Original Article

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Microbiological Analysis of Hospital Acquired Infections in Burn Patients

Manjushree L. Arlikar*, Ovhal Rajesh S., Nilekar Sandeep L.*****

Author Affiliation
*Junior Resident **Assistant Professor ***Associate Professor, Dept. of Microbiology, Swami Ramanand Teerth Rural Medical College, Ambajogai, Maharashtra 431517, India.

Corresponding Author
Manjushree L. Arlikar
Junior Resident, Dept. of Microbiology, Swami Ramanand Teerth Rural Medical College, Ambajogai, Maharashtra 431517, India.
E-mail:
doctormanjushree@gmail.com

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Abstract

Introduction: About 75% of the mortality associated with burn injuries is related to sepsis, especially in developing countries. Since it is desirable to carry out periodic reviews of bacterial flora of burn wounds and environmental surveillance to look for possible sources of wound contamination, so that preventive strategies could be modified as necessary; present study has been carried out. *Aims & Objectives:* To identify the pathogens causing hospital acquired infections in burn units and determine their antimicrobial resistance pattern. Also to assess & correlate the environmental conditions and risk factors associated with burn infections. *Setting and Design:* Observational study done in rural tertiary care centre for duration of two years. *Materials & Methods:* Patients with Total burn surface area (TBSA)>10% were enrolled in study excluding children less than 12 years of age. From patient; surface wound swab, normal skin swab, nasal swab and throat swab were collected on first, third, seventh, tenth, and fourteenth day post admission. Environmental sampling was done every three months. *Result:* Out of 112 patients included in study, 62 were infected with burn wound infections. Colonization rates were 62% on third day, 77% on 14th day. Rates of invasive infections were 4% on third day increasing to 61% on 14th day. Overall, *Pseudomonas aeruginosa* was the predominant isolate (28%) followed by *Staphylococcus aureus* (23%), *Klebsiella pneumoniae* (11%), Coagulase Negative Staphylococcus (CONS) (9%) & Acinetobacter species (9%). High level of environmental contamination was seen with *Pseudomonas aeruginosa*. *Conclusion:* The most common route of infection was cross-infection.

Keywords: Burn Wound Infections; Colonisation; Environmental Surveillance; Health Care Workers.

Introduction

Burn patients are at a high risk for hospital acquired infection as a result of the nature, the immune-compromising effects of burns, prolonged hospital stays and intensive procedures [1]. Sources of organisms are found in the patient's own endogenous flora, exogenous sources in the environment and healthcare personnel [2]. This observational study was carried out to provide original data on the evolution of microbial flora in burn wounds and its correlation to endogenous & exogenous flora. Environmental conditions & risk factors assessed to know their significance in hospital acquired infections. This will help to undertake appropriate preventive measures to control such infections.

Subjects and Methods

The study conducted at rural tertiary care centre in duration of two years. Oral informed consent obtained from patients or their relatives. All the patients admitted during that period meeting inclusion criteria were included in study. The following inclusion criteria used for the study: 1) Total burn surface area (TBSA) >10% , 2) Length of stay in hospital more than 48 hours, 3) Survival more than 48 hours, 4) Age >12 years and infected as per the criteria of the National Nosocomial Infections Surveillance (NNIS) System [3]. Exclusion criteria: 1) Patients with co-morbidities like Diabetes Mellitus, Chronic Obstructive Pulmonary Disease, AIDS, 2) Patient already having infection on admission. Clinical and demographic details, which included age, sex, burn injury details, all investigations done, procedures and treatment details were filled up in a detailed proforma in consultation with resident doctors. Total surface area burned (TBSA) was calculated by Lund and Browder chart [4]. From patient, surface wound swab, normal skin swab, nasal swab & throat swab collected on first, third, seventh, tenth and fourteenth day post admission and plated on blood agar, MacConkey agar and Sabouraud Dextrose Agar(SDA) agar (Himedia Laboratories, Mumbai). Signs of invasive infections were also noted on day of collection [5]. The samples were collected, processed for aerobic bacterial & fungal identification by standard conventional methods [6,7,8]. Environmental sampling: During the study period, environmental sampling has been done every three months. Settle plates and Swabs were collected from various areas in burn unit, instruments used for dressing, also nasal and skin swabs from Health care workers and caregivers and processed to identify source of infections [7,9]. All the clinical samples and environmental samples tested for antibiotic sensitivity test by Kirby Bauer's disc diffusion method as recommended by the CLSI [2,10]. The procedures followed were in accordance with the ethical standards of the institutional ethics committee. Data generated from this work were tabulated into Microsoft excel sheets and percentage were calculated. No special statistical method used in this study.

Results

This study included 112 cases; total 405 burn wound samples were collected from them on different day of follow ups. In addition, to determine source of isolate, we studied 336 endogenous samples, 36 HCWs samples and 102 environmental samples. 16 patients were discharged before tenth day due to recovery and no infections; so, 96 patients could be followed up to 14th day after admission. Those 16 patients were included as non infected patients.

Table 1 shows patient distribution according to isolation pattern for bacterial isolates from wound samples. 62% of the patients were colonized by the day three, which increased to 77% at the end of second week. 93% (105 cases) patients were colonised on at least one occasion. In 6.25% (7 cases) patients, wounds were sterile on all follow ups. Out of 105 colonised patients, 21 (20%) were multi-microbial on at least one follow up. 4% of the patients were infected on third day increasing to 61% on 14th day. Maximum burn wound infections occurred on seventh day (39 cases). 55% (62 cases) patients suffered from burn wound infection. Out of 62 infected patients, 18(29%) were multi-microbial on at least one follow up.

Table 2 shows colonizers and invading pathogens. *Staphylococcus aureus* is the most common colonizer constituting 34% of all the colonising isolates, while *Pseudomonas aeruginosa* is the most common invader constituting 28% of all isolates. Fungal isolates account for 12% of total isolates. Non albicans Candida spp. found to be the most common fungal isolate in invasive wound infection.

Table 3 shows pathogens isolated from burn wound samples at intervals post-burn. On day three, most of the wounds were colonised by *Staphylococcus aureus,* CONS & *Klebsiella pneumoniae.* Invasive infections occurred in four cases by *Staphylococcus aureus. Staphylococcus aureus* infections increased on day seven & day ten, but got decreased on day 14 due to super-infection of other bacteria or fungi. On day seven; CONS, *Klebsiella pneumoniae & Acinetobacter baumannii* complex started predominating but CONS soon decreased in frequency, while *Klebsiella pneumoniae* & *Acinetobacter baumannii complex* remained throughout. On day 14, *Pseudomonas aeruginosa* was predominant isolate, followed by *Klebsiella pneumoniae* & *Acinetobacter baumannii complex.* Sixty four patients were inhabited by same bacteria throughout follow up; while 21 patients showed different isolates on different days. In nine patients, super-infections occurred by other isolates.

Environmental Surveillance

Out of 36 samples collected from healthcare workers, 77.77% were found to be contaminated. Predominant isolate was *Staphylococcus aureus,* followed by CONS. From 78 different environmental

samples that have been processed, 35 (44.87%) were contaminated. Most commonly isolated bacterial species was *Pseudomonas aeruginosa*. Table 4 & 5 summarizes Healthcare workers (HCW) samples & environmental sample culture results, respectively. Table 6 describes source tracing through antibiogram patterns.

Antimicrobial susceptibility results of isolates are graphically presented in Figure 1 to 4. Methicillin Resistant Staphylococcus aureus (MRSA) were predominantly isolated from healthcare workers and environmental samples. Among patients' isolates, rate is 25.49%. Four environmental isolates and four wound isolates were found to be inducible clindamycin resistant. Among all isolates of *Pseudomonas aeruginosa*, 14 wound isolates & four environmental isolates were found to be Multidrug Resistant (MDR). Pan-drug resistant strains were isolated from seven wound samples and five environmental samples from different sites at different periods. Most effective drugs presently are colistin & polymixin B followed by meropenem, imipenem, amikacin, tobramycin. Twenty five percent of *Pseudomonas aeruginosa* isolates were Metallo-beta lactamase (MBL) producers as tested by imipenem-EDTA disk synergy test.

Table 1: Patient distribution according to isolation pattern for bacterial isolates from wound samples

	3rd day	7th day	10th day	14th day	Overall
No. of patients sampled	112	101	96	96	112
No. of patients colonised	69 (62%)	76 (75%)	75 (79%)	74 (77%)	105 (93%)
Polymicrobial wound colonization	5	12	17	1	21
No. of patients with invasive infection	4 (4%)	43 (43%)	59 (61%)	59 (61%)	62 (55%)
Polymicrobial wound infection	0	10	18	12	18
Sterile lesions	43	25	21	22	7

*Invasive infections include bacterial as well as fungal infections. Colonization include only bacteria, study of fungal colonization is beyond this study.

Table 2: Pathogens isolated from burn wound samples

Pathogens Isolated	Colonization(Co)	Invasive infection(In)
Pseudomonas aeruginosa	40(27.39%)	28(28%)
Staphylococcus aureus	51(34.93%)	23(23%)
CONS	12(8.21%)	9(9%)
Enterococcus spp.	3(2.05%)	0
Citrobacter freundii	7(4.79%)	5(5%)
Acinetobacter baumannii complex	12(8.21%)	9(9%)
Klebsiella pneumoniae	17(11.64%)	11(11%)
Proteus mirabilis	4(2.73%)	3(3%)
Candida albicans	-	2(2%)
Non-albicans Candida spp.	-	5(5%)
Mucor spp.	-	2(2%)
Aspergillus niger	-	2(2%)
Aspergillus flavus	-	1(1%)
Total no. of strains isolated	146	100

Table 3: Pathogens isolated from burn wound samples on different days

Pathogens isolated	3rd day		7th day		10th day		14th day	
	Co	In	Co	In	Co	In	Co	In
Pseudomonas aeruginosa	3	0	14	7	32	26	38	28
Staphylococcus aureus	40	4	34	17	21	17	7	6
CONS	13	0	11	9	4	3	0	0
Enterococcus spp.	3	0	0	0	0	0	0	0
Citrobacter freundii	2	0	3	2	6	4	4	3
Acinetobacter baumannii	1	0	8	6	10	9	12	9
Klebsiella pneumoniae	11	0	16	11	16	11	15	10
Proteus mirabilis	2	0	2	1	4	3	4	3
Candida albicans	-	0	-	0	-	1	-	2
Non-albicans Candida spp.	-	0	-	0	-	3	-	5
Mucor spp.	-	0	-	0	-	0	-	2
Aspergillus niger	-	0	-	0	-	0	-	2
Aspergillus flavus	-	0	-	0	-	0	-	1
Total strains isolated	75	4	88	53	93	77	80	71

Table 4: Culture results of samples from HCWs

Organism	Nail	Hands	Nasal	Total (%)
Pseudomonas aeruginosa	0	1	0	1(3.5%)
Staphylococcus aureus	4	2	6	12(42.85%)
CONS	5	4	2	11(39.28%)
Klebsiella pneumoniae	0	2	2	4(14.28%)
Total strains isolated	9	9	10	28(100%)
Sterile	3	3	2	8
Total samples collected	12	12	12	36

Table 5: Isolates found in environment of burn unit. (6 samples collected from each site during study period)

Sites	Strains Isolated
Settle plate Gen. ward	4 SAU, 1 CONS, 1 KL, 2 ACI
Settle plate Corridor	3 CONS
Settle plate Pvt. Room	4 PAE, 2 SAU, 2 CONS, 1 ACI
Settle plate Dressing	1 PAE, 2 SAU, 1 CONS
Bed	2 PAE, 1 SAU, 1 KL, 3 ACI, 2 CFR
Dressing bed	2 PAE, 1 SAU, 3 KL, 1 ACI
Cheatle forcep soln.	5 PAE, 1 KL
Oxygen mask	2 CONS, 1 KL, 1 ACI
Multidose vial	1 CONS, 1 ACI, 2 CFR
Bronchoscope	3 PAE
Hood	3 SAU
IV stand	3 SAU
Water for bath	2 PAE, 1 ACI, 2 PMI
Total	

SAU: *Staphylococcus aureus,* PAE: *Pseudomonas aeruginosa,* CONS: coagulase negative *Staphylococcus,* ACI: *Acinetobacter baumannii complex,* KL: *Klebsiella pneumoniae,* CFR: *Citrobacter freundii,* PMI: *Proteus mirabilis.*

Table 6: Results of antibiogram typing

Strains		Source
Pseudomonas aeruginosa	17.5%	cheatle forcep, thermometer
	10%	air in pvt room, dressing room, cheatle forceps soln.
	15%	Thermometer
	15%	dressing bed
	17.5%	endogenous flora
	25%	Unknown
*Staphylococcus aureus**	59%	HCW
	14%	Hood, IV stand
	6%	air in ward, dressing bed,
	6%	air in dressing room
	14%	endogenous flora
	8%	Unknown
CONS	100%	Endogenous flora
Citrobacter freundii	50%	patients bed and multidose vial
	50%	Unknown
Acinetobacter spp.	92%	air in general ward, dressing bed, water for bath
	8%	air in pvt room, multidose vial
Klebsiella pneumoniae	29.4%	gen ward dressing bed oxygen mask, Cheatle forcep solution
	23.5%	HCW
	29.4%	endogenous flora
	23.6%	Unknown
Proteus mirabilis	100%	Water for bath

*Total calculation more than 100% due to same strains isolated from more than one source.

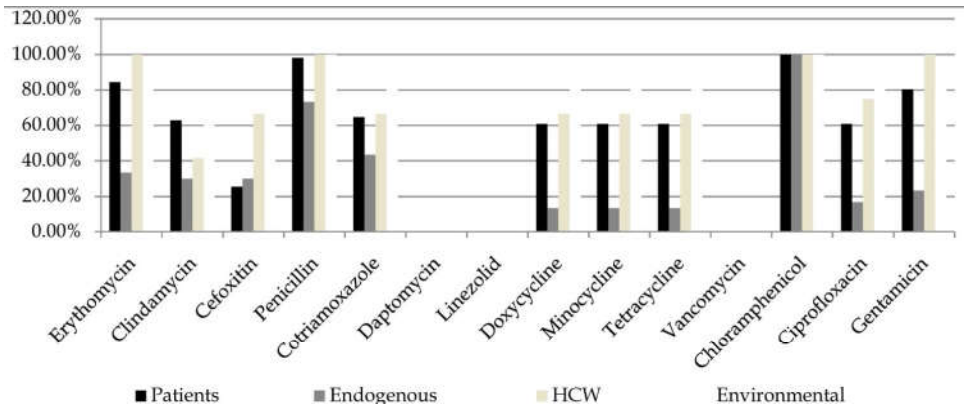

Fig. 1: Antibiotic resistance pattern of *Staphylococcus aureus* isolated from different sources

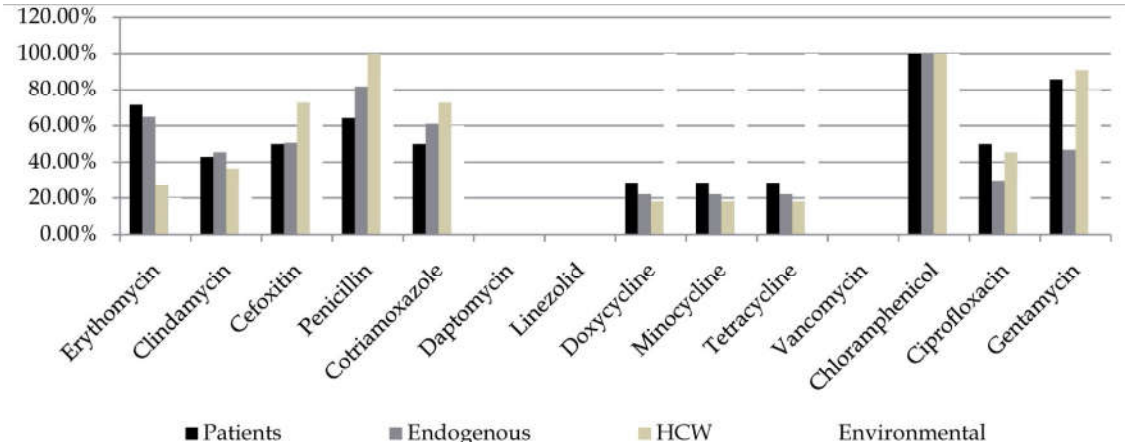

Fig. 2: Antibiotic resistance pattern of Coagulase negative *Staphylococci* isolated from different samples

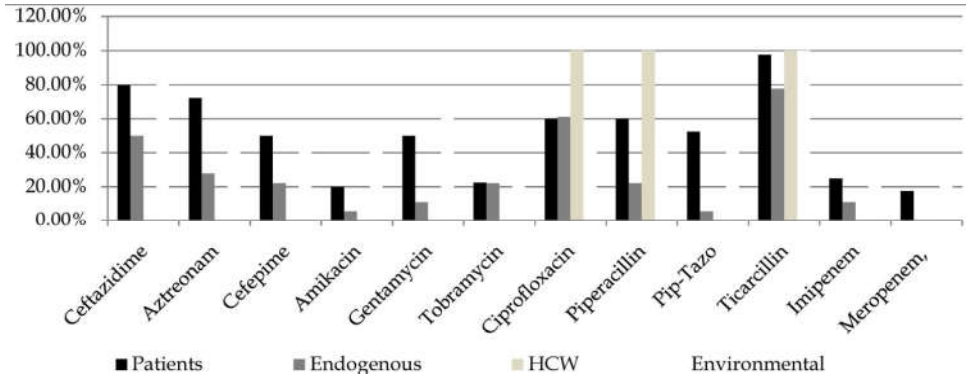

Fig. 3: Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from different sample

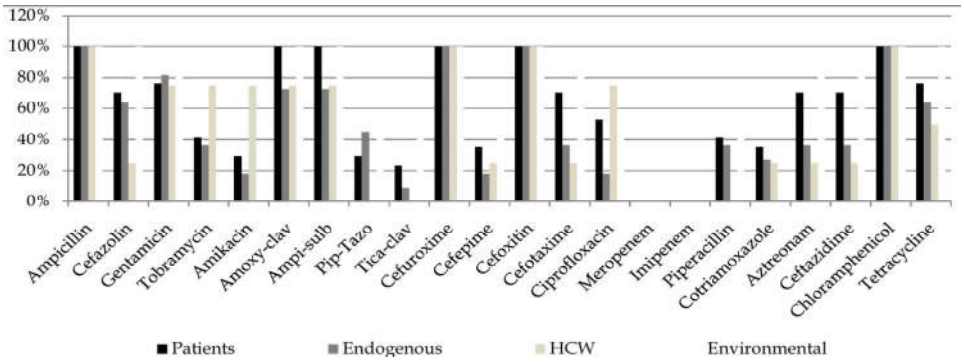

Fig. 4: Antibiotic resistance pattern of *Klebsiella pneumoniae* isolated from different samples

Discussion

The purpose of this study was to analyse the various hospital acquired infections in burn patients, to identify the most common burn pathogens, their sequential emergence in burn wound, antimicrobial resistance of bacteria that causing nosocomial infections and to identify the sources of these pathogens.

Overall, 93% of our study population were colonized at some point of time. After colonization, the organisms on the surface start to penetrate the burn eschar to a variable extent depending on their invasive capacity, local wound factors and the degree of patient's immune-suppression. If viable sub-eschar tissue becomes invaded, disseminated infection is likely to occur [11]. It clearly indicates that if invasion of bacteria has to be decreased; it is very important to maintain the wound sterile from beginning only. Initial colonization gave place for infection. We found that invasive infections started occurring on day seven in most of the cases, a similar finding as found in the study by Taneja *et al* [12].

There was predominance of gram positive bacteria in initial days of admission which got decreased & dominated by gram negative bacteria & fungi in second week.

Staphylococcus aureus (34.93%) was found to be the commonest colonizer followed by *Pseudomonas aeruginosa* (27.39%); finding similar with study by Taneja *et al* [12]. However, predominant bacteria causing invasive infection was found to be *Pseudomonas aeruginosa* (28%). *Pseudomonas aeruginosa* is the most commonly encountered source of chronic or acute burn wound infection in other studies [10,13,14]. The picture is slightly different in China, where *A. baumannii* and *Proteus mirabilis* are the most common causes of burn infection, with *P. aeruginosa* in third place [10,15].

The remarkably high prevalence of *Pseudomonas aeruginosa* in the burn wards may be due to the fact that the organism thrives in a moist environment [16]. *Pseudomonas aeruginosa* is known for its ability to resist killing by a variety of antimicrobials. The minimal nutritional requirements of *Pseudomonas*, as evidenced by its ability to grow in distilled water and its tolerance to a wide variety of physical conditions, contribute to its ecological success and ultimately to its role as an effective opportunistic pathogen.

On second week, fungal species started causing infections. There is a shift from commensal *Candida albicans* to more severe nosocomial pathogens, *non-albicans Candida*. This correlates with the study done by Sarabahi *et al* [17]. Literature showed that fungal colonization frequently associated with the multidrug resistant MRSA and *Pseudomonas* infections demanding treatment with newer generation of antibiotics like imipenem, vancomycin and linezolid [18,19]. In present study, 5 cases showed fungal colonization which were previously infected with various multidrug resistant bacteria. Among them, two were infected with MRSA and three with multidrug resistant *Pseudomonas aeruginosa, Klebsiella spp. and Citrobacter freundii.*

To trace the source of pathogens causing hospital acquired infections; we also studied endogenous flora of skin, nose and throat from patients, resident flora from healthcare workers and environmental samples.

Most of the isolates of *Pseudomonas aeruginosa* were traced to be from environmental source such as water for bath, air sampling, patient bed, cheatle forcep, dressing pad and bronchoscope; whereas *Staphylococcus aureus* was mainly through the healthcare workers. Coagulase negative *Staphylococcus* spp. from wound infections were found to be from patients' own endogenous flora. *Citrobacter freundii, Acinetobacter* spp. and *Proteus mirabilis* were traced to be from various environmental sources. For *Klebsiella pneumoniae,* all sources were found to be of equal importance (Table 6). Taneja *et al* found high contamination of air and surfaces with *Staphylococcus aureus.* They found no environmental sources for *Pseudomonas aeruginosa* which is contradictory to our findings [12].

In our study, we found most of the environmental samples and HCW samples contaminated. Hence, periodic surveillance and standard precautions to prevent cross infection should be carried out routinely. Hand hygiene for healthcare workers is essential to prevent cross infections. Their periodic trainings and periodic surveillance of their flora should be done. As in our study, surfaces and air is mostly contaminated, so thorough cleaning and fumigation is a must. Barrier precautions must be taken. Infected patients should be cared in separate wards by the separate healthcare workers. Dressing of uninfected patients should be done before infected patients. Regular disinfection of bed linens should be done to prevent cross transmission to other patients.

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