylococci persist in the colon, irrespective of the preparation method. The incidence of bowel and postoperative infection in colon and rectal surgery, in the absence of systemic intraoperative prophylaxis, may reach up to 50%.

The increasing prevalence of P. aeruginosa in recent years has been a subject of special attention. The prevalence of P. aeruginosa in postoperative wound infections is increasingly concerning in developing nations due to inadequate general hygiene practises, widespread use of substandard antiseptic and medicinal solutions for treatment, and challenges in clearly defining the responsibilities among hospital personnel.

MATERIALS AND METHODS

The investigation was carried out in the Department of Microbiology. The specimens obtained from hospitalised patients were subjected to conventional microbiological procedures for the purpose of isolating and identifying bacterial pathogens.[13] Aseptic postoperative wound swabs were obtained using two sterile cotton wool swabs per sample from several wards inside the hospital. One swab was used for Gramme staining, while the other was used for culturing.

The study involved the utilisation of various media and the execution of multiple tests, including blood agar, MacConkey agar, chocolate agar, nutrient agar, mannitol salt agar, Simmon citrate agar, peptone water, indole production test, motility test, methyl red test, voges proskauer test, catalase, coagulase, urease, and oxidase tests. The aforementioned media and reagents were acquired from HiMedia, located in Mumbai, India. The media were created in accordance with the instructions provided by the makers. All wound swabs obtained for bacteriology studies throughout the research period were processed using the recognised protocols for treating wound swabs. Gramme stain preparations were created using one swab, while cultures were treated using another swab. The plates were placed in an incubator and kept at a temperature of 37°C for a duration of 18 to 24 hours. The plates were examined the next day, with the option to prolong the observation period to 48 hours if no bacterial growth was seen during the first 24 hours. The isolated colonies underwent Gramme staining and biochemical testing to facilitate identification. Identification was conducted in accordance with the established biochemical tests.[13]

Antibiotic susceptibility testing

The antimicrobial susceptibility test was conducted on isolated and identified colonies of P. aeruginosa using commercially manufactured antibiotic discs (HiMedia) on Mueller Hinton agar plates. The test was performed using the disc diffusion technique, following the recommendations set by the Central Laboratory Standards Institute (CLSI).[14] No antibiotic testing was conducted on other bacterial isolates in this investigation since our primary objective was to determine the prevalence of P. aeruginosa. A control was established using the standard strain of P. aeruginosa. The antibiotics that were tested include: Amikacin (30 mcg), gentamycin (10 mcg), tobramycin (10 mcg), ampicillin (10 mcg), piperacillin (100 mcg), ticarcillin (75 mcg), levofloxacin (5 mcg), ciprofloxacin (5 mcg), norfloxacin (10 mcg), aztreonam (30 mcg), ceftazidime (30 mcg), cefepime (30 mcg), and cefoxitin (30 mcg). The following antibiotics are included: piperacillin-tazobactam (100/10 mcg), colistin (e-strip), imipenem (10 mcg), doripenem (10 mcg), and meropenem (10 mcg). An epsilon-test was conducted to determine the minimum inhibitory concentration (MIC) value for colistin. Hi-Media, located in Mumbai, India, provided us with dehydrated media and antimicrobial discs/e-strips.

Statistical Analysis

The SPSS version 25.0 was used for statistical analysis. A chi-square test was conducted, and a significance level of $p < 0.05$ was used to determine statistical significance.

RESULTS

A grand total of 110 wound swabs were obtained from the post-operative patients who were hospitalised in the surgery department. Out of the total, 100 specimens (90.91%) showed growth, whereas 10 samples (9.09%) were sterile. Pseudomonas aeruginosa was found in 22.73% of the samples, followed by Escherichia coli in 20%, Klebsiella pneumonia in 18.18%, Staphylococcus aureus in 16.36%, Proteus mirabilis in 4.55%, and Acinetobacter baumannii in 3.64%. A co-infection was identified in 5.45% of the samples. (Table 1)

Pseudomonas aeruginosa was found to be present in the largest number of infected wound swabs, accounting for 25 cases (22.73%). Additionally, a somewhat greater proportion of male patients (61.53%) tested positive for this bacterium. (Table 2). The results were found to be highly significant (p-value=0.01). The patients in which higher number of P. aeruginosa isolates were detected belonged to 60-80 years of age group (48 %). (Table 3)

However, the results were not found to be significant (p value=1.74). The abscess drainage was the most common type of post-operative wound (44%) followed by surgery of diabetic foot (28%) and Cesarean section (12%). (Table 4)

The results were not found to be significant (p value= 0.88). P. aeruginosa revealed maximum susceptibility to colistin (92%) followed by meropenem (76%) and imipenem (72%). (Table 5)

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

Microorganism	No. of Cases (110)	Percentage
P. aeruginosa	25	22.73
Escherichia coli	22	20
Klebsiella pneumoniae	20	18.18
Staphylococcus aureus	18	16.36
Proteus mirabilis	5	4.55
Acinetobacter baumannii	4	3.64
Escherichia coli + Proetus mirabilis	4	3.64
Escherichia coli + Klebsiella pneumoniae	2	1.82
No growth	10	9.09

Table 2: Gender wise distribution of P.aeruginosa isolates

Gender	Number of *P.aeruginosa* isolates (25)	Percentage(%)	p-value
Male	15	60	0.01
Female	10	40	

Table 3: Age wise distribution of P. aeruginosa isolates

Age group (years)	Number of *P.aeruginosa* isolates(25)	Percentage(%)	p-value
0 -20	2	8	1.74
20-40	6	24	
40-60	5	20	
60-80	12	48	

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

Type of Surgery	No. of specimens (110)	No. of *P.aeruginosa* isolated (25)	Percentage (%)	p-value
Abscess drainage	33	11	44	0.88
Diabetic foot	27	7	28	
Cesarean section	22	3	12	
Bone excision	14	2	8	
Mastiodectomy	8	1	4	

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Lipoma excision	6	1	4	

Table 5: Antibiotic Sensitivity Profile of P.aeruginosa

Antibiotic	Sensitive (%)	Intermediate sensitive (%)	Resistant (%)
Aztreonam	36	20	44
Amikacin	60	8	32
Cefepime	28	12	60
Colistin	92	4	4
Ceftazidime	36	20	44
Ciprofloxacin	56	16	28
Doripenem	72	4	24
Gentamicin	56	12	32
Imipenem	72	4	24
Levofloxacin	56	16	28
Meropenem	76	8	16
Norfloxacin	56	16	28
Piperacillin	24	12	64
Piperacillin Tazobactum	60	8	32
Ticarcillin	24	12	64
Tobramicin	56	12	32

DISCUSSION

The global issue of antimicrobial resistance in surgical site infections (SSI) is leading to extended hospitalisation periods for patients, as well as increased rates of death and morbidity. Pseudomonas aeruginosa is a significant contributor to surgical site infections and wound infections in gram-negative non-fermentative bacteria. It is the predominant bacterium found in surgical site infections (SSIs) and wound infections. The emergence of antibiotic-resistant strains has made treating both infections acquired in the community and those acquired in hospitals particularly difficult. Accurate identification of the organism and selection of suitable antibiotics based on AST values are crucial for initiating the proper treatment.[13]

The main objective of this research was to ascertain the prevalence of P. aeruginosa in post-operative wound infections and analyse its susceptibility to frequently prescribed antibiotics. In this investigation, 90.91% of the samples showed the presence of aerobic bacteria, which is similar to the findings of Ranjan et al,[5], who reported a 91% rate of positive cultures from wound swabs.

A research conducted by Agrawal et al,[14] found that 77.22% of the specimens tested were positive for culture. In the current investigation, P. aeruginosa 25(22.73%) was the predominant bacterial pathogen found in post-operative wounds. The findings we obtained were exactly the same as those reported by other authors.[5,15,16-18] Pseudomonas aeruginosa significantly adds to the morbidity and death associated with wounds worldwide. The virulence characteristics of this pathogen greatly increase its ability to colonise and infect host tissue, making it a clinically relevant disease among non-fermenters. In their separate research, Lilani et al,[19] identified Escherichia coli and Livermore DM et al,[20] identified Staphylococcus aureus as the most prevalent organisms. Research has shown that postoperative wound infection is widespread and manifests with a bacteriological profile that differs depending on geographic location, skin flora, clothes at the wound site, and the time elapsed between the formation of the wound and its bacteriological examination.[5] According to the findings of this investigation, P. aeruginosa was more prevalent in male patients compared to female patients, which aligns with the results published by Ranjan et al.[5] Nevertheless, Oguntibegri and Nwobu,[17] documented a greater incidence of P. aeruginosa among female patients in their research. Our investigation found that the age group of 60 to 80 years had the largest frequency of P. aeruginosa isolates among the patients. The findings of our study did not align with those of Ranjan and colleagues,[5] who observed a higher prevalence of P.aeruginosa isolates among individuals aged 20 to 40 years. Nevertheless, Oguntibegri & Nwobu,[17] documented a greater quantity of isolates containing P. aeruginosa in both young individuals (0-29 years), and older people. The findings from many research,[5,14,15,17,18] unequivocally demonstrate that variations in the occurrence of P. aeruginosa, depending on age and gender, may also be influenced by the immunological condition and underlying medical conditions of the individuals being studied.[21]

The current investigation indicated that the incidence of P. aeruginosa isolation was greatest among patients who had abscess draining, followed by those with diabetic foot and caesarean delivery. This finding is consistent with a study conducted by Anupurba et al.[18] This might be attributed to the extended hospitalisation period after the operation, which led to the establishment of bacteria and eventual infection. The antimicrobial susceptibility pattern shown by P. aeruginosa varied across different geographical locations, as indicated by several research. In this investigation, we discovered that 92% of Pseudomonas aeruginosa isolates were susceptible to colistin. These findings align with the data published by Oguntibegri and Nwobu,[17] who observed a 100% sensitivity to colistin. Nevertheless, our investigation revealed a significant

ISSN (O): 2687-5365; ISSN (P): 2753-6556

sensitivity to meropenem (76%), with colistin, Oguntibegri, and Nwobu,[17] reporting Gentamicin (75%) as the second most sensitive antibiotic, behind colistin. Ranjan et al. discovered that imipenem had a sensitivity rate of 76.9%, followed by meropenem at 70.4%. Agrawal et al. found that piperacillin-tazobactum had a sensitivity rate of 89.7%, followed by imipenem at 88.24%. Anupurba et al. found that cefoperezone sulbactum had a sensitivity rate of 74%, followed by ciprofloxacin at 58%. Kirkland KB et al. identified amikacin as the most sensitive drug, with a sensitivity rate of 78%. Pseudomonas aeruginosa has the ability to harbour plasmids that encode genes responsible for regulating antimicrobial resistance. Consequently, some strains of this bacterium have emerged that exhibit resistance to antibiotics that are often effective. In addition to their inherent resistance, additional significant factors contributing to high drug resistance in Pseudomonas aeruginosa isolates include the development of β-lactamase, reduced permeability due to the loss of porin protein Opr D, and the increased activity of multidrug efflux systems.[5]

The prevalence of Pseudomonas aeruginosa is mostly attributed to the widespread and uncontrolled use of antibiotics without adequate culture and drug susceptibility testing. This singular component is accountable for eradicating the typical microbiota and creating a favourable habitat for Pseudomonas to establish and thrive perpetually. The primary cause that may be responsible for the high risk of P. aeruginosa infection is the inherent resistance to antimicrobial drugs, the ability to adapt to different nutritional conditions, and the inadequate hygiene practises of workers engaged in wound dressing and patient care. In our investigation, we discovered that an extended hospitalisation period after surgery had a crucial influence in the development of Pseudomonas infections.

CONCLUSION

Post-operative wound infection not only burdens the patient, but also imposes a substantial burden on healthcare services in terms of morbidity, mortality, and financial costs. The prevalence of Pseudomonas infection seems to be extensive in healthcare facilities where cleanliness requirements are not strictly enforced, as shown in the recent study.

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