

BACTERIAL INFECTIONS IN BURN WOUND PATIENTS AT A TERTIARY TEACHING HOSPITAL IN ACCRA, GHANA

LES INFECTIONS CUTANÉES DES PATIENTS BRÛLÉS DANS UNE CHU D'ACCRA, GHANA

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SUMMARY. Intact human skin surface is essential for protection against infection, preservation of body fluid homeostasis and thermoregulation. Burn injury compromises the skin barrier and enables bacterial infection, hence delaying burn wound healing. This study aimed to determine the microbial profile of burn wounds, and resistance patterns of microbes with respect to the source of the injured patient's wound. Fifty wound swab samples were collected from fifty burn patients at the Korle-Bu Teaching Hospital, Accra (KBTH). Sterile swabs moistened with sterile saline were used to swab burn wounds. The swabs were plated on blood agar and MacConkey agar for 24 hrs at 37°C. Biochemical tests were carried out on the representative isolate on each plate, and antibacterial sensitivity pattern was determined using the Kirby-Bauer disc diffusion method. The study revealed that the main source of burns was gas flames (66%) and scalds (28%). Out of the 50 samples analysed, 86% were culture positive and 14% were culture negative for bacteria. The predominant organisms isolated were *Pseudomonas* sp. (30.2%) and *Acinetobacter* sp. (20.9%). *Proteus mirabilis* (2.3%) and *Staphylococcus aureus* (2.3%) were the least frequently isolated bacteria. Although *Pseudomonas* sp. showed varying resistance levels to gentamicin, cotrimoxazole and ciprofloxacin, all the *Acinetobacter* sp. were resistant to most of the tested antibiotics used. Resistant gram negative bacteria are the most common isolates associated with burn wounds in Accra, Ghana. Hence a careful selection of antibiotics to control the wound infection is required for proper management of burn wounds in order to help reduce morbidity and mortality.

Keywords: burn wounds, Accra, bacteria, antibiotic resistance, gas

RÉSUMÉ. L'intégrité cutanée est essentielle à la protection contre les infections, l'homéostasie circulatoire et hydro-électrolytique ainsi qu'à la thermorégulation. La brûlure détruit la barrière cutanée et permet l'infection locale, qui obère la cicatrisation. Le but de cette étude était d'évaluer le profil microbiologique des infections cutanées (bactéries et antibiogrammes). Cinquante écouvillons cutanés obtenus sur autant de patients du CTB du CHU Korle-Bu d'Accra. Les prélèvements étaient des écouvillonnages humides. Les échantillons ont été incubés sur gélose au sang et milieu de Mc Conkey pendant 24 h à 37°C. Les identifications bactériennes étaient réalisées par test biochimiques, les antibiogrammes par diffusion en milieu solide (méthode de Kirby-Bauer). Les 2/3 des brûlures étaient dues à du gaz enflammé, 28% étaient des ébouillancements. Quatre vingt six pour cents des cultures étaient positives, 14% négatives. *P. aeruginosa* était retrouvé dans 30,2% des prélèvements, *Acinetobacter* 20,9%, *P. mirabilis* et *S. aureus* bien moins fréquemment (2,3% tous deux). Les résistances de *Pseudomonas* à la gentamicine, au cotrimoxazole et à la ciprofloxacine étaient variables, quand *Acinetobacter* était souvent multirésistant. Les BGN résistants sont les bactéries les plus souvent isolées des brûlures infectées à Accra, Ghana. De ce fait, un choix rigoureux des antibiotiques en cas d'infection de brûlure est nécessaire, afin de réduire la morbidité et la mortalité.

Mots-clés: brûlure, Accra, bactéries, résistance, gaz

Introduction

Globally, burns are considered devastating forms of trauma in patients with serious thermal injury.^{1,2} They can be caused by scalds, thermal, electrical, gas or chemical agents.¹⁻⁴ Patients with serious burn injury require immediate specialized care in order to minimize bacterial infection, which is a major cause of morbidity and mortality in burn patients.⁵⁻⁸

Much progress has been made with respect to infection

control and burn wound management, however, burn wound infection still poses a major clinical challenge in most developing countries, where wound site infections are a major source of post-operative illness and mortality among burn patients.⁸ The consequential effect of burn wounds contaminated with pathogenic bacteria can delay wound healing, cause wound breakdown and herniation of the wound or complete wound dehiscence.⁹⁻¹⁰ Although in most cases the source of contamination is the patient's normal flora or exogenous con-

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tamination from contaminated wound dressing devices in or from the hospital environment, various groups of microorganisms have been reported to be associated with wound infections.¹⁰⁻¹¹ A study carried out by Patil et al.¹¹ in India revealed that *Pseudomonas aeruginosa*, Methylene Resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter* sp., Coagulase negative *Staphylococci*, *Enterobacter* sp. and *Escherichia coli* were commonly associated with the wounds of burn patients. Whilst some studies have reported *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella* sp. and *Escherichia coli* as predominant bacteria associated with burn wounds,¹²⁻¹⁴ the exact number of burn injuries is very difficult to estimate. Although some studies have shown that adult females and children (1-9 years) are at a greater risk of burn-related injuries than adult males,¹⁵⁻¹⁸ burn wound is an important cause of disability and mortality in all ages and in both developed and developing countries.^{19,20}

Antibacterial susceptibility patterns for microorganisms isolated from hospitalized patients are continuously evolving, and this can pose a major challenge for clinicians treating burn wound victims.^{21,22} Therefore, the present study was conducted to determine the microbial profile of burn wounds, the antimicrobial susceptibility patterns of the microbes with respect to the source of wound, age, and sex among burn injured patients at the National Reconstructive Plastic Surgery and Burns Centre (NRPSBC) of the Korle-Bu Teaching Hospital (KBTH).

Methodology

The investigation was a cross-sectional study carried out at the National Reconstructive Plastic Surgery and Burns Centre (NRPSBC) of the Korle-Bu Teaching Hospital (KBTH). KBTH is a leading national referral centre in Ghana and also serves as a referral centre for neighbouring countries.²³ All consenting burn patients admitted to the Burns Centre of KBTH from April – July, 2016 were recruited and included in the study.

Sample collection

A total of fifty wound swabs were collected, using a sterile cotton swab, from the wounds of burn patients admitted to the Burns Centre of the Korle-Bu Teaching Hospital (KBTH) from April to July, 2016. Without considering how long the patients were admitted for, all consenting patients were included in the study. Duration of patient admission ranged from 5 to 31 days. However, patients on antibiotic therapy for bacterial infection were excluded from the study. A single wound swab was taken from each patient prior to wound dressing with hydrocortisone. Swabs were taken from areas that appeared deep, with discharge, and the swabs were immediately transported to the Microbiology Unit of the School of Biomedical and Allied Health Sciences (SBAHS) for analysis. Data on age, gender and type of burn were also collected from the patients' clinical folder.

Laboratory analysis

On arrival at the laboratory, the wound swabs were immediately cultured onto blood (Oxoid) and MacConkey agar (Oxoid), then incubated at 37°C for 18-24hrs. After 24 hrs, the colonial morphology of the colour, shape and general appearance of the individual colony on each of the plates was examined.²⁴ A representative single colony on the blood and

Table I - Guidelines for interpreting antimicrobial susceptibility results

Antibiotic		Disk Content (µg)	Zone diameter (mm)	
Class	Type		S ≥	R <
Penicillins	Ampicillin +	10	14	14
Cephalosporins	Cefotaxime*	30	26	26
Cephalosporins	Ceftriaxone*	30	23	19
Cephalosporins	Cefuroxime+	30	19	19
Carbapenems	Meropenem*	10	23	19
Fluoroquinolones	Ciprofloxacin+	5	26	24
Aminoglycosides	Amikacin+	30	18	15
Aminoglycosides	Gentamicin*	10	16	16
Tetracyclines	Tetracycline*	10	15	16
Sulfonamides	Cotrimoxazole	25	16	16
Chloramphenicol	Chloramphenicol+	10	17	17

*CLSI (2012) break points, + EUCAST (2016) breakpoint

MacConkey agar was gram stained and tested with indole and citrate, and Triple Sugar Ion test (TSI), urease and oxidase were performed to identify which bacteria species were present.

Susceptibility testing

Briefly, stored isolates were subcultured onto horse blood agar plates (37°C, 18 h) and individual colonies were suspended in saline to a turbidity equivalent of 0.5 McFarland standard.²⁵ The suspensions obtained were then streaked on Mueller-Hinton agar plate using sterile swab sticks. The paper discs were gently but firmly placed on the inoculated plates using sterile forceps. The plates were incubated at 37°C for 24 hours after which zones of inhibition were measured and interpreted according to the Clinical and Laboratory Standard Institute.²⁵ The reference strains used for the determination of MIC values were *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213. Clinical and Laboratory Standard Institute susceptibility break points were used when available, while other break points were sourced from EUCAST (European Society of Clinical Microbiology and Infectious Diseases) Break Points for Enterobacteriaceae 2016²⁶ (Table I). Each gram negative isolate was tested using eleven antibiotics: Ampicillin (10µg), Chloramphenicol (10µg), Cefotaxime (30µg), Tetracycline (10µg), Ceftriaxone (30µg), Gentamicin (10µg), Cefuroxime (30µg), Meropenem (10µg), Amikacin (30µg), Cotrimoxazole (25µg) and Ciprofloxacin (5µg), and each gram positive isolate was tested using Amikacin (30µg), Gentamicin (10µg), Cefuroxime (30µg) Cotrimoxazole (25µg), Erythromycin (15µg), Vacomycin (30µg) and Oxacillin (1µg). These antibiotics were used because they are the most commonly used in the treatment of bacterial infections in Ghana.²⁷

Data analysis

The data obtained from the study were analysed using descriptive statistics generated with the help of Microsoft Excel. The quantitative data generated from the study were coded and fed into Microsoft Excel and analyzed using GraphPad Prism software, version 6. In all cases, P-values less than 0.05 were considered statistically significant. Paired student *t* test was used to test for significance between prevalence of burns in the different sex and age groups.

Ethical clearance

The study was approved by the Ethics Committee of the

School of Biomedical and Allied Health Sciences, University of Ghana: Ethics Identification Number SAHS/10403317/AA/MLS/2015-2016. Participation by the patients was voluntary in accordance with the Ethics Committee's guidelines.

Results

Out of a total of 50 samples collected from the burn patients, 18 (36%) were from males and 32 (64%) were from females (Table II). The majority of the patients were within the 21-30 age group (32%), followed by 0-10 (28%), 11-20 (18%), 31-40 (8%), 41-50 (8%), and 51-60 (4%), with the 61-70 age group having the least number of patients (2%). Gas (flame) was the cause of injury in 33 (66%) patients, scalds were responsible for 14 (28%) injuries and 3 (6%) patients had been injured by electricity.

Out of the 50 samples cultured, 43 (86%) were found to be positive for bacteria, while 7 (42%) showed no bacterial growth. In total, 9 different isolates were identified, with the predominant bacteria being *Pseudomonas* sp. (30.2%) (Table III). It was followed by *Acinetobacter* species (20.9%), *Proteus mirabilis* (16.3%), *Enterobacter* sp. (11.6%), *Klebsiella* sp. (7.0%), *Citrobacter* sp. (4.7%), *Klebsiella oxytoca* (4.7%), *Proteus vulgaris* (2.3%) and *Staphylococcus aureus* (2.3%).

Most of the *Pseudomonas* sp. were found to be resistant to ampicillin, cotrimoxazole, cefuroxime and ceftriaxone (Table III). All of the *Acinetobacter* sp. were resistant to ampi-

Table II - Demographic characteristics of recruited burns patients

Age Groups (Yrs)	No. of Patients (%) (n=50)
0 – 10	14 (28)
11 – 20	9 (18)
21 – 30	16 (32)
31 – 40	4 (8)
41 – 50	4 (8)
51 – 60	2 (4)
61 – 70	1 (2)
Gender of total patients	
FEMALE	32 (64)
MALE	18 (36)
Mechanism of burn injury	
Gas (Flame)	33 (66)
Scalds	14 (28)
Electricity	3 (6)

Table III - Prevalence of isolated bacteria and the antibiotic resistant profiles of gram negative bacteria from the burn wound patients

ISOLATE	No. Isolated (%)	Prevalence of Resistant Isolates										
		AMP	TET	COT	GEN	CRX	CHL	CTR	CTX	CIP	AMK	MEM
<i>Pseudomonas</i> sp.	13(30.2)	11	9	12	8	11	9	12	12	11	1	8
<i>Acinetobacter</i> sp.	9(20.9)	9	9	9	7	9	9	9	9	8	3	7
<i>Citrobacter</i> sp.	2(4.7)	2	1	1	0	1	0	2	1	0	0	0
<i>Enterobacter</i> sp.	5(11.6)	5	4	5	5	5	5	4	5	5	1	5
<i>Klebsiella</i> sp.	3(7.0)	3	3	3	2	2	2	2	2	3	0	2
<i>Klebsiella oxytoca</i>	2(4.7)	2	2	2	0	2	0	2	2	1	0	2
<i>Proteus vulgaris</i>	1(2.3)	1	1	1	1	1	1	1	1	1	0	1
<i>Proteus mirabilis</i>	7(16.3)	6	6	6	4	7	2	7	7	6	2	6

KEY: AMP=Ampicillin TET=Tetracyclin COT= Cotrimoxazole GEN=Gentamicin CRX=Cefuroxime CHL=Chloramphenicol CTR=Ceftriaxone CTX=Cefotaxime CIP=Ciprofloxacin AMK=Amikacin MEM=Meropenem

Table IV - Prevalence of isolated bacteria with respect to the age groups, sex, work status and causes of burns in patients

Females				
Ages (Yr group)	No. of Patients	Work Status	Cause of burns	Bacteria isolated
0-10	4	Student	Scalds	<i>Acinetobacter</i> sp. (1), <i>Pseudomonas</i> sp. (1), <i>Klebsiella</i> sp. (1), <i>Klebsiella oxytoca</i> (1)
11 -20	7	Student	Gas	<i>Pseudomonas</i> sp. (5), <i>Proteus mirabilis</i> (1), <i>Acinetobacter</i> sp. (1)
21-30	5	Student	Gas	<i>Proteus mirabilis</i> (1), <i>Klebsiella</i> sp. (1), <i>Proteus mirabilis</i> (1), <i>Enterobacter</i> sp. (2)
	4	Employed	Gas	<i>Proteus mirabilis</i> (1), <i>Pseudomonas</i> sp. (1), <i>Acinetobacter</i> sp. (2)
	1	Employed	Electrical	<i>Klebsiella oxytoca</i> (1)
31-40	4	Employed	Gas	<i>Proteus mirabilis</i> (1), <i>Pseudomonas</i> sp. (1), <i>Klebsiella</i> sp. (1), <i>Enterobacter</i> sp. (1)
41-50	3	Employed	Gas	<i>Proteus mirabilis</i> (1), <i>Pseudomonas</i> sp. (2)
51-60	1	Employed	Gas	<i>Proteus mirabilis</i> (1)
Total (%)	29(58)			
Males				
Ages (Yr. group)	No. of Patients	Work Status	Cause of burn	Bacteria isolated
0-10 years	8	Student	Scalds	<i>Acinetobacter</i> sp. (3), <i>Staphylococcus aureus</i> (1), <i>Citrobacter</i> (1), <i>Pseudomonas</i> sp. (1), <i>Proteus mirabilis</i> (1), <i>Enterobacter</i> sp. (1)
11-20	1	Student	Gas	<i>Pseudomonas</i> sp. (1)
21-30	1	Employed	Gas	<i>Proteus vulgaris</i> (1)
	1	Student	Gas	<i>Pseudomonas</i> sp. (1)
	1	Student	Electrical	<i>Citrobacter</i> sp. (1)
61-70	2	Employed	Gas	<i>Acinetobacter</i> sp. (2)
Total (%)	14 (28)			

cillin, cotrimoxazole, cefuroxime, ceftriaxone and tetracycline. Whilst most of the *Proteus mirabilis* were resistant to ampicillin, cotrimoxazole, gentamicin, cefuroxime and ceftriaxone, all of the isolated *Enterobacter* sp. were also resistant to ampicillin, cotrimoxazole, gentamicin, cefuroxime and ceftriaxone. *Klebsiella* sp. and *Klebsiella oxytoca* were both found to be resistant to ampicillin, tetracyclin, cotrimoxazole and ciprofloxacin. One *Staphylococcus aureus* was isolated in this study. The isolate was found to be resistant to oxacillin, erythromycin, amikacin and gentamicin (data not included).

Out of the 29 female burn wound patients, 16 were students and 13 were employed (Table IV). Whilst a lot of the female patients were found to have gas burns (12 students, 8 employed) often infected with *Pseudomonas* sp. or *Proteus mirabilis*, a few (4 students) had scalds as the source of injury. Paired student t test analysis of females with regards to type of burn and age group revealed that there was no significant association ($p = 0.3361$).

The 14 male patients with burn injuries were students (11) or employed (3). In contrast to the females, males had most often suffered scalds (8 students), with gas (2 students, 3 employed) and electrical (1 student) burns the other sources of injury. Whilst the scald wounds were often infected with *Acinetobacter* sp., *Staphylococcus aureus*, and *Citrobacter* sp., a few gas wounds were infected with *Pseudomonas* sp., and *Acinetobacter* sp.

Paired student t test analysis of males with regards to type of burn and age group revealed there was no significant association ($p = 0.2893$).

Discussion

This study reports for the first time prevalence of burn injury and associated resistant bacteria in patients in a teaching hospital in Accra, Ghana. In this present study, the incidence of burn injuries was higher in females: 32 (64%) compared to 18 males (36%). This is in conformity with a study conducted in India by Rao et al.⁶ which also reported a higher incidence of burn injuries in females (56.9%) than in males (43.1%). The relatively higher number of cases of burns in females may be due to their greater participation in kitchen activities. However, our findings are in contrast to a previous study by Ekrami and Kalantar²⁸ from India, which showed a higher prevalence (59.3%) of burn injuries in males than in females (40.6%). The studied patients ranged from 1 to 62 years of age, the 21-30 age group being the most affected age group - which correlates with the study by Chaudhary et al.²⁹ This could be due to the fact that the 21-30 group is the most active group, and most involved in outdoor activities.

Among the culture positive samples, 29 (58%) were from female patients and 14 (28%) were from male patients. Thirty-three (66%) of the studied patients had flame injury caused by gas, while 14 (28%) had scald injury and 3 (6%) had electrical injuries. This correlates with a study conducted by Shahzad et al.³⁰ in Pakistan, which reported the predominant burn agent to be gas flame (76%), followed by scald (14%), contact (6%), electrical (3%) and chemical (1%). This study revealed *Pseudomonas* sp., *Acinetobacter* spp, *Proteus mirabilis*, *Enterobacter* spp, *Klebsiella* sp., *Citrobacter* sp., *Klebsiella oxytoca* and *Proteus vulgaris* to be the most common gram negative bacteria species isolated from burn wounds, with *Staphylococcus aureus* being the only gram positive bacteria

sp. isolated. This is similar to a study carried out by Patil et al.,¹¹ which also revealed *Pseudomonas* sp, *Acinetobacter* sp., *Proteus mirabilis*, *Klebsiella* sp., *Citrobacter* sp, *Enterobacter* sp and *Escherichia coli* as the most common gram negative bacteria associated with burn wounds. This study showed a high incidence of gram negative organisms compared to gram positive organisms as previously reported in the study by Shahzad et al.³⁰ from Pakistan.

Out of nine different bacterial species isolated from 43 patients, *Pseudomonas* sp. was the most predominant bacteria associated with the burn wounds. The high prevalence of *Pseudomonas* sp. in this study may be due to the fact that the organism thrives well in a moist environment.³¹ Our findings correlate with the study by Lakshmi et al.³² from India, which also reported *Pseudomonas* sp. as the most common isolate in burn wounds with a prevalence of 33.6%. This study, however, is in contrast to studies conducted by Chaudhary et al.²⁹ in Nepal and Rao et al.⁶ in India, which reported *Staphylococcus aureus* as the most common isolate associated with burn wounds with a prevalence of 28.71% and 42% respectively. Another study by Srinivasan et al.³³ conducted in India also reported *Klebsiella* sp. (33.91%) to be the predominant isolate associated with burn wounds. The differences in isolated bacterial isolates in burn wounds may be due to a variation in treatment practices in the different geographical locations of burn victims.³⁴

The antimicrobial susceptibility pattern of the different gram negative isolates from the burn patients revealed that *Pseudomonas* sp. was resistant to amikacin, ceftriaxone, ciprofloxacin and gentamicin. Findings from this study are similar to those of Shahzad et al.,³⁰ who reported varying resistance levels to amikacin (35%), ceftriaxone (85%), ciprofloxacin (70%) and gentamicin (97%). This study also revealed a resistance to cefotaxime and tetracyclin in *Pseudomonas* sp. Our findings are similar to those of Rao et al.,⁷ who reported a resistance to cefotaxime (34.9%), and Mehedi et al.,³⁵ who revealed a resistance to tetracycline (65.57%) in *Pseudomonas* sp.. *Acinetobacter* sp. showed 100% resistance to both ceftriaxone and cefuroxime, which is similar to a study by Nahar et al.,³⁶ from Bangladesh, who also reported a high resistance of *Acinetobacter* species to ceftriaxone (100%) and cefuroxime (100%). Resistance of *Acinetobacter* sp. to gentamicin in this study is similar to that found in a study by Moradi et al.³⁷ in Iran, who also reported a resistance of *Acinetobacter* to gentamicin (78%). Moradi et al.³⁷ also reported resistance to cefotaxime (95%), which does not correlate with our findings of 100% resistance of *Acinetobacter* sp. to cefotaxime. *Proteus mirabilis* were resistant to tetracycline (100%), which is similar to a study by Mordi and Momoh³⁸ in Benin, who also reported a 100% resistance of *Proteus mirabilis* to tetracycline. *Enterobacter* sp. was resistant to cefotaxime (100%), which is in contrast to findings by Otta et al.³⁹ in India, who reported moderate resistance (50%) of *Enterobacter* sp. to cefotaxime.

Conclusion

Findings of the study suggest that multidrug resistant gram negative organisms are the most common isolates from burn wounds. Hence a careful selection of antibiotics to treat burn wound infection is required for proper management of these wounds in order to help reduce morbidity and mortality associated with multi-resistant bacteria.

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Availability of data and materials. The datasets used and/or analysed during this study are available from the corresponding author upon reasonable request.