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To ascertain the antimicrobial susceptibility profile of *Pseudomonas aeruginosa* and *Acinetobacter* species in burn wound samples from the inpatient department and burn ICU patients in tertiary hospital

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Abstract
This study was carried out in 226 patients, from whom 271 bacterial isolates were identified between January to April 2014. All the specimens were from the burn ward (165) and other were from burn ICU unit (106) of the tertiary hospital, New Delhi. Data was collected from laboratory patient records, isolates were graphed according to incidence of both *Pseudomonas aeruginosa* and *Acinetobacter species* in duration of January to April 2014 and their antimicrobial profile.
It was seen that the number of *Pseudomonas aeruginosa* isolates were greater than the number of *Acinetobacter* in all the four months. For result analysis and computation, antimicrobial profile of both *Acinetobacter species and Pseudomonas aeruginosa* obtained from multiple swabs were added together. It was also observed that most of the *P. aeruginosa* and *Acinetobacter* isolates were highly resistant to antibiotics. The total number of *P. aeruginosa* isolates were 152/271.Most of the *P. aeruginosa* isolates were highly resistant to antibiotics- ceftazidime (88.8%), amikacin (95.3%), ciproflox (96%), netilmicin (92.6%), cefoperazone + sulbactam (83.5%) meropenam (74.3%), piperacillin + tazobactam (61.8%). 60% of these isolates were sensitive to imipenam. The total number of *Acinetobacter* isolates were 119/271. *Acinetobacter* isolates collected were highly resistant to ceftazidime (100%), amikacin (99%), ciproflox (98.3%), cefoperazone + sulbactam (56. 3%), meropenam (94.9%), piperacillin + tazobactam (86.5%) and imipenam (61.3%). 64.4% of these strains were sensitive to netilmicin.

Keywords: Burn wound patients, antimicrobial susceptibility, New Delhi

1. Introduction
Burns are a serious global public health problem. Among all cases of trauma, burn cases have highest duration of hospitalization. The squeal of burns exerts even a more catastrophic influence on people in terms of suffering. The World Health Organization's (WHO) latest figures indicate that that nearly 1.95 lakh deaths every year are caused by burns [1]. Major causes of severe burn injuries are flame burns (37%) followed by liquid scalds (27%). In older patients (of 80 years and above), hot surface exposure is a major cause (22%) of burn cases [2]. Leading causes of death in burn victims are sepsis (47%), respiratory failure (29%), brain injury (16%) and shock (8%) [3]. The pathogenesis of colonization, infection and invasion of microorganisms is related to the disruption of the normal skin barrier. Determining the depth and extent of burn wound is important for the patient management. Burn wounds can be classified as First, Second and Third degree burns. The severity of burn wound depends on the temperature of the burning substance, the duration of contact, the skin depth in the area burned and blood flow to the skin. Burn injury causes immunologic dysfunction, manifested by impaired neutrophil function, reversal of T-helper to T-suppressor cell ratio, decreased lymphocyte count, and decreased production of IgG and IL-2. Variety of organisms has been isolated from burn wound colonization and infections. Aerobic bacterial isolates from burn wounds have ranged from gram positive organisms e.g. *Staphylococcus aureus*, coagulase negative *Staphylococci* and *Enterococcus species* to gram negative organisms *e.g. Acinetobacter* species, *Escherichia coli, Klebsiella pneumoniae*, *Enterobacter* species and *Proteus species.* Early assessment of the victim is crucial in order to prevent the rate of microbial infection. The major problem in the treatment of burn patients is the emergence of

antimicrobial resistance in bacterial pathogens. Multidrug resistance has been noted in gram positive organisms e.g. methicillin-resistant *S. aureus* (MRSA), and in gram negative bacilli e.g. *P. aeruginosa* and *Acinetobacter* species. This study was done to determine the antibiotic resistance in *Acinetobacter* species and *P. aeruginosa* species isolated from wounds of burn patients.

2. Materials and Methods

This study was carried out on 226 patients, from whom 271 bacterial isolates were identified between January to April 2014. All the specimens were from the burn ward (165) and other were from burn ICU unit (106) of the Safdarjung hospital, New Delhi. All the burn specimens were classified on the basis of degree of burn (in %), type of burn. All relevant specimens were collected as wound swabs from the patients in ward, ICU and were sent to the laboratory. All the specimens were inoculated on Mac Conkey (supports growth of gram negative organisms, inhibits gram positive organisms, demonstrates lactose use) and sheep blood agar (supports growth of most of gram positive and gram negative organisms, demonstrates hemolysis) plates. A cotton swab (containing patient's specimen) was used to inoculate the first sector, and then it was discarded into an appropriate container, while reusable loops, usually with nichrome or platinum wire (24 gauge), were flamed to incinerate any organisms on the loop. When cooled, the sterile loop was streaked through the initial sector and organisms were carried into the second sector where they were spread using a zigzag movement. In a similar manner, the organisms present on the loop were incinerated after the second sector is streaked, and the third sector is streaked. For a four quadrant plate, the process is carried an extra step. The plates were then incubated overnight at 37 °C. Identification of isolates was done by conventional biochemical methods according to standard microbiological techniques.

The methods used were: Gram's staining; Hanging drop method; enzymatic methods – Oxidase, Catalase, Coagulase; Biochemical sugar tests - Indole test, Triple Sugar Iron (TSI) test, and Citrate test. Antimicrobial susceptibility testing of isolates was performed by Kirby Bauer Disk Diffusion method according to CLSI (Clinical and Laboratory Standard Institute) protocol. This test is used to determine the resistance or sensitivity of aerobes or facultative anaerobes to specific chemicals, which can then be used by the clinician for treatment of patients with bacterial infections. The presence or absence of an inhibitory area around the disc identifies the bacterial sensitivity to the drug.

2.1 These were the following antibiotics discs used in the present study

Ceftazidime (Ca; 30 µg), amikacin (Ak; 30 µg), ciproflox (Cf; 5 µg), netilmicin (Nt; 10 µg), cefoperazone + sulbactum (CS; 75/25 µg), piperacillin + tazobactam (PT; 100/10 µg), imipenam (Imp; 10 µg) and meropenam (Mero, 10 µg).

3. Results and Discussion

The total number of patients in the study was 226 and the total number of isolates of both *Pseudomonas aeruginosa* and *Acinetobacter* species were 271. The percentage of the patient's specimens with a single organism growth was 72% and the remaining 28% of the total patient's specimens grew both *Pseudomonas and Acinetobacter species*. The percentage isolates of *Pseudomonas aeruginosa were* 29.6% in burn ICU patients and 70.3% in burn ward patients. Graphical representation in Figure 1 shows that number of *Pseudomonas aeruginosa* isolates were greater than number of *Acinetobacter* in all the four months. *Pseudomonas* isolate's % was 62%, 33%, 39%, 18% respectively in the month of January, February, March, and April. *Acinetobacter* isolate's % was 49%, 27%, 30%, 13% respectively in January, February, March, and April.

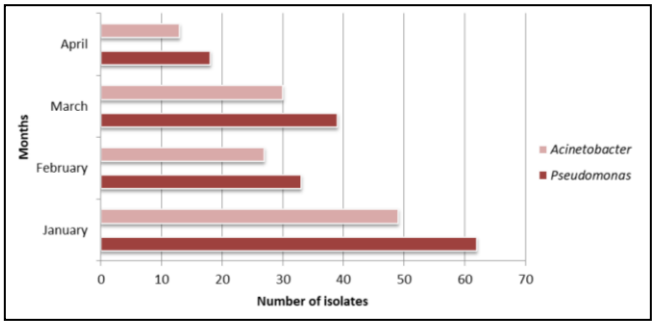

Fig 1: Graph showing monthly incidence of *Pseudomonas and Acinetobacter*.

The total number of *P. aeruginosa* isolates were 152/271.Most of the *P. aeruginosa* isolates were highly resistant to antibiotics- ceftazidime (88.8%), amikacin (95.3%), ciproflox (96%), netilmicin (92.6%), cefoperazone + sulbactam (83.5%) meropenam (74.3%), piperacillin + tazobactam (61.8%). 60% of the isolates were sensitive to imipenam. (Table 1)

Table 1: Antibiotic profiling of *P. aeruginosa* showing percentage of resistant and sensitive isolates

Antibiotics	***Pseudomonas***			
	No. of Resistant (R) isolates	**% of R isolates**	**No. of Sensitive (S) isolates**	**% of S isolates**
ceftazidime	135	88.8	17	11.2
amikacin	145	95.3	7	4.7
ciproflox	145	96	6	4.0
netilmicin	138	92.6	11	7.4
cefoperazone+sulbactam	127	83.5	25	16.5
piperacillin+tazobactam	94	61.8	58	38.2
imipenam	61	40.2	91	59.8
meropenam	113	74.3	39	25.7

The total number of *Acinetobacter* isolates were 119/271. *Acinetobacter* isolates collected were highly resistant to ceftazidime (100%), amikacin (99%), ciproflox (98.3%), Cefoperazone + sulbactam (56. 3%), meropenam (94.9%), piperacillin + tazobactam (86.5%) and imipenam (61.3%). 64.4% of the strains were sensitive to netilmicin (Table 2).

Table 2: Antibiotic profiling of *Acinetobacter* showing percentage of resistant and sensitive isolates

Antibiotics	*Acinetobacter*			
	No. of Resistant (R) isolates	% of R isolates	No.of Sensitive (S) isolates	% of S isolates
ceftazidime	119	100	0	0
amikacin	118	99	1	1
ciproflox	116	98.3	2	1.7
netilmicin	41	35.6	74	64.4
cefoperazone+sulbactam	67	56.3	52	43.7
piperacillin+tazobactam	103	86.5	16	13.5
imipenam	73	61.3	46	38.7
meropenam	113	94.9	6	5.1

Various studies have been conducted to document the spectrum of burn wound isolates and their antibiograms. *P. aeruginosa* has emerged as a predominant member of the burn wound flora (26.7% and 4.4% in two Indian studies).Similar study conducted by Department of Burns and General Surgery, Choithram Hospital and Research Center, Indore, India has also showed the highest incidence of *P. aeruginosa* (43%). Other gram negative includes polymicrobial (23%), *Citrobacter* (17%), *Acinetobacter baumannii* (11%) [4]. In present study, the incidence of *P. aeruginosa* isolates (56%) were higher than isolates of *Acinetobacter* (44%). In case of *Acinetobacter* isolates; the percentage in burn ICU (51.2%) was higher than incidence in burn ward (48.7%). *P. aeruginosa* isolates were much greater (70.3%) in burn ward than burn ICU (29.6%). Increasing antimicrobial resistance among burn wound isolates is a matter of concern, with limited treatment options available for multi-drug resistant strains. It is evident that *P. aeruginosa* has emerged as a great threat in burn wound infection and it is very important that antibiotic policy should formulate to keep a check on it.Study conducted by Department of Medical Microbiology, Faculty of Health Sciences, Mthatha, South Africa had shown 75% *of P. aeruginosa* were resistant to gentamicin [5].

In present study, most of the *P. aeruginosa* isolates were highly resistant to ceftazidime (88.8%), amikacin (95.3%), ciproflox (95.3%), netilmicin (90.7%), sulbactam (83.5%), meropenam (74.3%).60% of the *P. aeruginosa* isolates were sensitive to imipenam. *Acinetobacter* isolates collected were highly resistant to ceftazidime (100%), amikacin (99.1%), ciproflox (97.4%). 53.7% of the strains were sensitive to netilmicin.

4. Limitations

Among the gram negative pathogens, there is higher incidence of *P. aeruginosa* than *Acinetobacter* in the present study of burn wound infection. There were some limitations in this study:

a. Availability of limited clinical data obtained from hospital records tended to restrict the scope of the study in terms of site, cause of infection, severity of burns and treatment outcomes.
b. Percentage of mortality of burn patients could not be analyzed.
c. Etiology of incidence of multiple – organisms (in an individual patient) during the course of treatment could not be evaluated.

5. Conclusion

Overall it can be concluded that there is a very high level of resistance against commonly used antibiotics. In the present study, the total number of *P. aeruginosa* isolates were 152/271. Most of the *P. aeruginosa* isolates were highly resistant to antibiotics- ceftazidime (88.8%), amikacin (95.3%), ciproflox (96%), netilmicin (92.6%), cefoperazone + sulbactam (83.5%) meropenam(74.3%), piperacillin + tazobactam (61.8%). 60% of these isolates were sensitive to imipenam. The total number of *Acinetobacter* isolates were 119/271.*Acinetobacter* isolates collected were highly resistant to ceftazidime (100%), amikacin (99%),ciproflox (98.3%), cefoperazone + sulbactam (56. 3%), meropenam (94.9%), piperacillin + tazobactam (86.5%) and imipenam (61.3%). 64.4% of these strains were sensitive to netilmicin. Regular surveillance of burn wound organisms and their antibiotic resistance profile along with the proper management and prevention of further infection in the burn care unit will help in formulating antibiotic therapy, thus reducing mortality from septic events. By observing the variety of burn wound isolates and their increasing antimicrobial resistance, it's clear that there is an urgent need of regular microbiological surveillance, *in vitro* testing and monitoring of the parameters which would play an important role in guiding the proper antibiotic therapy in burn patients. Multi drug resistance can be prevent by using antimicrobials that target specific organisms and decreasing infection related complications [6].

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