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Research Article

Prevalence of *Pseudomonas sp.* and their antibiotic sensitivity profile in burn patients

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Abstract

Burns are one of the most severe wounds that damage extensively the first line of defense of the body, the skin, making them easily prone to infections, thus requiring a higher level of containment to prevent infection. Thus, this is a routine to keep abreast of the microbial profile of burn wounds in patients; hence in the present study the prevalence of the most commonly identified and suspected *Pseudomonas* sp. were identified using Gram-stain, motility, culture characteristics and biochemical reactions; they were screened for susceptibility to antibiotics using Kirby-Bauer Method. *Pseudomonas* sp. was found only next to *Klebsiella* sp. with a prevalence percentage of 29 %. All the isolated strains of *Pseudomonas* sp. are sensitive to colisitin.

Keywords: ESBL producers, Klebsiella pneumoniae, Escherichia coli, 3rd generation cephalosporins, wound infections

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1. Introduction

Skin is the largest organ in the human body and plays a crucial role in the first line of defense of the body against infectious microbes and establishment of normal microflora as opportunistic pathogens. Thus, the disruption of the epithelial integrity of the skin exposing the inner tissues is called wound [1]. This in turn opens a moist, warm and nutritive environment that is conducive for microbial colonization and proliferation [2]. In developing countries like India, large number of people die daily because of preventable and curable diseases like wound infections. These are of common occurrence in hospitals as nosocomial infections accounting for 70 - 80 % mortality [3, 4]. Thus, the importance of wound infections, in both economic and human terms, should not be underestimated [5].

Wound infections can be classified into two major categories: [8] exogenous wound infections and endogenous wound infections and/or abscesses. The potential wound pathogens popularly isolated are Gram-positive cocci (*Staphylococcus aureus*, *Streptococcus* species, coagulase-negative *Staphylococcus* species, *Enterococcus* species), Gram-negative bacilli (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus* species, *Enterobacter* species) and anaerobes (*Bacteroides* species, *Clostridium* species) [10].

Wound infection becomes a major scenario with the prevalence of resistant bacteria [13]. Rapid spread of resistant microbes affected the effectiveness of antimicrobials creating a worldwide medical problem [11]. This condition is serious in developing countries owing to irrational prescription of antimicrobial agents [14] that in turn will become pandemic. Thus, the measures to control this problem will include development of new antimicrobials, better infection control

program and more appropriate use of existing antimicrobial agents [15, 16, 17].

The objective of this study was to find out the prevalence of microflora and their sensitivity pattern to antibiotics with special reference to *Pseudomonas* species in burn patients. For this study, patient samples from burn wound infections was profiled for microbial flora and was assessed for prevalence of *Pseudomonas* species, their resistance and susceptibility to antibiotics to give a cue for antibiotic regime for prescription. This study will also make a record of development of any resistance by the strains of *Pseudomonas* species to antibiotics.

2. Materials and methods:

The study was conducted at burns unit of Kilpauk Medical College, Chennai, Tamil Nadu during the period from March 2009 to February 2010. The study included microbiological profile of swabs taken from the wounds of burn patients and descriptive statistical analyses of the data thus obtained.

2.1 Swabs:

The wound was wiped with sterile saline. The swab was rolled along the leading edge of the wound and transferred to a sterile test tube containing sterile saline. Two swab specimens were collected from each patient- one for smear examination and one for culture.

2.2 Specimen Processing:

Once the specimen reached the laboratory, smears were prepared from the swab on a clean glass slide. Smears were routinely examined using Gram stain method. Swabs were inoculated onto Blood agar plate and Mac Conkey agar plate. They were incubated aerobically at 37° C for 18 - 24 hours. *Pseudomonas* sp. was identified based on colony morphology, Gram stain, motility and biochemical reactions.

2.3 Antimicrobial susceptibility testing:

Routine disk susceptibility test of isolates of *Pseudomonas* sp. was performed by Kirby - Bauer method on Mueller-Hinton agar medium obtained from Himedia for a range of antibiotics. 25 ml of the prepared medium was poured onto a Petri dish of 90 mm diameter to obtain a thickness of 4 mm.

a. Preparation of 0.5 McFarland's turbidity standard for inoculum preparation:

0.05 ml of 1% barium chloride solution was added to 9.95 ml of 1% sulphuric acid in a test tube with constant stirring to maintain a uniform suspension. 4-6 ml barium sulphate suspension was transferred to a screw capped tube that is used for growing and diluting the bacterial inoculum. The tube was tightly sealed and stored in the refrigerator and shook vigorously before each use until all the deposit was raised into uniform suspension.

b. Preparation of inoculum and inoculation:

Morphologically similar colonies from agar medium were transferred to a test tube containing 1.5 ml of nutrient broth and incubated at 35 °C until the density is equivalent to 0.5 McFarland's standard; this was measured to contain 150 million microorganisms per ml. Within 15 minutes of preparation of the suspension, a sterile cotton wool swab was used to transfer the inoculum onto the MHA medium. The lid of the dish was left to stand for 3 to 5 minutes to dry before placing the antibiotic disks [19].

c. Antibiotic disks:

For Gram-negative bacilli, the antibiotic disks included Ampicillin 10 µg, Cotrimoxazole 25 µg, Ciprofloxacin 5 µg, Cefotaxime 30 µg, Ceftazidime 30 µg, Gentamicin 10 µg and Amikacin 30 µg disks was used; Imipenem disk was used for ESBL producers. Antibiotic disks were applied with sterile forceps and pressed gently to ensure even contact with the medium. The plates were inverted and incubated at 35 °C to 37 °C for 16 to 18 hours [7].

d. Reading zones of inhibition:

The diameters of the zones of complete inhibition was measured with the disc using a ruler and rounded off to the nearest whole millimetre. The Petri plate was held a few inches above a black non-reflecting background and illuminated with reflected light to record measurement on the backside of the inverted Petri plate. The size of the zones of inhibition was interpreted by indexing to the measurements standardized in the NCCLS Table 2 (Volume 20(1): 2000) as zone diameter interpretive standards and reported as susceptible, intermediate or resistant for the tested standard antibiotic disks.

3. Results and discussion:

3.1 Microbiological profile:

The prevalence of microflora from this study of 289 samples was distributed as follows: 34.86 % *Klebsiella* species, 28.94 % *Pseudomonas* species, 25.65 % *Staphylococcus aureus*, 5.92 % *Proteus* species, 2.63 % *Escherichia coli* and 1.97 % Coagulase-negative *Staphylococcus* species (Figure – 1).

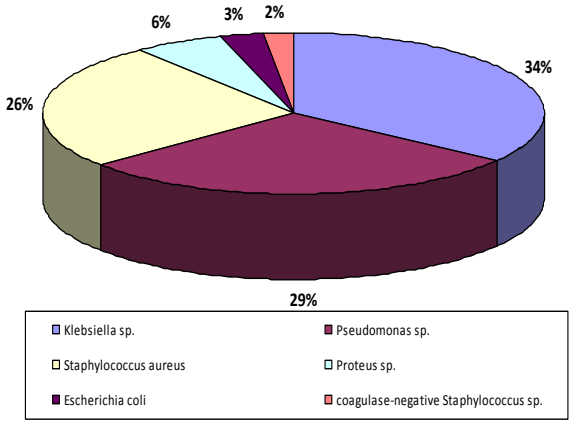

Table 1: Age and sex distribution of cases (N=289)

Age in years	Male	Female	Total
0-10	24	16	40
11-20	17	27	44
21-30	32	57	89
31-40	35	24	59
41-50	9	5	14
51-60	14	13	27
61-70	8	4	12
71-80	4	-	4
TOTAL	143 (49.48%)	146 (50.52%)	289

The distribution of burn patient cases was found to be predominant in women (50.52 %) than men; and the maximum cases were observed in the age group of 21 to 30 years.

In a study by NP Singh et al [21] the predominant isolate in burns was *Pseudomonas aeruginosa* – (31%) followed by *Staphylococcus aureus* (22 %) and *Klebseilla pneumoniae* (19%), which is in contrast to our study. A study by Manjula et al [22] showed contrary observation from the present study, in which *Pseudomonas aeruginosa* was the most common pathogen isolated from burn wounds (51.5%) followed by *Staphylococcus aureus* (11.15%). In another study by Ekrami and Kalantar et al [23]. *Pseudomonas aeruginosa* was the common pathogen. In a study by Shankar Srinivasan et al [24]. The predominant isolate in burn wounds was *Klebseilla pneumoniae* (33.91%). Similar contradiction was also observed in the study by Herjinder Kaur et al [25], where the predominant isolate was *Pseudomonas aeruginosa* (19%). In a study by Agnihotri et al [29] the predominant isolate was *Pseudomonas aeruginosa* (59%), followed by *Staphylococcus aureus* (17.9%) and *Klebsiella pneumoniae* (3.9%).

3.2 Antibiotic sensitivity pattern:

The pattern of antibiotic sensitivity showed that *Klebsiella* species, *Staphylococcus aureus*, *Proteus* species, *Escherichia coli* and coagulase-negative *Staphylococcus aureus* were resistant to commonly used antibiotics. *Pseudomonas* species, however, showed 100% sensitive to colistin (Table – 2).

Out of 22 isolates of *Pseudomonas aeruginosa*, 18 (81.81%) were sensitive to amikacin, 13 (59.09%) were sensitive to ciprofloxacin, 8 (36.36%) were sensitive to cefotaxime, 6 (27.27%) were sensitive to cephalexin, 12 (54.54%) were sensitive to Piperacillin/tazobactum, 22 (100%) were sensitive to imipenem, 11 (50%) were sensitive to gentamicin (Table 2).

Table 2: sensitivity pattern of pseudomonas aeruginosa

Antibiotics	*Pseudomonas aeruginosa* N = 22 (Percentage)
Amikacin	18 (81.81)
Ciprofloxacin	13 (59.09)
Cephatoxime	8 (36.36)
Cephalexin	6 (27.27)
Piperacillin / tazobactum	12 (54.54)
Imipenem	22 (100)
Gentamicin	11 (50)

The antibiotic sensitivity pattern of *Pseudomonas aeruginosa* in our study showed that18 (81.81 %) were sensitive to amikacin, 13(59.09 %) were sensitive to ciprofloxacin, 8(36.36 %) were sensitive to cephotaxime, 6 (27.27 %) were sensitive to

cephalexin, 12 (54.54%) were sensitive to piperacillin / tazobactum, 22 (100 %) were sensitive to imipenem and 11 (50 %) were sensitive to gentamicin. Similar results were observed in the study by Shankar Srinivasan et al [24] where the maximum sensitivity was for amikacin (62.3 %). In a study by Sanjay Dhar et al [27] the sensitivity pattern of *Pseudomonas aeruginosa* was 65 % to amikacin, 30 % to ciprofloxacin. In a study by Jyoti Sonawane et al [12] the antibiotic sensitivity pattern of *Pseudomonas aeruginosa* was 54.22 % to amikacin and 96.38 % to imipenem. In a study by Jonathan Osariemen Isibor et al [9] the sensitivity pattern of *Pseudomonas aeruginosa* was 35.7 % to ciprofloxacin, 71.4 % to gentamicin and 28.5 % to cephalexin. In another study by Fantahun Biadglegne et al [26] sensitivity of *Pseudomonas aeruginosa* to gentamicin was 67 %; while, a study by Prabhat Ranjan et al [19] showed susceptibility of *Pseudomonas aeruginosa* was 76.9 % to imipenem, 53 % to amikacin, 36 % to ciprofloxacin and 29.1 % to gentamicin. In the study by Shampa Anupurba et al [28], the prevalence of *Pseudomonas aeruginosa* in wound infection was 32 % and were sensitive to ciprofloxacin 58 %. In contrast to our study, Misra et al [20] reported that *Pseudomonas aeruginosa* was sensitive to cefotaxime (67 %), gentamicin (12 %), amikacin (60 %) and ciprofloxacin (47 %).

Conclusion:

The prevalence of microflora and their sensitivity pattern was studied in each burn ward facility, which clearly showed that microflora of burn wounds varied from place to place and from time to time. Two hundred and eighty nine patients with wound infections between March 2009 and February 2010 formed the study group. Specimens obtained from patients were cultured and *Pseudomonas aeruginosa* was identified to be prevalent at the rate of 11.71 % and was susceptible to imipenem and amikacin.

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