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Isolation And Antibiogram Profile Of Multiple Drugs Resistance Pseudomonas Aeruginosa From Burn Wound Infection In Western Nepal

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Abstract: Different microorganisms that resist multiple antibiotics is considered to be most threatening problems to public health now a days. The aim of this study was to evaluate antibiotic resistance of Pseudomonas aeruginosa in burn wound infection and the antibiotic susceptibility pattern of P. aeruginosa isolates.Wound pus was collected using a sterile swab from all age group excluding the patients already on antibiotic therapy. One hundred eighty four (184) samples were collected from the wound pus in Medicross Diagnositc Center, Butwal, Nepal from October , 2017 through March , 2018.Out of 184 samples, 48 Pseudomonas aeruginosa strains were isolated and tested for antibiotic susceptibility against different antibiotics. The pattern of antibiotic susceptibility suggested that 100% of the isolates were resistant to Tobramycin, and 94.1% were resistance to Cefoperazone and Meropenem whereas the least resistance was shown against ciprofloxacin (35.3%) followed by amikacin and gentamicin (47.1%) both. The percentage of multi drug resistance in Pseudomonas aeruginosa isolates was 100% since all these positive samples were resistant to at least 3 drugs in the following classes: β-lactams, carbapenems, aminoglycosides and fluoroquinolones. In P. aeruginosa infections, periodic antimicrobial resistance monitoring is fundamental to update the current activity level of commonly used anti-pseudomonal drugs to minimize the risk of drug resistance.

Keywords: Antibiotic Susceptibility, Burn Infection, Multi Drug Resistance, Pseudomonas aeruginosa,

1. INTRODUCTION

Opportunistic pathogen like Pseudomonas aeruginosa colonizes the burn wound easily [1].Infection of burn wounds is a serious problem leading to death and needs critical care and monitoring. Infection in burns is one of the most important factors determining the prognosis of burn disease [2]. Burn wound infections are largely hospital acquired and cross infection is very common [3]. According to data from the Centers For Disease Control and Prevention (CDC) and Prevention National Nosocomial Infection Surveillance System, in the USA, Pseudomonas aeruginosa was declared as the second most common cause of nosocomial pneumonia. In the patients with nososcomial urinary tract infection and nosocomial bacteraemia, Pseudomonas aeruginosa was the third and seventh most common cause respectively. Also, Pseudomonas aeruginosa was reported the third most common cause of nosocomial infections in intensive care units in Europe [4]. Pseudomonas aeruginosa are also called as Multiple Drugs Resistance (MDR) Pseudomonas aeruginosa as it is intrinsically resistant to many classes of drugs and also able to acquire resistance to all operational antibiotics which makes difficult in the treatment of patients with Pseudomonas aeruginosa infections [5]. Pseudomonas aeruginosa isolates associated with nosocomial infections are frequent multidrug-resistance (MDR) and are associated with high rates of resistance to anti-pseudomonal drugs [6]. Pseudomonas aeruginosa infections in burn patients are very common, life threating and is greatly responsible for morbidity and mortality worldwide in spite of having the advances in surgical care and wide variety of commercially available antibiotics. Multidrug-resistant Pseudomonas aeruginosa has been reported frequently as the cause of nosocomial outbreaks of infection in burn patients [7]. Patients infected with multi – drugs resistance microorganisms may suffer more and drugs of choice for treating infection does not work completely ,they required second or third choice of drugs that may more toxic and less effective. This means identification, isolation and antibacterial susceptibility patterns of isolates bacteria are most important for determining best antibiotic therapy in patients. This study investigates the susceptibility pattern of Pseudomonas aeruginosa from burn wound infection.

2. METHODS

2.1 Collection of Specimens

A retrospective study was conducted at Medicross Diagnostic Center , Butwal , Nepal During 10th October 2017 till 25 th March 2018. In this study 184 appropriate specimens from wound infection were collected from different age group of male and female excluding the patients already on antibiotic therapy consulting the patients who age group between 2 years to 90 years by applying strict aseptic condition during collection and processing of sample according to standard laboratory methods.The information of patients were recorded in laboratory entry log book including a range of data like as patient name , age , sex , address , contact number , date of sample collection. Ethical clearance was obtained for this study from Medicross Diagnostic Center , Butwal , Nepal.

2.2 Isolations and Identification of Bacteria

1. **Isolation**
   Pus sample were inoculated directly or by the help of sterile inoculating loop onto Nutrient agar and Macconkey agar and incubated at 37 º C for 24 hours in aerobic condition.

2. **Identification of Isolates**
   A.Microscopic Examination
   The suspected colonies were stained by using Gram Staining method and their shape,colour and arrangement were observed under light microscope also motility characters identified by hanging drops techniques.

B. Biochemical Test

All bacteriological isolates were examined and confirmed by different biochemical test according to Bergey's Manual of Systemic Bacteriology [8] – [11].

2.3 Antibiotic Susceptibility Test

Antibiotic Susceptibility Test was determined by applying Kirby Bauer Disc Diffusion method according to Clinical and Laboratory Standard Institute (CLSI) recommendation [12]. Total 12 antibiotics of Hi Media were used include Amikacin 30 (mcg) , Ciprofloxacin (5mcg), Gentamycin (10mcg), Cephotaxime (30 mcg) , Imipenem (10 mcg), Meropenem (10 mcg),Cefoperazone (75 mcg), Tobramycin (10 mcg), Piperacillin – Tazobactam (100/10 mcg) , Cefepime (30 mcg) , Ceftazidime (30 mcg), Norfloxacin (10 mcg).The zone of inhibition were measured in mm after incubation of plate at 35ºC -37ºC for 18 – 20 hrs and the organisms identified as sensitive or resistant.The result are expressed in percentage.Data was analysed by using Microsoft Office Excel , Word 2007 and presented by chart and diagrams.Quality control In this study Pseudomonas aeruginosa American Type Culture Collection (ATCC) 27853 was used as reference strain.

3. RESULT

184 clinical samples from the burn wound infection patient were processed out of which Pseudomonas aeruginosa was isolated from 48 samples. These isolated Pseudomonas aeruginosa formed large, opaque, irregular colonies with a pigmentation on Nutrient Agar whereas large, opaque, irregular with non-lactose fermenting colonies on MacConkey agar. Gram staining revealed pink coloured gram negative straight rods arranged singly. Hanging drop preparation showed motile bacilli. The catalase and oxidase test were positive and the isolate was oxidative in O/F test. These isolates grown on TSI medium showed alkaline slant/acidic butt (K/A) and the nitrate was reduced to nitrite in nitrate reduction test. Indoles, methyl red, Voges Proskauer test were negative but citrate test was positive in a IMViC test.

Fig. 1 shows the prevalence of Pseudomonas aeruginosa in burn wound infections which indicates that 26 % of the cases were positive i.e. out of 184 samples Pseudomonas aeruginosa was isolated only from 48 samples.

Fig. 2 shows the resistance pattern of positive samples with Tobramycin being 100% resistant, Cefoperazone and Meropenem being 94.1% resistant and the least resistant was shown by ciprofloxacin 35.3%.

Table 1 shows the multidrug resistance pattern of Pseudomonas aeruginosa from the positive samples i.e. all the 48 isolates were resistant to more than at least 3 drugs in the following classes: β- lactams (Cefotaxime, Imipenem, Meropenem, Cefoperazone Piperacillin-Tazobactum, Cefepime, Ceftazidime), carbapenems, aminoglycosides (Amikacin, Gentamicin, Tobramycin), and fluoroquinolones (ciprofloxacin, Norfloxacin). So all the isolates were Multidrug Resistance Pseudomonas aeruginosa.

Fig 3 shows the incidence of Multi drug resistance of the positive samples. Out of 184 samples 48 samples were isolated as Pseudomonas aeruginosa and 100% were Multi Drug Resistant (i.e. MDRPA). Amikacin (47.1%), Ciprofloxacin (35.3%), Gentamicin (47.1%), Cefotaxime (76.5%), Imipenem (88.2%), Meropenem (94.1%), Cefoperazone (94.1%), Tobramycin (100%), Piperacillin-tazobactam (82.4%), Cefepime (64.7%), Ceftazidime (70.6%), Norfloxacin (70.6%). All of total 48 isolates with Pseudomonas aeruginosa are resistance to multiple drugs from minimum four antibiotics to maximum 10 antibiotics . So all isolates are 100 % resistance with multi drugs.

Fig. 1: *Prevalence of Pseudomonas aeruginosa in samples.*

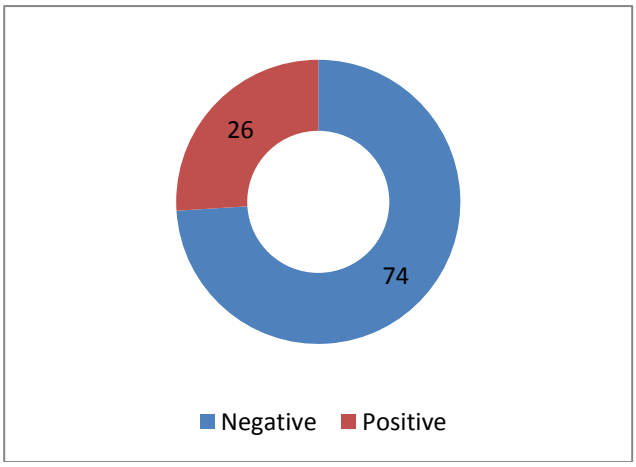


Fig. 2: *Resistance pattern of positive sample*

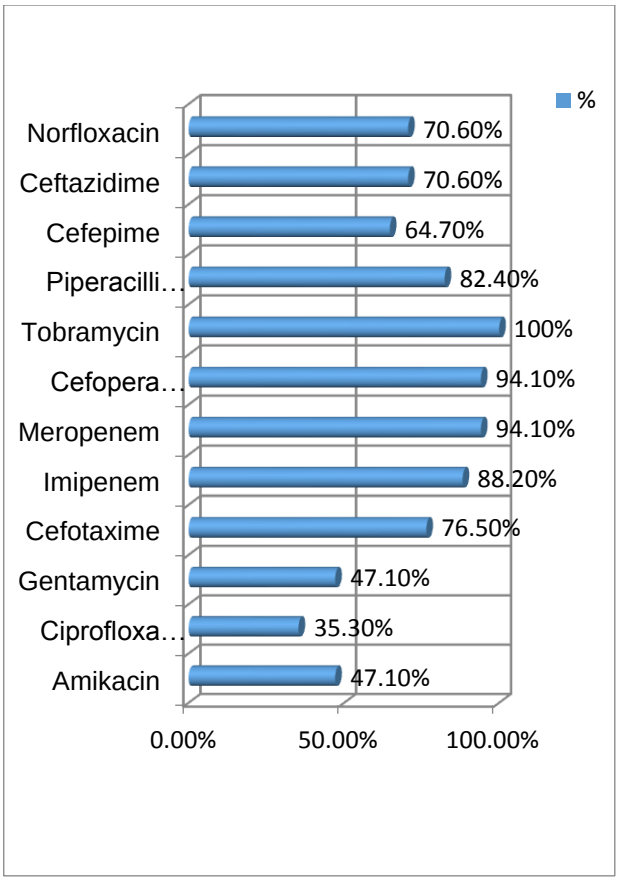
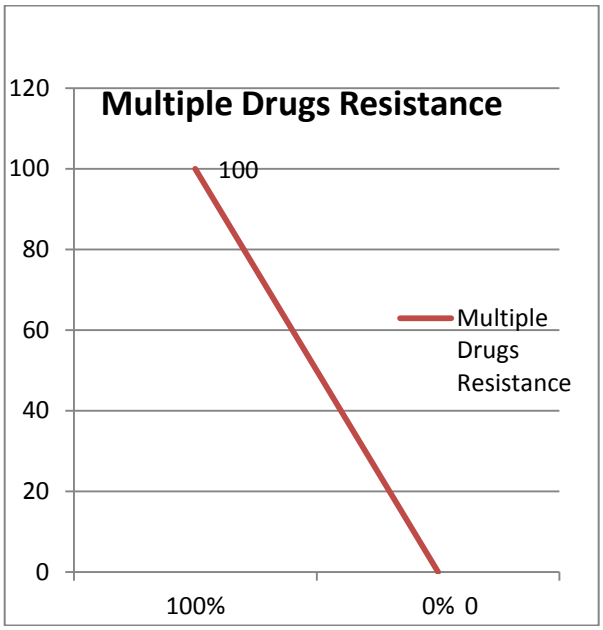


Table 1: *Incidence of Multidrug resistance of sample*

Number of antibiotics - resistance	Isolation number
4	Isolate 22,25,35,41,32
5	Isolate 2,5,12,17,40,46,24,3
6	Isolate 4,10,9,13,23,29,45
7	Isolate 1,7,15,18,26,31,38,43,27,37
8	Isolate 11,16,21,34,42
9	Isolate 6,8,14,20,30,36,44,47
10	Isolate 19,28,39,48,33,39

Fig. 3*: Incidence of Multidrug resistance of sample*



4. DISCUSSION

Infection due to Multiple Drugs Resistance Pseudomonas aerugonisa (MDRPA) has become a challenge in clinical practice. The risk factors like illness, severity of disease, bedridden state, invasive devices used in hospitals and exposure to antibiotics particularly β-lactams and fluoroquinolones are associated to MDR Pseudomonas aerugonisa infection [13]. In developing countries, P. aeruginosa isolates have substantial effect on the mortality and morbidity in hospitalized burn patients. In Iran (1998), P. aeruginosa was responsible as the leading cause of nosocomial infections in burn unit showing 97.7% susceptibility to amikacin, 95% resistance (2003), 45% resistance (2009) for thisantibiotic [14]. It is very important to know the antimicrobial resistance pattern of Pseudomonas aeruginosa which is useful in effective treatment by helping physician in the choice of antibiotics. The study also

emphasizes on that the antibiogram study which is essential before prescribing the antibiotics by physician against Pseudomonas aeruginosa isolates. In a number of recent studies carried, Imipenem, member of class carbapenems, has been reported to be very effective against P. aeruginosa. Compared to the present study (20.6%), a low occurrence of imipenem resistance (9.90%) were reported in a tertiary care hospital in Malaysia. The level of drug resistance pattern is different between the hospitals in the same country, the reason being the differential usage of antibiotics in the respective hospitals. In a study carried in one of the hospital in India, the incidence of imipenem resistance was 7.2% compared to a higher resistance detected in another hospital and established the fact that imipenem was used as a reserve drug [15]. There is considerable variations in the result of study which depend upon various factors like geographical condition, time variations, others local factors like financial problems, lack of well education and the techniques used for study. That is why commonly isolated microorganisms from particular disease and its antibiotics susceptibility test to appropriate use of antibiotics are very important for the effective treatment of the disease.

5. CONCLUSIONS

The purpose of the present study was to describe the isolation and identification of Pseudomonas aeruginosa in burn patients and to study antibiotic susceptibility patterning of Pseudomonas aeruginosa. Out of 184 sample, 48 clinical samples showing positive culture isolated with Pseudomonas aerugonisa . The percentage of Multi drug resistance P. aeruginosa from the positive samples was 100% since all these positive samples were resistant to at least three drugs in the following categories which are carbapenems, β-lactams, fluoroquinolones, and aminoglycosides.The percentage of resistance by antibiotics: Amikacin (47.1%), Ciprofloxacin (35.3%), Gentamicin (47.1%), Cefotaxime (76.5%),Imipenem (88.2%), Meropenem (94.1%), Cefoperazone (94.1%), Tobramycin (100%), Piperacillin-tazobactam (82.4%), Cefepime (64.7%), Ceftazidime (70.6%), Norfloxacin (70.6%).The study suggest therefore the establishment of a critical antibiogram of the most frequently isolated microorganism for appropriate use of antibiotics in the effective treatment of suffer patients.

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