ORIGINAL ARTICLES

CHANGES IN BACTERIAL FLORA OF BURN WOUNDS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERNS AT A TERTIARY HOSPITAL IN GHANA

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Abstract

Objective: To determine the time-related changes in burn wound bacteria and to determine the antibiotic susceptibilities of these bacteria.

Methodology: The study was carried out over a 4-month period from September 2017 at the Burns Unit of the Korle-Bu Teaching Hospital in Accra. Wound swabs were taken weekly from burn patients on admission and each patient was followed-up for a month. The swabs were cultured, and antibiotic susceptibility testing done on isolated pathogens.

Results: A total of 214 wound swabs were taken from 59 patients enrolled with an overall isolation rate of 65%. Gram negative isolates predominated each week throughout the period of monitoring. The commonest bacterial isolate was *P. aeruginosa* which formed 51.8% of all isolates, followed by coagulase negative staphylococci 13.7%, *S. aureus* 10.1% and other Gram-negative bacilli. Sixty-four percent of *S. aureus* were resistant to cefoxitin (MRSA), and100% resistant to penicillin. Resistance to the cephalosporins and fluroquinolones was generally high among the Gram-negative bacteria. *P. aeruginosa* had moderate resistance to the anti-pseudomonal antibiotics. Resistance to amikacin among the Gram-negative bacteria was low.

Conclusion: Burn wounds are colonized by pathogenic bacteria, some highly antibiotic-resistant. There were no significant time-related changes in bacterial flora of burn wounds.

Key words: *Burn wounds, bacterial colonization, infection, antimicrobial resistance*

Introduction

Burn wounds, though sterile immediately following thermal injury, rapidly become colonized by bacteria, some pathogenic, and capable of causing wound infection, with its serious consequences.

The bacteria colonizing burn wounds initially come from the endogenous flora of the patient[1]. These are later replaced by more antibiotic resistant ones from the hospital environment and from the hands of healthcare personnel [1-3]. The open burn wound also increases the environmental contamination, making other patient's wounds an additional source of bacterial colonization and possible infection of the wounds of a freshly burnt patient. Outbreaks of cross colonization and infection are thus a major challenge on burn units[4]. Colonization of wounds often serves as a precedent to infection[5]. Depending on the numbers and virulence of colonizing bacteria, critical colonization may be achieved, beyond which wound infection becomes established[6].

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The burn patient is highly susceptible to wound infection because of multiple factors. First, loss of skin integrity allows micro-organisms to access and invade viable tissue. The highly proteinaceous avascular tissue, eschar, also serves as a favourable niche for micro-organisms to proliferate. Being avascular, the eschar, additionally prevents cells of the immune system and antibiotics from gaining access to these organisms promptly, thus giving them the opportunity to invade tissue and cause wound infection. Additionally, significant thermal injury causes a state of immunosuppression, allowing even bacteria with low virulence to cause wound infection in the burn patient. Bacteria colonizing burn wounds often exist in biofilms which make them difficult to eradicate[3,7].

Routine surveillance cultures are important to monitor the changing microbiota of burn wounds and determine their antimicrobial susceptibilities so that appropriate empirical treatment can be given in the event of burn wound infection. Surveillance cultures also help to monitor the effectiveness of current wound treatment and detect any cross-colonizations which occur quickly so that further transmission can be, prevented. Admission cultures are important, especially for patients transferred from other facilities, as they may be colonized by multidrug-resistant organisms and serve as reservoirs for cross-transmission to other patients in the unit[8].

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Most studies carried out in this area have been cross-sectional. This study, however, aimed at providing data on the common bacteria that colonize burn wounds during their management in the hospital environment, as well as their antibiotic susceptibility patterns. Such data is useful in determining the pathogenic bacteria to target during hospital management of burn patients.

Materials and Methods

Study Site and Design

The study, which was a prospective, longitudinal, observational study was carried out at the Burns Unit of the Korle-Bu Teaching Hospital, a tertiary hospital in Ghana. Patients admitted to this unit are typically started on systemic antibiotics right after admission. There are no specific local guidelines directing the use of antibiotics at the unit. Burn patients admitted to the unit from September to December 2017 who gave written or verbal consent to the study were enrolled using a convenient sampling method. Basic demographic information and information relating to burn injury were obtained from patients, close relatives and/or hospital records.

Specimen Collection

Wound assessment (with respect to wound site(s), appearance, discharge, and odour) was done during each wound dressing. Total body surface area burnt was estimated using Lund and Browder charts. Irrigation of wounds was done using normal sterile saline solution and it was ensured that no antimicrobial agent was applied to wounds before specimens were taken. Wound swab specimens were taken with sterile cotton-tipped swab-sticks for bacteriological analysis during first wound dressing, and subsequently, weekly over a 4-week period. Depending on which body areas where affected, swabs of burn wounds were taken from the head and neck, left upper limb, right upper limb, left lower limb, right lower limb, trunk, and perineum. The Levine technique of swabbing wound surfaces was used: A sterile cotton swab tip was rotated in a 1cm square area of wound tissue for a period of 5 seconds, using gentle pressure to release tissue exudate[9].

Laboratory procedures

Isolation of Specific Aerobic Pathogens

The swab specimens were cultured under aerobic conditions on blood agar and MacConkey agar for bacterial isolation.

Bacterial Identification

Preliminary identification of bacterial isolates was done using colonial morphology and characteristics such as pigmentation and haemolytic pattern on blood agar and by Gram staining. This was followed by conventional biochemical tests on bacterial isolates from primary cultures for final identification. Gram negative rods were identified using oxidase test, indole test, motility test, hydrogen sulphide (H_2S) production test, triple sugar iron (TSI) reactions, citrate utilization test, and urease test. Gram-positive cocci were identified by catalase test, oxidase test and coagulase test [10].

Antibiotic susceptibility testing

Antibiotic susceptibility testing was done on all significant bacterial isolates. This was done using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton Agar[11]. Ceftazidime (CAZ, 30 µg), cefuroxime (CXM, 30 µg), ceftriaxone (CRO, 30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LVX, 5 µg), gentamicin (GM, 10 µg), amikacin (AN, 30 µg), meropenem (MEM, 10 µg), penicillin (AM, 10 µg), and cefoxitin (FOX, 30 µg) from Becton Dickinson BBL, USA, were used in accordance with the Clinical Laboratory Standard Institute (CLSI) recommendations [12]. *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as control strains.

Data management and analysis

All data obtained from patients was entered into Epi Info version 7 and exported into the Statistical Package for Social Sciences (SPSS) version 20 for statistical analysis. Comparison of frequencies, mean and median values were made. Comparison of proportions was done using Chi-square test. The level of significance was determined at $p<0.05$.

Patient Consent

Written informed consent was obtained by the signature of the patient or the signature of the guardian/legal representative (in case a patient is a minor or is too ill to give consent) on the informed consent form or, if the patient or guardian/ legal representative is illiterate, their consent certified with a fingerprint accompanied by the signature of an impartial witness. For children of appropriate age, assent was obtained in addition to parental consent.

Ethical Approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and in compliance with ICH-GCP, ISO 14155-1 and -2, and the applicable laws and regulations of Ghana. Ethical approval of this study was obtained from the Institutional Review Board of the hospital (approval number: KBTH-STC/IRB/00047/2016).

Results

Patient and wound characteristics

A total of 59 patients were enrolled in the study. These were made up of 39 (66.1%) males and 20 (33.9%) females, giving a male-female ratio of about 2:1. The ages of patients ranged from 3 months to 65 years with median and mean ages of 6 and 18.4 years respectively (Table 1). Forty-nine percent of all patients were children aged 5 years and younger.

Other patient and wound characteristics are summarised in Table 1.

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Antibiotic Use

All 59 patients enrolled had been put on systemic antibiotics (intravenous) from the time of admission as part of normal clinical practice at the burns unit. Cefuroxime was used either alone or with other antibiotics for 51 (86.4%) patients. Other antibiotics commonly used included ceftriaxone, ceftazidime and metronidazole.

Table 1: Demographic characteristics of patients

Characteristic	Value
Age (median)	6 years (49% ≤ 5 years)
Sex	
Male	39 (66.1%)
Female	20 (33.9%)
Type of burn	
Thermal	57 (96.6%)
Chemical	2 (3.4%)
Duration of burns before enrolment (median)	3 days
TBSA (mean)	23.8%
Burn thickness	
Superficial partial	38 (64.4%)
Mixed	20 (33.9%)
Full	1(1.7%)

Bacteriological profile of burn wounds

A total of 214 wound swabs were taken from the 59 patients enrolled. Of these, 139 yielded positive cultures, giving an overall isolation rate of 65%.

Qualitative bacterial analysis

The most predominant bacterial isolate from patients' wounds was *P. aeruginosa* which formed 72/139 (51.8%) of all isolates. This was followed by coagulase negative staphylococci (CoNS) 19/139 (13.7%), *S. aureus* 14/139 (10.1%), *Citrobacter* species 11/139 (7.9%), *Enterobacter* species 6/139 (4.3%), *Klebsiella* species 4/139 (2.9%), *E. coli* 2/139 (1.4%), *P. mirabilis* 1/139 (0.7%) and other Gram-negative bacteria which altogether formed 10/139 (7.2%) (Figure 1).

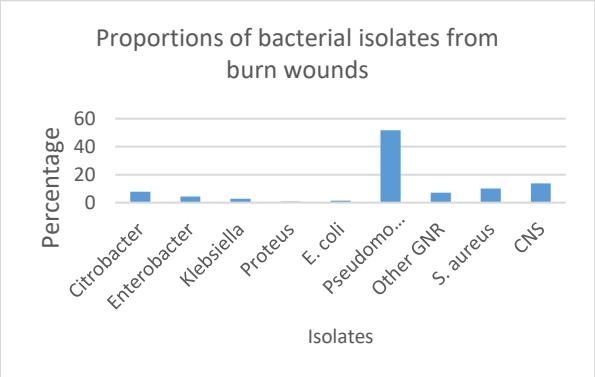

***Figure 1**: Proportions of bacterial isolates from burn wounds*

P. aeruginosa was isolated from all wounds clinically suspected to be infected. Two of these wounds additionally had *S. aureus* isolated.

Forty-four percent (44%) of patients had their wounds colonized by bacteria, without any signs of wound infection. Eight patients (13.6%) had clinical signs of wound infection.

Time related changes in bacterial isolates

Gram negative isolates predominated each week throughout the period of wound monitoring. They formed 66/90 (73.3%) of isolates in week one, and 22/29 (75.9%), 16/17 (94.1%), and 2/3 (66.7%) of isolates in weeks two, three, and four respectively. *P. aeruginosa* was the most predominant isolate each week.

Table 2: Percentage resistance of bacteria to systemic antibiotics

Organism (*n*) [N=120]	Percentage Resistance (%)				
	Gentamicin	Amikacin	Ciprofloxacin	Levofloxacin	Penicillin
Citrobacter (11)	54.5	18.2	27.3	27.3	NT
E. coli (2)	0	0	100	100	NT
Enterobacter (6)	66.7	16.7	50	33.3	NT
Klebsiella (4)	100	25	50	25	NT
P. mirabilis (1)	0	0	0	0	NT
P. aeruginosa (72)	52.8	34.7	48.6	47.2	NT
Other GNR (10)	60	10	60	40	NT
S. aureus (14)	42.9	NT	NT	NT	100
Organism (*n*) [N=120]	Percentage Resistance (%)				
	Cefuroxime	Ceftriaxone	Ceftazidime	Cefoxitin	Meropenem
Citrobacter (11)	54.5	54.5	36.4	NT	27.3
E. coli (2)	100	100	100	NT	0
Enterobacter (6)	50	50	50	NT	33.3
Klebsiella (4)	75	75	50	NT	25
P. mirabilis (1)	0	0	0	NT	0
P. aeruginosa (72)	NT	NT	56.9	NT	37.5
Other GNR (10)	100	100	100	NT	100
S. aureus (14)	NT	NT	NT	64.3	NT

Systemic Antibiotic susceptibility

Nine out of fourteen isolates (64.3%) of *S. aureus* were resistant to cefoxitin (Methicillin Resistant Staphylococcus aureus), and all 14 (100%) isolates were

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resistant to penicillin. Resistance to the cephalosporins was relatively high among the Gram-negative organisms, especially *E.coli* and *Klebsiella* species. There was relatively high resistance to the fluoroquinolones among *E. coli* isolates. *P. aeruginosa* had moderate resistance to the antibiotics it was tested against. Resistance to amikacin among the Gram-negative organisms was low (Table 2).

Discussion

Bacteriological profile of burn wounds

Predominance of *P. aeruginosa* among other bacterial isolates on burn wounds in the current study has also been reported by several other authors[13-16]. Some authors however reported a predominance of *S. aureus* [17,18]. The frequent association of *P. aeruginosa* with the hospital environment due to its ability to thrive in water containing only trace nutrients and to withstand disinfectants makes it a ready colonizer of burn wounds. The isolation of *P. aeruginosa* from all wound specimens of clinically diagnosed wound infections indicates the capability of the organism to cause invasive infections especially in immunocompromised individuals such as burn patients[19].

In the present study, there were no significant time-related changes in the bacterial isolates, as *P. aeruginosa* predominated each week throughout the study period. Proportions of the other bacterial isolates however changed over time but not significantly (p=0.175). This observation was also made by other authors who found P. aeruginosa to predominate isolates from burn wounds over the entire 4-week period of assessment[20,21]. It was however not in conformity with the trend observed by some authors, who found Gram positive organisms to predominate initially after burn injury and their replacement by Gram negative bacteria over time[14,22]. In our study P. aeruginosa may have heavily contaminated the immediate environment of the patients and served as a ready source of the organism for wound colonization. The persistence of *P. aeruginosa* on wounds after the first week, unlike the other bacterial isolates makes it necessary for antimicrobial interventions to target this organism mainly, especially, after the first week of admission.

Antibiotic susceptibility

Generally, there was significant resistance by burn wound isolates to the commonly used antibiotics at the burns unit. Most significant amongst these were methicillin-resistant *S. aureus* (MRSA) (64.3%), and the Gram-negative bacteria, notably, *Klebsiella* species and *E. coli* to the cephalosporins. Routine prophylactic use of systemic antibiotics may have contributed to this observation by causing selective pressure for resistant strains of bacteria. Resistance to the cephalosporins may have been because of the high usage of cefuroxime for burn patients on the ward. The mechanism of resistance may be by the production of extended-spectrum ß-lactamases (ESBL) or Amp-C ß-lactamases especially by *Klebsiella* and *E. coli*, since there was less resistance to the carbapenems. Those resistant to meropenem may additionally have produced carbapenemases. One author reported a high prevalence of ESBL-producing isolates from various samples taken from patients at the same hospital[23]. MRSA rate of 64.3% is very high compared to the 28% reported by another author at the same study site two years earlier[24]. The implication of this level of resistance to cefoxitin is that no beta-lactam antibiotic would be able to clear infections caused by a significant 64.3% of S. aureus isolates. For these, other antibiotics like the glycopeptides would have to be used. Almost half of all *P. aeruginosa* isolates would not respond to treatment by any of the commonly used antibiotics at the unit. This observation calls for concern as P. aeruginosa was the most predominant bacterial isolate at the unit. These high levels of antibiotic resistance by burn wound bacteria have been reported by other authors[13,25].

Resistance to amikacin was low. For serious or life-threatening infections of burn patients at this unit therefore, amikacin and/or vancomycin may be the most effective antibiotics for treatment. A limitation of this study is that it was conducted at a single centre. A multicentre study would have been more appropriate in determining the time-related changes of bacterial flora on burn wounds as the environmental contamination of these organisms play a significant role.

Conclusions

Burn wounds are heavily colonized by antibiotic resistant bacterial pathogens which may cause difficult to treat, invasive wound infections with Pseudomonas aeruginosa being the most predominant. This study however did not show significant time-related changes in the type of bacteria found on the wound surfaces.

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Declarations

Author Contributions

All authors contributed to the conceptualization of the study design, drafting of the manuscript and revision for important intellectual content. NOA was responsible for data acquisition and statistical analysis. All authors have approved the final report.

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Transparency declarations

None.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

References

1. Erol S, Altoparlak U, Akcay MN, Celebi F, Parlak M. Changes of microbial flora and wound colonization in burned patients. *Burns: J Int Soc Burn Inj*. 2004;30:357-361.
2. Saaiq M, Ahmad S, Zaib MS. Burn wound infections and antibiotic susceptibility patterns at pakistan institute of medical sciences, islamabad, pakistan. *World J Plast Surg*. 2015; 4:9-15.
3. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. *Clin Microbiol Rev*. 2006; 19:403-434.
4. Rafla K, Tredget EE. Infection control in the burn unit. *Burns: J Int Soc Burn Inj*. 2011; 37:5-15.
5. Halstead FD, Lee KC, Kwei J, Dretzke J, Oppenheim BA, Moiemen NS. A systematic review of quantitative burn wound microbiology in the management of burns patients. *Burns*: *J Int Soc Burn Inj*. 2017.
6. World Health Organization. Wound and lymphoedema management WHO/HTM/NTD/GBUI/2010.1. Geneva: World Health Organization, 2010. http://whqlibdoc.who.int/publications/2010/9789241599139_eng.pdf.
7. Nasirmoghadas P, Yadegari S, Moghim S, Esfahani BN, Fazeli H, Poursina F, et al. Evaluation of biofilm formation and frequency of multidrug-resistant and extended drug-resistant strain in Pseudomonas aeruginosa isolated from burn patients in Isfahan. *Adv Biomed Res*. 2018; 7.
8. Weber J, McManus A. Infection control in burn patients. *Burns : Journal of the Int Soc Burn Inj*. 2004; 30:A16-A24.
9. Levine NS, Lindberg RB, Mason AD, Jr., Pruitt BA, Jr. The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. *J Trauma*. 1976; 16:89-94.
10. Mahon CR, Lehman DC, Manuselis G. Textbook of diagnostic microbiology. Saunders, Elsevier; 2015.
11. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. 2009.
12. CLSI. Performance standards for antimicrobial susceptibility testing. 26th ed. 2016.
13. Fadeyibi IO, Raji MA, Ibrahim NA, Ugburo AO, Ademiluyi S. Bacteriology of infected burn wounds in the burn wards of a teaching hospital in Southwest Nigeria. *Burns : J Int Soc Burn Inj*. 2013; 39:168-173.
14. Mehta S, Singh K, Sawhney N, Singh VA, Goyal S. Time related changes in pathogenic bacterial patterns in burn wound infections and their antibiotic sensitivity traits. *Bangladesh J Med Sci*. 2017; 16:295-301.
15. Vaez H, Beigi F. Antibiotic susceptibility patterns of aerobic bacterial strains isolated from patients with burn wound infections. *Germs*. 2016; 6:34-36.
16. Gupta M, Naik AK, Singh SK. Bacteriological profile and antimicrobial resistance patterns of burn wound infections in a tertiary care hospital. *Heliyon*. 2019; 5:e02956.
17. Chen Y-Y, Wu P-F, Chen C-S, Chen I-H, Huang W-T, Wang F-DJBid. Trends in microbial profile of burn patients following an event of dust explosion at a tertiary medical center. *BMC Infect Dis.* 2020; 20:193. doi: 10.1186/s12879-020-4920-4
18. Upreti N, Rayamajhee B, Sherchan SP, Choudhari MK, Banjara MR. Prevalence of methicillin resistant Staphylococcus aureus, multidrug resistant and extended spectrum β-lactamase producing gram negative bacilli causing wound infections at a tertiary care hospital of Nepal. *Antimicrob Resist Infect Control*. 2018; 7:1-10.
19. Alhazmi A. Pseudomonas aeruginosa–pathogenesis and pathogenic mechanisms. *Int J Biol*. 2015; 7:44-67.
20. Rajbahak S, Shrestha C, Singh A. Bacteriological changes of burn wounds with time and their antibiogram. *Sci World*. 2014; 12:70-76.
21. Belba MK, Petrela EY, Belba AG. Epidemiology of infections in a burn unit, Albania. *Burns: J Int Soc Burn Inj*. 2013; 39:1456-467.
22. Altoparlak U, Erol S, Akcay MN, Celebi F, Kadanali A. The time-related changes of antimicrobial resistance patterns and predominant bacterial profiles of burn wounds and body flora of burned patients. *Burns: J Int Soc Burn Inj*. 2004; 30:660-664.
23. Obeng-Nkrumah N, Twum-Danso K, Krogfelt KA, Newman MJ. High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. Am J Trop Med Hyg. 2013; 89:960-964.
24. Amissah NA, van Dam L, Ablordey A, Ampomah OW, Prah I, Tetteh CS et al. Epidemiology of Staphylococcus aureus in a burn unit of a tertiary care center in Ghana. *PloS ONE*. 2017; 12:e0181072.
25. Rezaei E, Safari H, Naderinasab M, Aliakbarian H. Common pathogens in burn wound and changes in their drug sensitivity. *Burns : J Int Soc Burn Inj*. 2011; 37:805-807.

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