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Incidence of Multidrug Resistant Pseudomonas Aeruginosa Isolated From Burn Patients Tertiary Care Hospital, Jamnagar, Gujarat, India.

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Abstract:
Background: *Pseudomonas aeruginosa is a ubiquitous micro-organism that can rapidly acquire resistance to different broad-spectrum antibiotics. Multidrug resistant (MDR) Pseudomonas aeruginosa is an emerging cause of mortality and morbidity in burn patients, which causes 4-60% nosocomial infections in different parts of the world.[1]*
Objectives:
- *To isolate Pseudomonas aeruginosa from pus samples of burns patients.*
- *To assess the rates of antibiotic resistance and multidrug resistance among Pseudomonas isolates.*

Material & Methods: *This study was carried out in the Department of Microbiology, Shri M. P. Shah Government Medical College, Jamnagar, Gujarat from September 2014 to June 2015 from burns ward, Guru Govind singh Govt. Hospital Jamnagar. A total of 391 swab samples were tested. The Pseudomonas aeruginosa isolates were identified by their direct microscopy examination,colony morphologies, pigment , Gram's staining patterns, oxidase test, catalase test, Oxidation fermentation test, Triple sugar iron agar test, Arginine dihydrolase and Sugar fermentation test. Antibiotic sensitivity was determined for Amikacin (30mcg), Netilmycin(30mcg),Gentamycin(10mcg),Ceftazidime(30mcg),Piperacillin(100mcg), Piperacillin + Tazobactum(100mcg/10mcg), Aztreonem (30mcg), Imipenem (10mcg), Meropenem (10mcg), Colistin (10mcg), Polymixin B(300units), Ciprofloxacin(5mcg).*
Results: *Incidence of Pseudomonas aeruginosa from burns patients is 36.45%. Incidence of Multidrug Resistant (MDR) Pseudomonas aeruginosa is 36.48%. Antibiotic susceptibility tests showed high level resistance toGentamycin(93.24%), Ciprofloxacin(89.18%), Amikacin(85.13%), Aztreonem(83.78%), Ceftazidime(81.08%), Netilmicin(75.67%), Piperacillin(72.97%), Piperacillin+Tazobactum(56.76%), Imipenem(14.86%). Resistance to Colistin and Polymixin B were 0%.*
Conclusions: *We conclude that Pseudomonas aeruginosa with high level resistance to Aminoglycosides, Fluoroquinolones & Penicillin group of drugs. Infection in burns continues to be a great problem which is not yet solved and posses a challenge to the microbiologist and the surgeon. Based on the result of the antibiotic susceptibility testing of the various isolates antibiotics are to be administered only on clinical suspicion of sepsis.*
Keywords: *Burn, Swab samples, Pseudomonas aeruginosa, Multidrug Resistance,*

I. Introduction

Pseudomonas is a non-fermentative gram negative bacterium, which is widely distributed in nature. It is responsible for about 10% -20% of nosocomial infections which are seen as septicemia in burn and wound infections, cystic fibrosis intensive-care units (ICUs), etc [1].

Pseudomonas is a ubiquitous micro-organism that can rapidly acquire resistance to different broad-spectrum antibiotics. Multidrug resistant (MDR) Pseudomonas is an emerging cause of mortality and morbidity in burn patients, which causes 4-60% nosocomial infections in different parts of the world [1].

The morbidity and mortality associated with Pseudomonas are mainly attributed to inadequate empirical therapy and/ or delay in the initiation of appropriate therapy [2,3]. Burns Patients with Pseudomonas infection have increased need for debridement and they frequently require re-grafting due to loss of skin grafts or allografts [4].

MDR Pseudomonas elaborates inactivating enzymes that make beta-lactams and carbapenems ineffective, such as extended spectrum beta lactamases (ESBLs) and metallo-β-lactamases (MBLs) [5]. MDR Pseudomonas is defined as a bacterium which is resistant to anti-microbial agents which are included in three or more anti-Pseudomonal anti-microbial classes (carbapenems, fluoroquinolones, penicillins /cephalosporins and aminoglycosides) [6]. Pseudomonas spp. Continues to be a leading cause of nosocomial infection which is often

life threatening. Natural resistances to this make the treatment problematic. Indiscriminate use of antibiotics has led to the emergence of multiple drug resistance strains [7].

II. Material And Methods

Study duration & sample size: This study was carried out in the Department of Microbiology, Shree M.P. Shah Govt. Medical College and G.G.G. Hospital, Jamnagar, Gujarat from September 2014 to June 2015. The study group comprised 391 swab samples of 203 patients, who were admitted in burns ward in Guru Gobind Singh Govt. Hospital, Jamnagar.

Collection of Samples: Pus samples from burns patients received in Microbiology Department, Shree M.P. Shah Medical College and G.G. Hospital, Jamnagar were tested to isolate Pseudomonas aeruginosa to detect antimicrobial resistance pattern. The pus samples were collected onto sterile cotton wool swabs aseptically. All samples were transferred to laboratory soon after being obtained. Total 391 swab samples were tested during study period. The samples were cultured and smear made for direct microscopy.

1st Day Follow Up:

1) Direct Microscopy:

Smears were made on glass slide from pus samples for gram staining technique. Smears were allowed to air dry, heat fixed and stained by gram staining technique. The stained slides were examined microscopically under oil immersion lens for pus cells and bacteria.

Result of Direct Microscopy:

From all the smears gram positive and gram negative results were observed. Pseudomonas aeruginosa appear pink –red bacillus, 1.5 – 3 µm × 0.5 µm.

2) Culture:

All the samples, either gram negative bacilli or gram positive cocci in smear examination were inoculated on MacConkey agar and Blood agar plates. The culture plates were incubated aerobically for 24 hrs at 37°c. Growth and cultural characteristics were observed next day.

2nd Day Follow Up:

Colony characteristics:

It grows well on ordinary media, producing large, opaque, irregular colonies with a distinctive , musty or earthy smell. On MacConkey media, it produced non-lactose fermenting, Pale colonies. Many strains are hemolytic on blood agar. They also produce pigments best known is pyocyanin. From colony, gram staining and motility by hanging drop preparation were also done. On hanging drop preparation method Pseudomonas aeruginosa is actively motile by a polar flagellum.

Biochemical Reaction:

Oxidase test was positive, Catalase test was positive, Arginine dihydrolase was positive. Oxidation fermentation test shows that test was oxidative for Pseudomonas aeruginosa. In triple sugar iron agar test, it was alkaline slant/ alkaline butt(K/K reaction)**.** In Sugar fermentation test - Glucose is utilized oxidatively, forming acid only. Indole, MR, VP and H2S test are negative.

Antibiotic Sensitivity Testing:

Anti-microbial sensitivity testing was done according to the CLSI (Clinical and Laboratory Standards Institute) guidelines for Amikacin(30mcg), Netilmycin(30mcg), Gentamycin(10mcg), Ceftazidime(30mcg), Piperacillin(100mcg), Piperacillin+Tazobactum(100mcg/10mcg), Aztreonem(30mcg), Imipenem(10mcg), Meropenem(10mcg), Colistin(10mcg), Polymixin B(300units), Ciprofloxacin(5mcg).

Ethical clearance: it is a retrospective analysis of samples tested for routine laboratory diagnosis; hence ethical clearance is not necessary.

III. Results

Sex wise distribution of patients suggests that out of 203 patients 78(38.42%) were male and **125(61.58%)** were **female**. Age wise distribution of patients suggests that highest cases were found in age group of **21 – 40 (53.20%)** followed by age group 41 – 60 (24.63%). Table-1 shows age group infected with Pseudomonas aeruginosa, highest in the age group **21 – 40 (52.70%)** followed by 41 – 60 (28.38%). Table-2 shows that incidence rate of Pseudomonas aeruginosa & incidence rate of MDR Pseudomonas aeruginosa, isolated from burns patients which are **36.45% & 36.48%** respectively. Table-3 shows antibiotic resistant

pattern of Pseudomonas aeruginosa. It showed high level resistance to Gentamycin(93.24%), Ciprofloxacin(89.18%), Amikacin(85.13%), Aztreonem(83.78%), Ceftazidime(81.08%), Netilmicin(75.67%), Piperacillin(72.97%), Piperacillin+Tazobactum(56.76%), Imipenem(14.86%). Resistance to Colistin and Polymixin B were 0%.

IV. Discussion

There has been rapid emergence of MDR P.aeruginosa in recent times, which is an important concern for clinicians who treat these infections. In the present study, incidence rate of Pseudomonas aeruginosa is **36.45%**. A study carried out by Navendu et al. in 2006 showed that **35.75%** of Pseudomonas aeruginosa among infected burns patients, which is almost similar to present study [8]. A study carried out by Heggers et al. in USA in 1998 showed that **51%** of the burns patients are having infection of Pseudomonas aeruginosa, which has higher rate of infection as compare to present study [9].

Pseudomonas aeruginosa shows highest resistance to gentamicin(93.24%). A study done by Navendu et al., Saha et al., Indu at al., showed 81.6%, 80.77% & 81.03% resistance to gentamicin respectively[8,11,12]. Present study shows that resistance to ceftazidime is 81.08%, similar study done by Indu et al.obtained 70.68% of resistance [12].According to Srinivasan et al. P. aeruginosa was 100% resistant to ceftazidime[10].

Present study shows that resistance to ciprofloxacin is 89.18%, similar study done by Ashwin et al. & Saha et al. showed 90.62% & 92.16% of resistance respectively[13,11]. Present study shows that resistance to imepenem is 14.86%, similar study done Indu et al., obtained 18.9% of resistance to imepenem[12]. Present study shows that resistance to colistin is 0%, similar study done by Indu et al. obtained 0% of resistance [12].

In the present study, **MDR Pseudomonas rate** (resistance to anti-microbial agents which are included in three or more anti-Pseudomonal anti-microbial classes (carbapenems, fluoroquinolones, penicillins /cephalosporins and aminoglycosides [6]) is **36.48%**. A study done by Unan et al.,[14] in Turkey reported rates of MDR, which were as high as **60%**, whereas study done by Sabir et al., in Pakistan detected lower rates of MDR that was **22.08%** [15]. However, rates of our study are comparable to a study done in Egypt, where Gad et al., observed **36%** MDR P. aeruginosa [16]. It is also comparable to a study done by Indu et al. that was **36.2%** [12].

Pseudomonas aeruginosa has gradually become a major cause of nosocomial infections which occur in burn patients and which requires immediate and effective implementation of infection control strategies, to combat its spread. Environmental sources may play a significant role in spread of MDR among hospitalized patients. In current times, antibiotics with anti Pseudomonal activity which are available include the aminoglycosides, ticarcillin, ureidopenicillins and ciprofloxacin. Combination treatments are generally recommended for suspected Pseudomonas infections. It has been reported that the choice of cabapenem, cefepime or piperacillin+ tazobactum, ciprofloxacin and gentamicin combinations with amikacin or tobramycin, in current times, appears to provide the widest potential antimicrobial activity against MDR P. aeruginosa [16].

The lack of any new compounds in the near future indicates that national and local surveillance efforts are essential, to provide clinicians with correct information for choosing right antimicrobial therapy. Rigorous monitoring for MDR among Pseudomonas isolates is very important, because outbreaks caused by strains which are resistant to potentially useful agents, including carbapenems, have been reported elsewhere [17-19].

V. Conclusion

We conclude that Pseudomonas aeruginosa with high level resistance to Aminoglycosides, Fluoroquinolones & Penicillin group of drugs. Infection in burns continues to be a great problem which is not yet solved and poses a challenge to the microbiologist and the surgeon. Restriction of 'selected antibiotic usage' and/or infection control policies must be tailored for each institution, to combat the rapid emergence of MDR P. aeruginosa in burn patients. The lack of newer antimicrobial agents with activities against P. aeruginosa, makes periodic studies on the antimicrobial esistance patterns very important.

VI. References

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Table – 1 Age Group Infected With Pseudomonas Aeruginosa Strains

Age group (Years)	No. of Pseudomonas aeruginosa strains	Percentage (%)
0 – 20	9	12.16%
21 – 40	39	52.70%
41 – 60	21	28.38%
>60	5	6.76%

Table – 2 Incidence Rate of Pseudomonas Aeruginosa & Incidence Rate Of Mdr Pseudomonas Aeruginosa Isolated From Burns Patients

Total patients	Pseudomonas aeruginosa isolated	Incidence rate	MDR Incidence rate
203	74	36.45%	36.48%

Table – 3 Antibiotic Resistant Pattern of Pseudomonas Aeruginosa

Name of antibiotics	Resistance (%)
Piperacillin (PI)	72.97
Ceftazidime (CAZ)	81.08
Gentamicin (GEN)	93.24
Piperacillin+Tazobactum(PT)	56.76
Aztreonem (AT)	83.78
Imipenem (IMP)	14.86
Amikacin (AK)	85.13
Ciprofloxacin (CIP)	89.18
Netilmicin (NET)	75.67
Polymixin B (PB)	0
Colistin (CL)	0