Original Article

Nosocomial Burn Wound Infections due to Non-Fermenting Gram Negative Bacteria: Our Experiences From A Tertiary Care Center In North India

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ABSTRACT

Background: Nosocomial infections contribute to up to 50% mortality in burn patients. Non fermenting Gram negative bacteria, being ubiquitous in nature, can easily colonize the burn site and subsequently cause infections. The objective of the study was to understand the role of two of the most common non-fermenters, *Pseudomonas aeruginosa* and *Acinetobacter baumanii* in nosocomial burn wound infections and determine their antimicrobial resistance pattern.

Methods: Wound swabs were collected from burn patients and cultured using standard microbiological techniques. Isolates of non-fermenters, *Pseudomonas aeruginosa* and *Acinetobacter baumanii*, were identified by conventional biochemical tests. The antibiotic susceptibility testing of these isolates were carried out by disc diffusion method.

Result: A total of 248 and 70 clinical strains of *Pseudomonas aeruginosa* and *Acinetobacter baumanii* were isolated, respectively. Out of these, 67.62% and 72.05% isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were MDR. *Pseudomonas* showed highest resistance to gentamicin (83.3%), followed by ceftazidime (80.18%), and Netilmicin (76.89%). Among *Acinetobacter baumanii* isolates, resistance to cephalexin (98.5%) was highest, followed by gentamicin (94.6%) and cefotaxime (94.12%).

Conclusion: The increasing antibiotic resistance shown by these important pathogens leaves us with fewer option to treat severe life-threatening infections, stressing the need of a continuous antibiotic surveillance program and stringent implementation of infection control practices.

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Introduction

Infections are an important cause of morbidity and mortality in patients with burns. Wound infections are one of the most common sites of nosocomial infections in burn patients with prevalence of about 60%, followed by blood stream infections (20%), urinary tract infections (20%) and pneumonia (10%). [1] Burn wound infections can lead to scarring, bacteremia, sepsis and multi-organ dysfunction, contributing to 75% mortality in burn patients. [2,3] The occurrence of nosocomial burn infections depends on several factors such as the burn severity, immune status, prolonged stay, invasive procedures and overcrowding leading to cross infections. [4]

Disruption of skin and vasculature with resulting compromise in the immune status is the usual pathogenesis. [5,6] Hence the infected burn wound is a frequent source of sepsis. [3] *Pseudomonas aeruginosa*, *Acinetobacter*, *Staphylococcus aureus*, *Klebsiella spp.* and other Gram negative bacteria are commonly incriminated pathogens in burn patients. [1]

Pseudomonas aeruginosa is known to frequently colonize the inanimate surfaces like floors, bed rails, sinks in the hospital and is transmitted by hands of health care workers. [7] Its resistance to commonly used disinfectants and antibiotics are the contributing factors. Hence, *Pseudomonas* infection is challenging to treat and is associated with a high mortality rate. [8]

Acinetobacter baumanii is also rapidly emerging nosocomial pathogen in various countries including India, especially in ICUs, burn units and surgical wards. [4] Like *Pseudomonas*, *Acinetobacter baumanii* tends to exist in the hospital environment due to its ability to adhere on inanimate surfaces and is also inherently resistant to several antibiotics. Hence, this species is notorious for causing infections where there is a breach in natural immunity like in the burn wounds. [9]

Burn units are often the sites of major and prolonged outbreaks with resistant organisms.[10] Both *Pseudomonas aeruginosa* and *Acinetobacter baumanii* can be problematic due to their inherent resistance to several drug classes as well as the easy development of acquired resistance. [5] Multidurg resistant *Pseudomonas aeruginosa* (MDR Pa) in burn units is often associated with significant morbidity and mortality; mostly due to lack of treatment options.[7] Previous studies reveal the fact that the bacterial profile of burn wound infections changes over time in any given burn unit probably due to cross infections, change in antibiotic use, and overcrowding.[11] Hence, it is important to regularly monitor the bacterial species responsible for burn wound infections and their antibiotic susceptibility pattern, to aid use of appropriate antibiotics both for empirical therapy and specific treatment.

Thus, the present study was conducted to determine the role of non-fermenting Gram negative bacteria, namely *Pseudomonas aeruginosa* and *Acinetobacter baumanii* in the burn wound infection and assess their prevalent antibiotic susceptibility pattern.

Materials And Methods

The study included 1294 burn wound patients admitted in the burns unit of a 1600-bedded tertiary care hospital in New Delhi, India during the year 2012. Swabs and/or pus discharge were collected from the apparently infected site after decontaminating the wound. The swabs/pus were inoculated onto blood agar and MacConkey's agar. In addition, pus specimen was inoculated in Brain Heart infusion broth and incubated for 24hrs at 37˚C; subsequent subculture was done on blood agar and MacConkey's agar if primary plates failed to show bacterial growth. The plates were incubated at 37˚C for 24-48 hours in ambient air. The identification of *Pseudomonas aeruginosa* and *Acinetobacter baumanii* was done using the standard biochemical tests. The antibiotic susceptibility testing was carried out by the modified Stokes disc diffusion method against gentamicin, amikacin, ciprofloxacin, piperacillin/tazobactam, impenem, meropenem, netilmicin, polymixin B and colistin. *Pseudomonas aeruginosa* isolates were also tested against aztreonam, ceftazidime and tobramycin; and *Acinetobacter baumanii* was also tested against cephalexin, cefotaxime, ceftriaxone and amoxicillin (HiMedia Ltd., India).

Pseudomonas aeruginosa isolates were identified as MDR Pa (Multi-drug resistant *Pseudomonas aeruginosa*), if the isolate was resistant to drug(s) in three or more antimicrobial classes; XDR Pa (Extensively drug resistant *Pseudomonas aeruginosa*) when resistant to all tested antibiotics except one or two classes; and PDR Pa (Pan drug resistant *Pseudomonas aeruginosa*) when resistant to all drug classes. [12] MDR *Acinetobacter spp*. was defined as the isolate resistant to at least three classes of antimicrobial agents: all penicillins and cephalosporins (including inhibitor combinations), fluroquinolones, and aminoglycosides; XDR (Extensively drug resistant) *Acinetobacter spp*. was defined as isolate that was resistant to the three classes of antimicrobials described above (MDR) and also to carbapenems; and PDR (Pan drug resistant) *Acinetobacter spp*. was the XDR *Acinetobacter* spp. that was also resistant to polymyxins and tigecycline.[13]

Result

Wound swabs/ pus specimens were collected from the infected wound sites of 1,294 patients and transported

to the Microbiology laboratory immediately. The study population included 711 males and 583 females with male: female ratio of 1.22. The median age of these patients was 32 years (range=11–76 years).

On culture, 171 specimens showed no growth; while rest of the wound swabs had bacterial growth, including 160 samples with poly-microbial growth; yielding a total of 883 bacterial isolates. These included 248 (28.08%) isolates of *Pseudomonas aeruginosa* and 70 (7.92%) of *Acinetobacter baumanii*. Therefore, the non fermenting Gram negative bacteria constituted 36% (318/883) of the total isolates. Gram positive cocci constituted 15.96% (141/883) of the isolates, while 47.88% (424/883) were members of Family Enterobacteriaceae.

Pseudomonas isolates showed highest resistance towards gentamicin (83.33%) followed by ceftazidime, netilmicin (Table 1). Most of the *Acinetobacter* isolates were highly resistant to amoxicillin, cephalexin, ceftriaxone and cefotaxime, (100%, 98.51%, 90.91% and 94.12% respectively). Approximately, 50% and 16% of the isolates were resistant to imipenem and Meropenem, respectively (Table 2).

We found that 67.62% *Pseudomonas* and 72.05% *Acinetobacter* isolates were multidrug resistant (MDR); while approximately 17% strains of both *Pseudomonas* and *Acinetobacter* isolates were extensively drug resistant (XDR). None of *Pseudomonas* or *Acinetobacter* strains were Pan drug resistant (PDR) (Table 3).

Table 1: Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa*.

Antimicrobial agent	Susceptible (%)	Intermediate susceptible (%)	Resistant (%)
Ceftazidime	16.13	3.69	80.18
Piperacillin + Tazobactam	65.82	18.99	15.19
Aztreonam	30.77	9.62	59.62
Imipenem	85.25	5.74	9.02
Meropenem	70.42	10.83	18.75
Ciprofloxacin	25.16	3.87	70.97
Gentamicin	16.22	0.44	83.33
Amikacin	25	8.97	66.03
Netilmicin	21.23	1.89	76.89
Tobramycin	17.84	4.87	77.29
Polymyxin B	93.78	1.91	4.31
Colistin	92.13	2.81	5.06

Table 2: Antibiotic susceptibility pattern of *Acinetobacter baumanii*.

Antimicrobial agent	Susceptible (%)	Intermediate susceptible (%)	Resistant (%)
Amoxicillin	0	0	100
Cephalexin	1.49	0	98.51
Ceftriaxone	0	9.09	90.91
Cefotaxime	0	6.25	94.12
Piperacillin/tazobactam	37.5	27.5	35
Imipenem	37.14	14.29	48.57
Meropenem	47.37	36.84	15.79
Ciprofloxacin	17.14	5.71	77.14
Gentamicin	5.41	0	94.6
Amikacin	6.45	4.84	88.71
Netilmicin	32.35	9.52	61.76
Polymixin B	92	0	8
Colistin	84.85	0	15.15

Table 3: Patterns of drug resistance in isolates of *Pseudomonas* and *Acinetobacter*.

Drug resistance	Pseudomonas aeruginosa (n= 244)	Acinetobacter baumanii (n=68)
MDR	165 (67.62)	49 (72.05)
XDR	41 (16.80)	12 (17.64)
PDR	0 (0)	0 (0)

Note: *MDR: multidrug resistant; XDR: extensively drug resistant; PDR: Pan drug resistant Figures in parentheses indicate percentages.*

Discussion

The prevalence of non-fermenting Gram negative bacteria in our study, namely *Pseudomonas aeruginosa* and *Acinetobacter baumanii* was found to be 28.8% and 7.92%, respectively. Few other Indian studies involving burns patients have shown wide isolation rates of *Pseudomonas aeruginosa* ranging from 18.2 to 59%, as well as that of *Acinetobacter baumanii* ranging from 7.2-28.6% (Table 4)**.** Our result is comparable to the Tamil Nadu based study done in 2011 where the prevalence of *Pseudomonas aeruginosa* in burn wound infections was 28%;[16] however, *Acinetobacter* was not isolated in that study. Results of the previous studies from Varanasi, Mumbai and Delhi corroborate with our findings.[3,17,20]

However, global studies report slightly higher isolation rate of *Pseudomonas aeruginosa* and *Acinetobacter baumnaii* as compared to those reported by Indian authors, with the isolation rates ranging from 28-58.3% and 10.4-62.3% respectively (Table 5). In western countries, *Pseudomonas aeruginosa* is found to be the most prevalent cause of burn wound infection followed by *Acinetobacter*.

In our study, *Pseudomonas aeruginosa* isolates showed high resistance to resistant to gentamicin (83.33%). Previous studies done in Turkey and Baghdad revealed the lower resistance to gentamicin (40% and 45% respectively).[5,22] However, in Iran 100% sensitivity to gentamicin was seen.[1] Resistance to amikacin in our study was again much higher than reported in other studies done elsewhere in India, where majority of the isolates were sensitive.[3,15] International studies too revealed a much lower resistance rate, except in the Baghdad based study where the amikacin resistance rate was 84.6%.[13] High resistance was also seen towards netilmicin, tobramycin and ciprofloxacin (76.89%, 77.29% and 70.97%, respectively). This is in contrast to the studies

TABLE 4: Indian scenario of the role of *Pseudomonas* and *Acinetobacter* in burn wound infection cases.

Place of study	Author	Period of study	Pseudomonas spp. (%)	Acinetobacter spp.
Pune[14]	Bhatt P *et al*	2013-14	54.9	18.6
Karnataka[15]	Bhat AS *et al*	2013	18.2	28.6
TN[16]	Valarmathi S *et al*	2011	28	-
Mumbai[3]	Srinivasan S *et al*	1994-2006	31.84	-
Chandigarh[4]	Mehta M *et al*	2002-05	51.5	14.23
Varanasi[17]	Anupurba S *et al*	2004-05	32	-
Chandigarh[18]	Agnihotri N *et al*	1997-2002	59	7.2
Delhi[19]	Sharma S *et al*	1992-1994	53.9	-
Delhi[20]	Singh NP *et al*	1997-2002	31	-
Delhi[21]	Revathi G *et al*	1993-97	36	1.1

TABLE 5 International scenario of the role of *Pseudomonas* and *Acinetobacter* in burn wound infections cases.

Place of study	Author	Period of study	Pseudomonas spp. (%)	Acinetobacter spp. (%)
Turkey[22]	Yolbaş I *et al*	2008-09	25.8	62.3
USA[23]	Keen EF *et al*	2003-08	20.04	22.2
Baghdad[2]	Alkaabi SAG	2011-11	48.14	14.81
Iran[1]	Ekrami A	2003-04	37.5	10.4
Turkey[10]	Oncul O *et al*	2004-05	57	21
Iran[24]	Azimi L *et al*	2010	40	17
Baghdad[5]	Hussien IA *et al*	2010-11	58.3	-

done in Iran and Baghdad, which showed 0% and 34.3% resistance against tobramycin.[1,5] The resistance rate for ciprofloxacin in our study was much higher as compared to a Karnataka based study where most of the isolates (95.8%) were sensitive.[15] Similarly, in Turkey and Baghdad, 22.8-29% resistance was seen.[5,22] However, as the resistance pattern differs among different institutions, another study in Baghdad revealed 84.6% of ciprofloxacin resistance.[2]

In our study, resistance to ceftazidime was 80.18%, higher as compared to the studies done in abroad, where the resistance of 55-60.25% has been reported.[2,5] Resistance against rest of the antibiotics, imipenem, meropenem, piperacillin + tazobactam , polymyxin B and colistin was lower, though still comparable to most of the international studies.[1,7,10,22]

The resistance to gentamicin in *Acinetobacter baumanii* isolates in our study was 94.6%, as against 55.6% and 96% in studies done abroad.[9,23] An Iranian study revealed 100% sensitivity of *Acinetobacter* to this drug. [1] Other drugs against which high resistance was shown by *Acinetobacter* were amikacin (88.71%), ciprofloxacin (77.14%), ceftriaxone (90.91%), cephalexin (98.51%), amoxicillin (100%), and netilmicin (61.76%) while the rest of the antibiotics were relatively more effective. Resistance to amikacin, ciprofloxacin and ceftriaxone was also high in other studies, similar to ours.[2,9,22] Amoxicillin resistance reported from Vietnam and Baghdad was also 100%, similar to our study.[2,9]

Although there is no consensus regarding the definition of the terms 'multidrug resistant', 'extensively drug resistant' and 'pan drug resistant' used for these bacteria, we used the previously described and commonly used definitions to report the results of our study.[12.13] Approximately two-thirds of the *Pseudomonas* strains and three-fourths of the *Acinetobacter baumanii* strains were resistant to three or more anti-microbial classes defined for each bacteria, making them MDR. Seventeen percent strains of both showed extensive drug resistance. Pan drug resistance was not seen in our study. A USA based study had reported MDR Pa as 15% and MDR *Acinetobacter* as high as 53%; while there was only 1 isolate each of Pan DR Pa and Pan DR *Acinetobacter*.[23] A Turkish study has previously shown 7.7% and 13.2% pan drug resistance in these isolates from burn wound infections.[10] These are much low as compared to our study; however, comparison with these studies may not be accurate due to lack of standard definitions for these terminologies; thus emphasizing the urgent need for clear-cut definitions.

Conclusion

To conclude, burns units in every hospital should determine the bacterial profile of the burn wounds and the antibiotic susceptibility pattern at regular intervals, and should also study the trend in the nosocomial spread of the pathogens. This will guide in the administration of empirical antibiotic therapy before the culture results are obtained. There should be a strict infection control policy in place with a stringent antibiotic stewardship program in order to ensure optimum treatment of the patient and to curb the menace of antibiotic resistant organisms. In addition, clear definitions of Multi drug resistance, Extensive drug resistance and Pan drug resistance should be available, to optimize inter-study comparisons.

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Competing Interests

None Declared

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