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Original Article

Evolution of bacterial flora in burn wounds: key role of environmental disinfection in control of infection

Neelam Taneja[1], PS Chari[2], Malkit Singh[1], Gagandeep Singh[1], Manisha Biswal[1], Meera Sharma[1]

[1]Department of Medical Microbiology, [2]Department of Burns and Plastic Surgery, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh, India

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Abstract: Bacterial flora in burn patients undergoes change over period of time and is dependent upon many factors. Study of burn flora is not only helpful in locating entry of multidrug resistant bacterial strains into the unit's usual flora but also in determining current antibiotic susceptibilities. Since no studies are available from India that have studied sequential emergence of different microorganisms in burn wound, present study was carried out to study evolution of bacterial flora in burn wounds and its correlation with invasive wound infection. Environmental sampling was also carried out for possible sources of infection. Patients with 20-70% of total burn surface were enrolled and followed up for entire duration of stay. Clinical & treatment details were noted. Surface wound swabs were collected on first, third, seventh, tenth and fourteenth day post admission. Environmental sampling was done every three months. Of 215 wound swabs collected from 71 patients, 72 were sterile and 143 yielded 214 isolates. Colonization rates were 33% on first day, 94% on 7th day and 100% by 14th day. 42% swabs grew gram negative bacteria. Overall *Staphylococcus aureus* was the predominant isolate (45%) followed by *Pseudomonas aeruginosa* (13.9%), beta hemolytic Streptococci (9.4%). Maximum invasive infections were seen at the seventh day. A high level of environmental contamination was seen with *S. aureus*, a substantial portion being MRSA. Better control of environmental contamination and disinfection along with rigorous hand washing and barrier precautions are recommended to prevent infection of wounds.

Keywords: Burns, infections, environmental, surveillance, disinfection

Introduction

It has been estimated that at least 50% of all deaths caused by burns are the result of wound infection [1]. Burn wounds are especially prone to infection because of loss of protective covering and presence of highly nutritive serum. The incidence of wound infection in burn patients appears to be correlated with both the extent and depth of injury as well as length of time the wound remains open [2]. Altered microbial ecology following burn injury is the result of the interaction of endogenous & exogenous microbial flora with injury induced physical and immunologic host defects [3]. The bacterial flora undergoes a change over a period of time [4] and is dependent upon length of hospitalization, environmental contamination, endogenous bacterial flora of patients and dressing procedures [5]. The determination of antibiotic susceptibility of the predominant isolated organisms or targeted organisms aids in recognizing the problems of cross contamination or introduction of multi-drug resistant bacterial strains into the unit's usual flora [5]. It is therefore desirable to carry out periodic reviews of the bacterial flora of burn wounds in all centers so that preventive strategies could be modified as necessary. No studies are available from India to date that have studied sequential emergence of different microorganisms in burn wound patients. Therefore, the present study was carried out in the burn unit of 1900 bed tertiary care referral centre in North India to study the evolution of bacterial flora of burn wounds. Environmental surveillance was also carried out to look for possible sources of wound contamination.

Subjects and methods

Patients presenting with 20-70% of total body surface area burned (TBSAB) were enrolled in

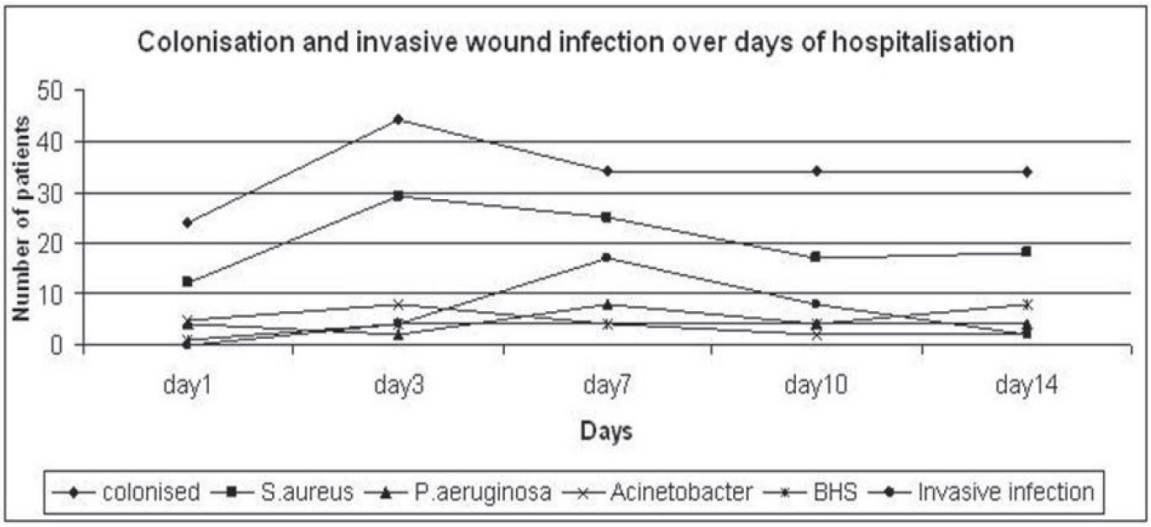

Figure 1. Graphic representation of colonization with Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter and beta hemolytic streptococci (BHS) and development of invasive wound infection over a period of 14 days.

this study. Details of the burn unit have been described elsewhere [6]. Patients referred from other hospitals and nursing homes were not included. Patients were visited daily by the hospital infection nurse, and followed up to death/discharge. 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 (TBSAB) was calculated by Lund and Browder chart [7]. Following investigations were done routinely and repeated as often as required: hemoglobin, total and differential leucocyte counts, renal and liver function tests, arterial blood gas analysis, urine microscopy, urine culture, blood culture, wound swab culture, tracheal aspirate culture. A surface wound swab was collected from the site marked on first, third, seventh, tenth, and fourteenth day post admission. Fifty ml sterile saline was poured over wound surface, a wet cotton tipped swab was collected and plated on blood and MacConkeys medium (Himedia Laboratories, Mumbai). The swabs were processed for aerobic organisms by standard conventional methods and antibiotic sensitivity tested by Kirby Bauers disc diffusion method as recommended by the CLSI [8]. The following definition based on CDC case definitions [9] was used to define invasive wound infection: 1. Change in burn wound appearance or character such as rapid eschar formation, discoloration of the eschar or edema at wound margin and 2. At least one of the following: a. Organisms cultured from blood in absence of other identifiable infections. b. Two of the following-fever>38°C or hypothermia, hypotension, oliguria, hyperglycemia at previously tolerated level of dietary carbohydrate or mental confusion.

Environmental sampling

During the study period an environmental sampling was done every three months. Samples were collected from various areas. Nasal swabs were collected from patients as well nursing staff to look for nasal carriage of *S. aureus*.

Results

Results of wound swab cultures

In all, 215 wound swabs were collected from 71 patients of which 72 were sterile & 143 yielded 214 isolates. Single isolates were obtained from 89 sample and multiple isolates from 54 samples. **Table 1** summarizes the findings of bacteriological cultures and correlation with gross appearance of wound and invasive wound infection. On first day post admission itself, 33% patients were colonized; *S. aureus* accounting for 50% of isolates, however, gram negative bacteria like *P. aeruginosa, Acinetobacter, Klebsiella, Enterobacter and E. coli* were isolated from 14 of 24 patients. By seventh day 94% of patients were colonized and 100% of patients were colonized by four-

Table 1. Result of gross wound appearance, invasive wound infection, colonization of wounds & mortality

Result	Post admission time of sampling				
	1st day	3rd day	7th day	10th day	14th day
Number of patients (n) sampled	71	64	36	36	24
Number of patients colonized	24 (33%)	44 (68.7%)	34 (94%)	34 (94%)	24 (100%)
Grossly clean wound	71 (100%)	61 (95.3%)	17 (47.2%)	18 (50%)	06 (25%)
Invasive wound infection	0 (nil)	04 (09.0%)	17 (50%)	8 (23.5%)	02 (8.3%)
Polymicrobial wound colonization	6 (25%)	15 (34%)	08 (23.5%)	13 (38.2%)	14 (58.3)
Patients died (n=16)*	0 (nil)	01 (6.25)	06 (37.5)	02 (12.5)	04 (25)

*Three died after 14th day.

Table 2. Results of bacterial cultures from wounds on different days

Organism	1st day	3rd day	7th day	10th day	14th day
No. of patients colonized	24	44	34	34	24
S. aureus (n=101)	12 (50%)	29 (65.9%)	25 (73.5%)	17 (50%)	18 (75%)
Pseudomonas (n=22)	4 (16.7%)	2 (8.3%)	8 (23.5 %)	4 (11.8%)	4 (16.7%)
Acinetobacter (n=21)	5 (20.8%)	8 (59.2%)	4 (11.8%)	2 (5.9%)	2 (8.3%)
β Hemolytic Streptococci (n=21)	1 (4.2%)	4 (9.1%)	4 (11.8%)	4 (11.8%)	8 (33.3%)
Klebsiella (n=15)	2 (8.3%)	6 (13.6%)	3 (8.8%)	2 (5.9%)	2 (8.3%)
Enterobacter (n=9)	2 (8.3%)	4 (9.1%)	3 (8.8%)	–	–
Proteus mirabilis (n=8)	–	2 (4.5%)	4 (11.8%)	1 (2.9%)	1 (4.2%)
E.coli (n=7)	1 (4.2%)	2 (4.5%)	1 (2.9%)	2 (5.9%)	1 (4.2%)
Enterococci (n=7)	2 (8.3%)	3 (6.8%)	1 (2.9%)	1 (2.9%)	–
Cirtobacter (n=2)	–	–	–	1 (2.9%)	1 (4.2%)
α-haemolytic Streptococci (n=1)	–	–	–	1 (2.9%)	–

n = number of isolates.

teenth day. **Table 2** shows the microorganisms isolated on different days. Out of 214 isolates 92(41.4%) were gram-negative bacteria. Overall *S. aureus* was the predominant isolate throughout (45.4%) followed by *P. aeruginosa* (13.9%), *Beta-hemolytic streptococci* (9.4%), *Acinetobacter spp* (9.4%), *Klebsiella* (6.3%), *Enterobacter* (4.3%), *Proteus* (3.6%), and *E.coli* (3.1%). All beta-hemolytic streptococci were group A.

Figure 1 shows the graphic representation of colonization, invasive wound infection and acquiring *S. aureus, P. aeruginosa* over a period of 14 days. Maximum invasive wound infection occurred at seven days (range 3 to 21 days, median 7 days). Two infections occurred after 14 days. The peak of the invasive wound infection coincided with the peak of acquiring *P. aeruginosa* and peak of colonization coincided with peak of acquiring *S. aureus*. Antibiotic susceptibility results of isolates are shown in **Table 3**.

Results of environmental surveillance

MRSA was grown from disinfectant solution, air samples, bath and medicine trolleys, bed mattresses, a nurse's locker and nasal swab of a patient. However none of the health care worker carried MRSA in their nose. Some of the sterile dressing material and open saline bottles used for irrigation of wounds were found contaminated (**Table 4**).

Discussion

The microbial component of the burn wound is the variable most easily influenced by therapy [3]. The organisms involved in these infections can be either endogenous or exogenous in origin. The exogenous sources of cross transmission and outbreaks in ICUs may include other patients, colonized health care personnel, contaminated food, supplies, hospital equipment and air [10]. Colonization precedes infection as also shown in our study (median day of coloni-

Table 3. Results of Antibiotic resistance of bacterial isolates from burn unit

Organism	Met	G	Cip	Net	Tmp-Sxt	E	Cef	Ak	Cefta	Pip
S. aureus (N=51)	13 (25.5%)	25 (49.2%)	12 (23.5%)	7 (13.7%)	21/30 (70%)	21/37 (56.7%)	12/40 (30%)	NT	NT	NT
P. aerugino-sa (N=17)	NT	11 (64.7%)	2/17 (11.7%)	12/17 (70.5%)	NT	NT	NT	3/17 (17.6)	8/17 (47%)	07/17 (41.2%)
Acinetobcter (N=13)	NT	7/13 (53.8%)	4/13 (30.7%)	3/13 (23.1%)	3/8 (37.5%)	NT	5/13 (38.5%)	2/13 (15.4%)	NT	NT
Klebsiella (N=6)	NT	5/6 (83.3%)	2/6 (33.3%)	2/5 (40%)	NT	NT	4/6 (66.6%)	3/6 (50%)	NT	NT
Proteus (N=4)	NT	3/4 (75%)	3/4 (75%)	1/4 (25%)	4/4 (100%)	NT	3/4 (75%)	1/4 (25%)	NT	NT
Enterobacter (N=4)	NT	4/4 (100%)	2/4 (50%)	0/4 100%)	NT	NT	2/4 (50%)	NT	NT	NT

N=number of isolates tested, NT-not tested, Met-Methicillin, G-Gentamicin, Ak-Amikacin, Cef-Cefotaxime, Cip-Ciprofloxacin, Tmp-Sxt-trimethoprim sulfamethoxazole combination, Net-Netilmicin, E-Erythromycin, Pip-Piperacillin, Cefta-Ceftazidime.

Table 4. Results of environment sampling, nasal and hand swabs

Surface tested	Total sample taken	Organism isolated & no. of samples contaminated or showing growth of pathogens
Medicine trolleys	05	*S. aureus* including MRSA (5), *Acinetobacter spp.* (5)
Bath trolleys	10	*S. aureus* including MRSA (10), *Acinetobacter spp.* (10)
Tap handles	08	MSSA (3)
Air conditioning grille	05	MSSA (3)
Wash basin	04	*Acinetobacter spp., Klebsiella spp., Enterobacter spp.* (4)
Refrigerators	05	*Citrobacter spp.* (1) *Enterobacter* spp. (1)
Sterile dressing material	13	*E.coli* (1) *Enterobacter spp. (*1)
Silver sulphadiazene cream & saline bottles	13	*Proteus mirabilis* (1) *Enterobacter spp. (*1)
Artery forceps	05	(0)
Disinfectant solution- Betadine	10	MRSA (1)
Bed mattresses	12	*S. aureus* including MRSA (12)
Nurses lockers	03	MRSA (1)
Electric switches	04	(0)
Nasal swab-patients	20	MSSA (7), MRSA (1), βHS (1)
Nasal swab-staff	10	MSSA (2)
Hand swab/Intact skin swab-Patients	20	MSSA (8), *Pseudomonas aeruginosa* (1), *Acinetobacter spp.* (1), βHS (2)
Hand webs-staff	10	MSSA (1)
Air	20	30-150 cfu of air including MRSA
Water	05	Satisfactory on all occassions
Total	182	90

cfu-colony forming units, MSSA-Methicillin sensitive *S. aureus*, MRSA-Methicillin resistant *S. aureus*, βHS-Beta hemolytic streptococci.

zation 3 days, and median day of wound infection 7 days). Burn wound is highly susceptible to colonization; colonizing microorganisms can easily multiply to reach high densities on the wound [11]. In the present study, it was found that colonization started almost immediately on admission (46.5% on first day), more than 90% of patients was colonized by the 7th day.

The results are similar to other studies [4, 12] where by the end of first week 80.6%-87% patients were colonized. An important finding of our study is an apparent change in the microbiology of burns wounds away from the traditionally important gram-negative rods to gram-positive cocci. *P. aeruginosa* which used to be the predominant colonizer and infective agent of burn wound have become uncommon due use of topical antibiotics [13]. In India many of the centers still have *P. aeruginosa* has been reported as the predominant organism from many centers in India [5, 14-16] however, at our center similar to developed countries *S. aureus* was the predominant organism throughout. Several studies have shown that flora of individual burn wound changes over time; gram-positive organisms are generally replaced by gram negative ones after the first week. In one study, *P. aeruginosa* did not appear in burn wounds until an average of 21 days after admission [17]. In our study this trend was not seen. *P. aeruginosa* was the second most common colonizer and colonization remained at the same level over a period of two weeks. In epidemiological studies of MRSA, it has been shown that the majority of MRSA positive burn patients, both the air and the environment surfaces become heavily contaminated [18]. This was also true for our burn unit. Most of the MRSA infections are due to failure to prevent cross-transmission in hospital [13, 19]. Contrary to the finding in the literature about beta hemolytic streptococci becoming a rarity [20], this organism, which is a serious threat to skin grafting, was the most common colonizing organism. A significant number of contaminated wounds showed a polymicrobial etiology with multiple isolates (54/143, overall 33%), 25% on first day, 34% on 3rd day, 32.5% on 7th day, 38.2% o 10th day, 58.3% on 14th day. Polymicrobial infection is increasingly being reported in burn patients [21]. The relationship between the colonizing organisms and the ones causing invasive wound infection was studied. Blood stream isolates were taken into account as we had not taken the burn biopsies. In 10 patients the organism isolated from blood culture were the same as that isolated from wound swab. Though *S. aureus* was the predominant colonizer, surprisingly *P. aeruginosa* and other gram-negative organisms were more frequently isolated from blood culture. This may be due to the higher invasiveness of *P. aeruginosa* or due to high resistance in this organism. The results of antibiotic sensitivity patterns give serious cause for concern because many of the isolates were resistant to commonly available antibiotics. Gross clean appearance of the wounds had very poor correlation with wound colonization, but correlated well inversely with invasive wound infection.

In 16 patients, nasal swab culture yielded *S. aureus* (MRSA in only 1). Antibiogram of these 16 strains did not match with *S. aureus* isolated from wound surface. None of the nursing staff had MRSA in their nose and none of the isolates matched in antibiogram with that isolated from wound surface. Another study with larger number of patients combined with epidemiological typing methods is warranted to know the exact source of *S. aureus*. However the source of *S. aureus* in burn patients may be exogenous. This finding is corroborated by high environment contamination of surfaces and air with *S. aureus* including MRSA. Since a high level of contamination of air and bath trolleys was seen it is obvious that control measures should be directed against contamination of environment with *S. aureus*. No environmental sources were found for *P. aeruginosa*. We suspect the gut of the patient to be a source as *P. aeruginosa* can colonize guts of 30% of the hospitalized patients [5]. Some of the sterile material used for dressing and disinfectants were also found contaminated. It is very important that all the dressing material be sterile and that dressings be done taking all sterile precautions.

Patient housing in single bed in a room with a separate sink facility to wash hands and change in staffing pattern has been shown to prevent infection and reduce mortality [22]. Cohort separation has been found to be a practical way of elimination endemic resistant gram-negative organisms from burn [3]. We recommend nursing of severely burned patients in a purpose built burn unit rather than general surgical ward and cohort nursing to reduce the cross infection problem. Particular nurse/nurses can look after uninfected patients. Barrier precautions should be taken at all times of patient handling. Disinfection of the bath trolleys in between two patients and routine disinfection of the surfaces is highly desirable as also better compliance to hand washing. Infected and uninfected patients should be cared for by separate groups

of nurses and residents should do the dressing of uninfected patients first and then proceed to infected patients.

Competing interest statement

The authors declare that they have no competing financial interests.

Address correspondence to: Dr. Neelam Taneja, Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh, India. Pin: 160012. Phone: + 91 172 2755160; Fax: + 91 172 274 4401; E-mail: drneelampgi@yahoo.com

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