BACTERIAL AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND THE PREVALENCE OF SEPSIS AMONG BURN PATIENTS AT THE BURN UNIT OF CIPTO MANGUNKUSUMO HOSPITAL

PRÉVALENCE DU SEPSIS, PROFIL BACTÉRIOLOGIQUE ET ANTI-INFECTIEUX CHEZ LES PATIENTS BRÛLÉS HOSPITALISÉS DANS LE CTB DE L'HÔPITAL CIPTO MANGUKUSUMO

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SUMMARY. Infection is a major cause of mortality and morbidity among burn patients. An effective measure to reduce infection is routine monitoring of bacterial infection and antimicrobial susceptibility patterns at the burn unit. This will help to create a burn centre-specific empirical antibiotic therapy protocol. A retrospective, descriptive study was conducted at the Cipto Mangunkusumo Hospital (RSCM) Burn Unit between September-November 2016. Data regarding bacterial culture isolates, antimicrobial susceptibility spectrum, and the number of burn patients diagnosed with sepsis were collected. There were 36 patients with positive bacterial cultures, with the isolates changing continuously between *Klebsiella pneumonia* (17%), *Pseudomonas Aeruginosa* (12%) and *Acinetobacter baumannii* (11%). High resistance was found for 10 antimicrobials, particularly cephalosporins. The three bacteria were only sensitive to carbapenem, aminoglycosides and tigecycline. Fourteen patients were diagnosed with sepsis (38.9%), 10 died. Two major sepsis-causing bacteria were *P. aeruginosa* (33.3%) and *K. pneumoniae* (28.9%). Bacterial isolates in our setting changed every month. Almost all bacterial isolates are multi-drug resistant, highly resistant to the empirical therapy given (ceftriaxone), leading to outbreaks of sepsis and increased mortality rates. Carbapenem (imipenem, meropenem and doripenem) and aminoglycosides (amikacin) combination was the selected empirical therapy.

Keywords: burn infection, antibiotic susceptibility, multi-drug resistance, sepsis

RÉSUMÉ. *L'infection est une cause majeure de morbi-mortalité chez les brûlés. Une des mesures de contrôle est le suivi microbiologique systématique dans l'unité, permettant de définir l'antibiothérapie probabiliste à ce niveau. Une étude rétrospective a été réalisée entre septembre et novembre 2016 dans le CTB de l'hôpital Cipto Mangukusumo (HCM, Indonésie). Les données concernant les patients infectés, les bactéries en cause et leur antibiogramme ont été colligées. Des résultats bactériologiques positifs ont été retrouvés chez 36 patients, 14 d'entre eux étant septiques (10 décès). On trouvait le plus souvent Klebsiella pneumoniæ (17%), Pseudomonas æruginosa (12%) ou Acinetobacter baumannii (11%), sans série d'isolement de l'une ou l'autre bactérie. La résistance était très fréquente vis-à-vis de 10 antibiotiques parmi lesquels les céphalosporines, les trois principales bactéries n'étant régulièrement sensibles qu'au carbapénèmes, aux aminosides et à la tigécycline. Pseudomonas æruginosa (33,3%) et Klebsiella pneumoniæ (28,9%) étaient les principaux responsables de sepsis. La répartition de l'écologie variait en permanence. Presque toutes les bactéries résistait à le ceftriaxone, qui était jusqu'alors la base de l'antibiothérapie probabiliste, avec pour conséquence une épidémie septique et une surmortalité. Nous avons alors choisi une antibiothérapie probabiliste basée sur les carbapénèmes (imipénème, méropénème, doripénème) et l'amikacine.*

Mots-clés: *brûlure, infection, antibiogramme, bactéries multirésistantes, sepsis*

Introduction

In many countries, infection is the major cause of death among burn patients.[1] Burn injury compromises the function of the skin as a physical barrier against microbes. Damage to the skin enables microorganisms to infiltrate the human body, which then results in infection.[2] Furthermore, sepsis is the cause of 75% of all deaths in patients with severe burns.[2] Studies have shown that the occurrence of sepsis in burn patients is caused by a depression in the immune response (cellular and humoral) and massive systemic inflammatory response (SIRS).[3] Additional contributors to the occurrence of sepsis in burn patients is high cutaneous bacterial load, the possibility of gastrointestinal bacterial translocation, prolonged hospitalization, and invasive diagnostic and therapeutic procedures.[4,5] However, with the increasing prevalence of drug-resistant

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pathogens worldwide, the treatment of infections in burn patients is becoming more difficult.[6] Therefore, to effectively reduce the incidence of infection and sepsis, an antimicrobial stewardship program needs to be developed. Every burn unit should routinely identify and track the pattern of microbial colonization at the unit.

The majority of the microorganisms isolated in many burn units are gram-negative pathogens.[7] Gram-negative bacteria have a higher probability of developing resistance to antimicrobials compared to gram-positive bacteria.[7] The most commonly isolated gram-negative bacteria in burn units worldwide include *Pseudomonas aeruginosa* (74%), *Escherichia coli* (35%), *Acinetobacter baumannii* (24%), *Coagulase-negative staphylococci* (21%) and *Enterococcus spp.* (14%).[8,9] Nevertheless, the spectrum of microbial colonization varies between burn units, and also varies from time to time and from place to place.

Based on our knowledge, there is currently limited literature on the bacterial profile and antimicrobial susceptibility pattern and its association with mortality and sepsis in Indonesia. Therefore, this study was conducted to investigate the prevalence of drug-resistant bacterial isolates in the burn unit at Cipto Mangunkusumo Hospital (RSCM), and its association with the incidence of sepsis and mortality in the unit, thus creating a guide for developing a burn centre-specific antimicrobial empiric therapy for patients at the RSCM burn unit.

Methods

Setting

The burn unit at Cipto Mangunkusumo Hospital (RSCM) is located in Jakarta, the capital city of Indonesia. This hospital burn unit is one of the referral centres for burn injuries in Indonesia, receiving a variety of burn cases from all over Indonesia since 2010.[10] The mean annual admission to the RSCM Burn Unit from 2013 to 2015 was 138 patients.[11] Furthermore, this burn unit is equipped with its own intensive care unit (ICU) and operating theatre, and follows a multidisciplinary treatment approach. In addition, it is separated into the ICU with 2 beds and the high care unit (HCU) with 6 beds. This unit is primarily managed by a consultant of plastic surgery and the plastic surgery residents of Universitas Indonesia (UI).[10]

Study design

This is a retrospective, descriptive study which reviewed the data of burn patients hospitalized at the RSCM Burn Unit between September 2016 and November 2016. Data was collected from medical records over a 3-month period (September – November 2016), and then reviewed. All burn patients, identified from the medical records, were included in the analysis of this study. Patient data collected included bacterial culture isolates, the anti-bacterial drug susceptibility spectrum (resistance and sensitive) identified in the patients at the RSCM burn unit, and the total number of burn patients diagnosed with sepsis.

The cultures were taken from wound swab, urine, blood, tissue and sputum. In RSCM, all patients admitted to the burn unit routinely undergo wound swab and blood cultures and sensitivity tests. Wound swab is commonly obtained from areas of deepest burn injury. For urine culture, samples were only obtained if there was a suspicion of a urinary tract infection (UTI) due to prolonged use of a urinary catheter. On the other hand, tissue cultures were performed only if the burn patient was scheduled to undergo surgery. The tissue is obtained by excising a selected burn wound from the patient. Sputum culture was only conducted if the burn patient was intubated with an endotracheal tube. All samples obtained from the wound, urine, blood, tissue and sputum were cultured to isolate the bacterial infection, and tested subsequently on different antibiotics to determine susceptibility to antibiotics (resistance and sensitivity).

Other than the culture results, this study calculated the total number of burn patients diagnosed with sepsis over the 3-month period. At the RSCM burn unit, sepsis is diagnosed based on the Third International Consensus of Sepsis[12] and the International Guidelines of Sepsis by the AHA (American Hospital Association).[13] Burn patients were diagnosed with sepsis if those with known/suspected infection exhibited two or more of the signs and symptoms described below:

- Body temperature <36°C or >38°C
- Patient heart rate >90/minute
- Respiratory rate >20/minute
- P_aCO_2 32 mmHg
- Leukocyte count <4000 or >12,000/mL

Results

There were 36 burn patients admitted to the RSCM Burn Unit over the 3-month period from September to November 2016. The baseline characteristics of patients included in this study are summarized in *Table I*. The patients' age ranged between 1-71 years old, with a mean age of 34.1 years. Furthermore, the majority of the patients were male (n=23; 63.9%). Most of the patients were admitted to the HCU (n=21; 58.3%) and 15 patients were treated in the ICU (41.7%). The most common cause was gas burns (n=15; 41.7%). In the prior 3-month period, patients at the RSCM burn unit were hospitalized for a duration of 3 to 60 days, with an average of 16.47 days (median=14). The total number of deaths that occurred during hospitalization at the RSCM burn unit was 10 patients (27.8%).

Table I - Patient demographics

Characteristics	Total (n=36)
Age (Range 1-71 years old)	
Mean	34.1
Median	35.5
Gender	
Male	23 (63.9%)
Female	13 (36.1%)
Mortality	10 (27.8%)
Type of burn	
Fire	5 (13.9%)
Chemical	9 (25.0%)
Gas	15 (41.7%)
Scald	4 (11.1%)
Electrical	3 (8.3%)
TBSA (Range 4.5 – 80%)	
Mean	38.7
Median	32.5
Hospitalized in	
ICU	15 (41.7%)
HCU	21 (58.3%)
Duration of hospitalization (Range 3-60 days)	
Mean	16.47
Median	14

Table II - Types of bacteria isolated at the RSCM Burn Unit, September – November 2016

Type of Organism	Number of isolates (n=89)	Blood	Wound swab	Sputum	Tissue	Urine
No growth	30 (34%)	27	2	0	1	0
Gram Negative	47 (52.8%)	3	19	15	9	1
Klebsiella pneumoniae	15 (17%)	3	2	7	3	0
Pseudomonas aeruginosa	11 (12%)	0	7	1	3	0
Acinetobacter baumannii	10 (11%)	0	5	3	2	0
Enterobacter aerogenes	6 (7%)	0	3	2	1	0
Enterobacter cloacae	2 (2%)	0	0	1	0	1
Proteus mirabilis	2 (2%)	0	2	0	0	0
Escherichia coli	1 (1%)	0	0	1	0	0
Gram Positive	12 (13.48%)	1	6	1	2	2
Enterococcus faecalis	5 (6%)	0	1	0	2	2
Staphylococcus epidermidis	3 (3%)	0	3	0	0	0
Bacillus sp.	2 (2%)	1	1	0	0	0
Staphylococcus saprophyticus (MRSS)	2 (2%)	0	1	1	0	0
Total		31	27	16	12	3

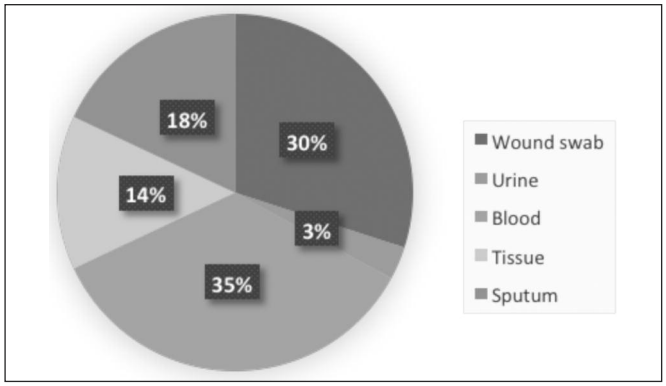

Fig. 1 - Spectrum of specimen cultures in the RSCM Burn Unit, September - November 2016.

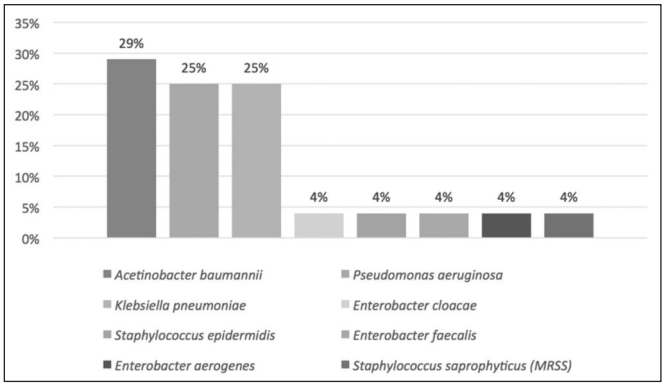

Fig. 3 - Bacterial isolates pattern in the RSCM Burn Unit, October 2016.

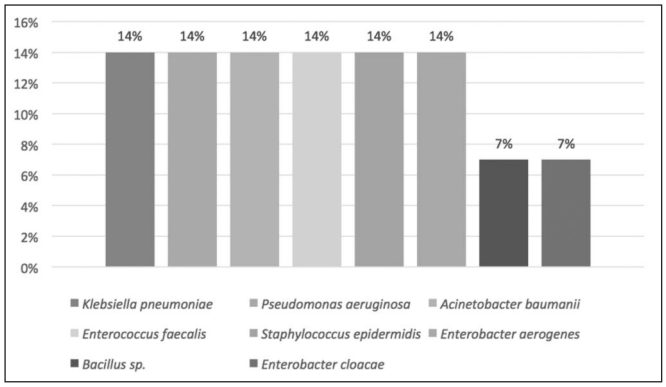

Fig. 2 - Bacterial isolates pattern in the RSCM Burn Unit, September 2016.

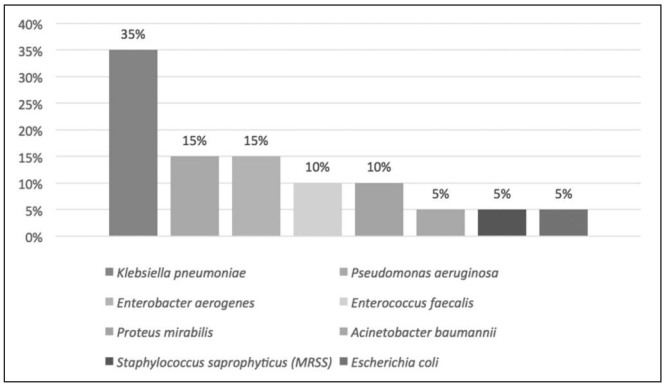

Fig. 4 - Bacterial isolates pattern in the RSCM Burn Unit, November 2016.

Bacterial isolates

The spectrum of specimens collected from the burn patients from September to November 2016 is described in *Fig. 1*. As shown, most of the samples were obtained from the blood (35%) and wound swabs (30%).

Various types of bacteria were isolated as shown in *Table II*. Out of the 31 blood cultures performed, only 4 samples were positive for bacterial growth – *Klebsiella pneumoniae* and *Bacillus spp.* The majority of the bacteria isolated from the burn unit were obtained from the wound swab (n=26) and sputum (n=16) samples. Among all of the specimens obtained from burn patients in RSCM (September – November 2016), the majority of the isolated bacteria were gram-negative bacteria (52.8%), whereas only a limited number of gram-positive bacteria were found (13.48%). There were three types of gram-negative bacteria that dominated the burn unit – *Klebsiella pneumoniae* (n=15; 17%), *Pseudomonas aeruginosa* (n=11; 12%) and *Acinetobacter baumannii* (n=10; 11%). On the other hand, the most commonly found gram-positive bacteria was *Enterococcus faecalis* (n=5; 6%).

On a month-to-month basis (September–November), the pattern of bacterial colonization at the RSCM burn unit changed constantly, as shown in *Figs. 2, 3 and 4*. Six major bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa,*

Table III - Patterns of antibiotic resistance among common organisms at the RSCM Burn Unit

Antibiotics (n=9)	Organisms		
	Klebsiella pneumoniae (n=15)	*Pseudomonas aeruginosa* (n=11)	*Acinetobacter baumannii* (n=10)
Cephalosporin			
Ceftriaxone	10 (67%)	10 (91%)	9 (90%)
Cefoperazone / Sulbactam	5 (33%)	8 (73%)	2 (20%)
Carbapenem			
Doripenem	8 (53%)	7 (64%)	7 (70%)
Meropenem	3 (20%)	8 (73%)	7 (70%)
Imipenem	2 (13%)	8 (73%)	7 (70%)
Aminoglycosides			
Gentamicin	11 (73%)	8 (73%)	8 (80%)
Amikacin	4 (27%)	8 (73%)	7 (70%)
Tetracycline			
Tetracycline	9 (60%)	9 (82%)	9 (90%)
Glycylcycline			
Tigecycline	2 (13%)	10 (91%)	5 (50%)

Table IV - Pattern of antibiotic sensitivity among common organisms at the RSCM Burn Unit

Antibiotics (n=9)	Organisms			
	Klebsiella pneumoniae	*Pseudomonas aeruginosa* (n=15)	*Acinetobacter baumannii* (n=11)	(n=10)
Carbapenem				
Imipenem	12 (80%)	3 (27%)	2 (20%)	
Meropenem	11 (73%)	3 (27%)	2 (20%)	
Doripenem	10 (67%)	3 (27%)	2 (20%)	
Aminoglycosides				
Amikacin	9 (60%)	3 (27%)	2 (20%)	
Gentamicin	2 (13%)	3 (27%)	1 (10%)	
Glycylcycline uan				
Tigecycline	1 (7%)	0	1 (10%)	
Cephalosporin				
Cefoperazone/ Sulbactam	3 (20%)	2 (18%)	3 (30%)	
Ceftriaxone	0	1 (9%)	0	
Tetracycline				
Tetracyclin	0	0	0	
Colistin				
Polymixin B	0	4 (36%)	0	

Acinetobacter baumannii, Enterococcus faecalis, *Staphylococcus epidermidis* and *Enterobacter aerogenes)* were found at relatively similar levels, approximately 14%, at the burn unit in September. However, starting from October, *Acinetobacter baumannii* (29%) were the most commonly found isolates among burn patients in the RSCM burn unit. This was followed by *Klebsiella pneumonia* (25%) and *Pseudomonas aeruginosa* (25%) as the second most common isolates in burn patients. Simultaneously, the incidence of other isolates (*Enterobacter cloacae, Staphylococcus epidermidis, Enterobacter faecalis, Enterobacter aerogenes, Staphylococcus saprophyticus)* decreased, with only 4% positive bacterial colonization incidence. Nevertheless, in November the occurrence of *Klebsiella pneumoniae* (35%) colonization increased by 10%, becoming major bacterial isolates in RSCM, followed by *Pseudomonas aeruginosa* (15%). In contrast, the colonization of *Enterobacter aerogenes* increased from 11% to 15% compared to the previous month. To conclude, our study found that the most commonly isolated bacteria at the RSCM burn unit alternated on a monthly basis between *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Antimicrobial susceptibility pattern

In this study, 12 different antibiotic spectrums (cephalosporin, penicillin + beta lactamase inhibitor, monobactam, carbapenem, penicillin, sulfonamide, chloramphenicol, aminoglycosides, tetracycline, glycylcycline, quinolones, macrolide) were tested against 11 different organisms isolated at the RSCM burn unit. Most of the bacteria isolated, except for *Bacillus sp,* were resistant to all 12 antibiotic spectrums tested. In general, most of the gram-negative bacteria were resistant to cephalosporin, tetracycline and quinolone antibiotics. On the other hand, gram-positive bacteria were mostly resistant to quinolones and penicillin. Nevertheless, both gram-negative and gram-positive bacteria at the RSCM burn unit were still sensitive to carbapenem antibiotics (imipenem, meropenem, doripenem), though *Enterococcus faecalis* was the only gram-positive bacteria still sensitive to carbapenems. In addition, the

Table V - Bacteria etiology of sepsis in burn patients (September – November 2016)

Organism	No. of Isolates* (n=45)	Wound swab	Tissue	Sputum	Blood
Pseudomonas aeruginosa	15 (33.3%)	7	6	2	0
Klebsiella pneumoniae	13 (28.9%)	2	4	5	2
Acinetobacter baumannii	5 (11.1%)	2	1	2	0
Enterobacter aerogenes	3 (6.7%)	1	1	1	0
Enterobacter cloacae	3 (6.7%)	0	0	3	0
Staphylococcus saprophyticus	2 (4.4%)	1	0	0	1
Proteus mirabilis	2 (4.4%)	1	0	1	0
Enterococcus faecalis	1 (2.2%)	0	1	0	0
Staphylococcus aureus	1 (2.2%)	0	1	0	0
Total	45	14	14	14	3*
Total of patients diagnosed with sepsis = 14 patients (n=36; 38.9%), with 10 patients (76.9%) demised due to sepsis (all hospitalized in the ICU)					

gram-negative bacteria isolates were highly sensitive to amikacin and gentamycin from the aminoglycoside antimicrobial spectrum. However, none of the gram-positive bacteria were sensitive to this antibiotic spectrum. Gram-positive bacteria were found to be highly sensitive to glycopeptide antibiotics (vancomycin and teicoplanin), except for *Bacillus spp*.

In *Table III* and *Table IV*, the pattern of antibiotic resistance and sensitivity of key antibiotics among the three major organisms (*Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter baumannii*) at the RSCM burn unit are illustrated.

From *Table III*, there is a clear pattern of antimicrobial resistance identified among these 3 major bacteria. *Pseudomonas aeruginosa* were shown to be highly resistant to all antimicrobial spectrums tested (cephalosporin, carbapenem, aminoglycosides, tetracycline, and glycylcyline). Moreover, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* were shown to be highly resistant to ceftriaxone (67%; 91%; 90%) and tetracycline (60%; 82%; 90%). Nearly all *Pseudomonas aeruginosa* isolates were resistant to tigecycline (91%). Among these, *Klebsiella pneumonia* was least resistant to tigecycline (13%) and imipenem (13%). On the other hand, cefoperazone/sulbactam (20%) was still effective against *Acinetobacter baumannii.*

Table IV shows that the 3 major bacteria isolated at the RSCM burn unit (*Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii*) were sensitive to 5 antibiotics including imipenem (80%; 27%; 20%), meropenem (73%; 27%; 20%), doripenem (67%; 27%; 20%), amikacin (60%; 27%; 20%) and tigecycline (13%; 27%; 10%). In addition, it shows that the 3 major bacteria isolated at the RSCM burn unit were highly sensitive to several antibiotics. For instance, most of the *Klebsiella pneumoniae* isolates were sensitive to imipenem, approximately 80% (n=12). Contrastingly, *Pseudomonas aeruginosa* was more sensitive to polymixin B from the collistin antibiotic class (n=4; 36%). Lastly, *Acinetobacter baumannii* bacteria were most sensitive towards cefoperazone/sulbactam (n=3; 30%).

Sepsis

Table V describes the 14 patients admitted to the RSCM Burn Unit between September and November 2016 that were diagnosed with sepsis (38.9%). Among the 14 patients, 10 (76.9%) died at the RSCM burn unit due to sepsis. All of the patients were being treated in the ICU. Out of the 45 culture samples taken from the 14 patients, two major gram-negative bacteria were identified as the cause of sepsis at the RSCM burn unit including *Pseudomonas aeruginosa* (n=15; 33.3%) and *Klebsiella pneumonia* (n=13; 28.9%). Other organisms that caused sepsis were *Acinetobacter baumannii* and *Enterobacter aerogenes*, found in 5 (11.1%) and 3 (6.7%) septic patients, respectively.

Discussion

Infection is a major problem that commonly occurs in burn patients.[2] The higher susceptibility to infection among burn patients is usually caused by an impaired immune system as destruction of the skin, which serves as a barrier to infection, occurs; there is also a high level of systemic inflammatory response (SIRS).[3] One major principal in the management of burn infections is the appropriate use and choice of antimicrobial therapy.[14] Unfortunately, over the last 10 years the pattern of antimicrobial sensitivity and bacterial infection profile in many burn units has changed significantly. Most of the burn units in developing countries including the Middle East, Africa and Asia have reported an increase in the occurrence of multidrug resistant bacteria such as *Pseudomonas, Acinetobacter* and *Enterobacter*, partly due to the ineffective use of antimicrobials.[15,16] This has led to an uncontrollable increase in sepsis complications and the incidence of multi-organ failure among burn patients in many burn centers.[17,18] Therefore, constant monitoring of bacterial cultures and antimicrobial susceptibility patterns are required to provide a guide for the burn centres to select the most appropriate empirical antimicrobial therapy, and to prevent the emergence of antimicrobial resistance in the burn unit.

In the present study, among the 88 samples (n=36 patients), there were three predominant gram-negative bacterial isolates (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii*) in the RSCM burn unit (September - November 2016). *Klebsiella pneumoniae* (17%) was the most commonly isolated pathogen at the RSCM burn unit (September-November 2016). This 3-month bacterial colonization pattern is similar to a previous report regarding the bacterial profile at the RSCM burn unit.[19-22] A study conducted by the RSCM Division of Infectious Disease from 2013 to 2016 reported that *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii* were the three major bacteria isolated, the incidence of each changing from one month to the next. *Pseudomonas aeruginosa* was the most common pathogen found at the RSCM Burn Unit in 2016 (January to

August), 2015 (January to December) and 2014 (June to December).[19-21] On the other hand, in 2014 (January to June) and 2013 (January to December), *Acinetobacter baumannii* was the most common isolated pathogen at the RSCM burn unit.[21,22] Based on these reports and the current study, it can be concluded that the pattern of bacterial profile at the RSCM burn unit alternates between the 3 most common pathogens – *Acinetobacter baumannii,* shifting to *Pseudomonas aeruginosa* after some time, then changing into *Klebsiella pneumoniae*.

According to several previous studies, *Pseudomonas aeruginosa* is the major etiologic agent of burn infections. The main source of *Pseudomonas aeruginosa* is commonly found in the sink.[23] The presence of this bacteria in the sink is attributed to the existence of nutrients in the plumbing system and moist environment, particularly in the drains, which allows colonization of this bacteria.[24] *Pseudomonas aeruginosa* is considered to be the most significant cause of infection among burn patients as it is able to grow on moist surfaces, especially burn wounds, which represent the ideal environment for infection and colonization of this bacterium.[25] Moreover, *Pseudomonas aeruginosa* is known to be highly pathogenic among immune-compromised patients, a condition that is common in burn patients.[25] This study has shown a similar result with *Pseudomonas aeruginosa* being the second most common pathogen (12%) isolated at the RSCM burn unit. In addition, this study shows that most of the bacteria are isolated from the burn wound with 7 out of 11 samples taken from wound swab, the most ideal environment for colonization.

Klebsiella pneumoniae was found to be the predominant pathogen of bacterial colonization among burn patients in RSCM (17%), particularly in November 2016 (35%). Previous research has reported that *Klebsiella pneumonia* is becoming one of the top five causative organisms of hospital-acquired infections (HAI) among burn patients.[26] One of the factors that contribute to the increase in *Klebsiella pneumoniae* prevalence is the lack of new antimicrobial agents that are able to actively combat this gram-negative microorganism. According to one study, the outbreak of *Klebsiella pneumoniae* infection among burn patients is also associated with the body area burned, particularly the head/neck and back, which necessitates mechanical ventilation (facilitating pulmonary colonization); it is also associated with higher TBSA and full-thickness burns.[26] This is similar to our study in which most of the patients colonized with *Klebsiella pneumoniae* were being treated in the ICU, and they were on mechanical ventilation. In addition, most of these bacteria were isolated from sputum samples (n=7). However, there were several patients in our burn unit being treated in the HCU who were also colonized with *Klebsiella pneumoniae*. This may be attributed to the high TBSA (>20% TBSA) that occurs in the burn patients being treated in our unit. A high TBSA is associated with an increased risk factor of *Klebsiella pneumoniae* colonization (mean 38.7%; median 32.5%). Nevertheless, further studies are required to re-assess the significance of mechanical ventilation, higher TBSA, and full-thickness burns as factors that cause an increased risk of *Klebsiella pneumoniae* colonization in our burn unit.

Based on our results, gram-negative colonization is predominant in the RSCM burn unit, particularly *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Over the last decade, this pattern of bacterial colonization has become more prominent in the Middle-East and South-East Asia, particularly countries with a tropical climate.[27] In a recent study by Bahemia et al.[28] in South Africa, gram-negative bacteria were one of the three most commonly isolated organisms from blood and central venous catheter cultures in the adult burn units, including *Acinetobacter baumannii* (n=178)*, Pseudomonas aeruginosa* (n=98) and *Klebsiella pneumoniae* (n=100).[28] Similar findings were reported from a burn unit in Pakistan where the most common isolates were *Pseudomonas aeruginosa* (35.29%), *Klebsiella pneumoniae* (20.58%) and *Acinetobacter baumannii* (6.86%), obtained from the culture of tissue specimens.[29] Other burn centres in Australia, Iran, Singapore and Turkey have also documented similar results with *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* being the most common pathogens responsible for burn wound infections.[30-34]

Gram-negative pathogens (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia Coli, Enterobacter spp*. and *Proteus spp*.) have been identified as the main cause of morbidity and mortality among burn patients across many burn centres.[35] Based on previous studies, one of the risk factors that increase the incidence of gram-negative bacterial colonizations among burn patients is prolonged hospital stay.[34] Many studies have claimed that gram-positive bacteria are usually more prominent during the first week of hospitalization in the burn centre. In contrast, gram-negative bacteria commonly presents after a longer period of hospitalization, usually at least more than one week after admission.[27,34,36] This is similar with the findings of our study which showed that 52.27% of the colonizations were caused by gram-negative bacteria, whereas out of the 16 patients, only 13.64% had gram-positive bacterial isolates. This finding may be attributed to the condition that most of the patients selected for this study were hospitalized in our RSCM burn unit for more than 1 week (mean 16.47 days; median 14 days).

In addition, studies have shown that gram-negative infection in burn patients are considered as HAI.[37] According to the CDC (Centres of Disease Control and Prevention), most hospitals worldwide have a higher prevalence of HAI, including ventilator associated pneumonia, urinary tract infections, and blood stream infections, which are caused by gram-negative bacteria.[38] *Klebsiella pneumoniae, Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacter spp.* are the major gram-negative pathogens responsible for HAI in hospitals all around the world.[39] Studies have claimed that burn patients are more susceptible to nosocomial infections in the hospital due to the typical feature of losing their first line of defence against invading bacteria, the presence of avascularized tissue which is favourable for microbial growth, and alterations in different components of their immune system.[40] According to a report on the bacterial profile of infections detected in RSCM in 2016, the majority of the bacteria that cause nosocomial infections in RSCM were *Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii* and *Pseudomonas aeruginosa.*[19] This is similar with our findings which show that the three major gram-negative bacteria commonly isolated in RSCM burn unit are *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*, similar to the bacteria causing hospital acquired infections in other divisions of RSCM. Therefore, it could be inferred that most of the patients at the RSCM burn unit are infected by HAI causing bacteria.

Other than the bacterial profile of burn infections in this unit, this study has also identified the pattern of antimicrobial

resistance and sensitivity to the drugs commonly used in this institution. Based on the results, almost all of the microorganisms in RSCM burn unit, including the gram-negative and gram-positive bacteria, are multi-drug resistant. The three major pathogens (*Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii*) found at the RSCM burn unit are resistant to 10 antibiotic spectrums (cephalosporin, penicillin + beta lactamase inhibitors, monobactam, carbapenem, sulfonamide, chloramphenicol, aminoglycosides, tetracycline, glycylcyline and quinolones) with a higher resistance rate found in *Pseudomonas aeruginosa*. Moreover, these bacteria are only sensitive to a limited number of antibiotics such as carbapenem (imipenem, meropenem, and doripenem), aminoglycosides (amikacin and gentamicin), and cephalosphorin (cefoperazone/ sulbactam). According to Magiorakos et al., these micro-organisms can be classified as multi-drug resistant (MDR) bacteria, considering that all of these bacteria are not susceptible to at least one antimicrobial agent among three or more antimicrobial spectrums or class.[41]

Based on previous reports, there has been an increasing prevalence of micro-organisms that are multi-drug resistant among burn patients over the past few years. One of the reasons for this condition is the high susceptibility of burn patients to nosocomial infections, and the increased use of broad-spectrum antibiotics.[31] One study has reported that *Pseudomonas aeruginosa* may easily become resistant to antibiotics due to the low permeability of their outer protein membrane, the overexpression of efflux pumps, and failure of antimicrobial therapy due to the existence of antibiotic modifying enzymes.[42] This is reflected in our results as the highest resistance rates were found to be in *Pseudomonas aeruginosa,* with nearly 100% of the isolates resistant to 10 antibiotics spectrum. This is followed by *Acinetobacter baumannii,* with around 70% to 90% of the isolates resistant to 10 antimicrobial classes. This finding is similar to previous studies that report the high resistance rate of *Acinetobacter baumannii* among their burn patients, primarily attributed to prolonged hospitalization.[6,28,43] One study by Park et al. showed that multi-drug resistant bacteria commonly occur due to the prolonged use of cephalosporin antibiotics among patients, particularly their use as prophylaxis treatment.[44] Similarly, *Klebsiella pneumoniae,* which has just become one of the major infection-causing bacteria in our burn unit, is found to have high resistance, with approximately 50% of the isolates showing resistance. Plasmid mediated resistance due to the usage of broad spectrum antibiotics has been considered as one of the factors associated with this phenomenon.[45] Cross-resistance or cross-contamination, which allows the transmission of resistant plasmids among all bacterial isolates, is also correlated with the increasing incidence of resistance among these three major microorganisms in our setting.[31] However, further studies are required to investigate the molecular epidemiological tests on micro-organism resistance to antimicrobials in our burn unit.

In this study, the high rate of resistance to cephalosporins is found in all of the bacteria isolated from our setting. This high resistance may contribute to the high mortality and morbidity rate among our burn patients, considering that the empirical therapy used in our setting is mainly cephalosporin antibiotics, particularly ceftriaxone. This is reflected by the outbreak of mortality and sepsis among the patients admitted to our burn unit from September to November 2016. Fourteen out of the 36 patients admitted to this burn unit were diagnosed with sepsis (38.9%) and 10 of them died (76.9%). Two major bacteria considered to be the etiologic agent of sepsis in these patients are *Pseudomonas aeruginosa* (33.3%) and *Klebsiella pneumoniae* (28.9%). Furthermore, most of these patients were being treated in the ICU. This is similar to previous studies that report that *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* infections are significantly associated with increased mortality among burn patients.[46] Many studies have demonstrated that bacterial pneumonia is one of the most common causes of sepsis and death among burn infections, commonly caused by *Klebsiella pneumoniae* or *Pseudomonas aeruginosa.*[46]

Considering the high mortality rate in our setting, it may be deduced that the ineffective therapy of burn patients is a result of high resistance to the empirical antibiotics being used. Therefore, our study has concluded that the RSCM burn unit needs to change the empirical therapy to carbapenem antibiotics, instead of the current regimen, as the bacterial isolates still demonstrate a high sensitivity to this group of antibiotics. However, a monotherapy approach is not recommended for the treatment of burn infections due to its ineffectiveness in targeting all of the bacteria in burn patients, and its low success rates in increasing survival.[6,47] Therefore, a combination of carbapenem antibiotics with another antibiotic is suggested. According to Tumbarello et al., a combination of carbapenem with tigecycline and polymixin B is significantly correlated with a decreased risk of death among burn patients. This triple regiment of empirical therapy has shown to have a clinical success rate of 73% in their burn setting.[47] On the other hand, the combination of carbapenem with aminoglycosides has demonstrated lower success rates, although still successfully lowering mortality rates.[47] Considering the bacterial isolates in this research are less sensitive to tigecycline, and the use of polymixin B is not registered at the RSCM burn unit, the combination of carbapenem with aminoglycosides may be selected. Amikacin was selected considering the higher sensitivity pattern demonstrated by all microbial isolates towards this drug, compared to other aminoglycosides. In addition, other than combination therapy, this empirical therapy needs to be used only when sepsis has been clinically confirmed or is suspected in the patient, as an effort to prevent the emergence of carbapenem resistance in the future. Prophylactic use of this empirical therapy is not suggested as it will increase the possibility of resistance.[48] Strict infection control in our burn unit also needs to be comprehensively implemented by all health workers, including hand hygiene training and washing procedures, use of barrier (gown and gloves) for contact precautions, and routine environmental cleaning of the burn unit. This needs to be followed by regular surveillance and monitoring of the micro-organism infection profile at the burn unit to find the most appropriate empirical antibiotic regimen for future use.

Limitations

This study has several limitations. First of all, due to the retrospective method of reviewing the laboratory results on the cultures of all patients admitted to our burn unit, it may affect the accuracy of the data collected for this study. However, several measures have been taken to ensure that the data collected is accurate. In addition, in our study limited samples (n=36) were included and this may affect the significance of the results obtained compared to other burn centres. Statistical analysis

was also required to accurately identify the relationship between the bacterial isolates and pattern of antimicrobial resistance and sensitivity, and the incidence of sepsis and death. In this study, further details regarding the time samples collected from each patient was not included, which may be required in future studies to analyze the impact of length of stay on microbial colonization in burn patients. Moreover, the setting of this study was a public hospital in a developing country which means that the infection control protocol is very limited considering this facility is over-crowded and the number of staff is very limited. As a result, the practice of infection control is very challenging. This affects the generalizability of our findings to other hospitals in developed countries which may have a stricter infection control approach. Furthermore, considering this is a tertiary referral hospital, most of the patients admitted to our burn unit have been transferred from other hospitals. Therefore, most of the patients have a high possibility of being infected by pathogens outside our burn unit. Our study also does not control other potential con-founders which could affect the results, considering the retrospective design. Most of the patients hospitalized in our unit have several co-morbidities including diabetes, chronic renal disease, and other diseases that could affect susceptibility of the patients to infection by pathogens. However, this was not considered in our study as the objective of this study was not to associate infection with patient co-morbidities. Therefore, future studies should be conducted prospectively to control all variables that could affect the conclusion of the study.

Conclusion

The types of bacteria commonly isolated at the RSCM burn unit varied continuously every month between *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Acinetobacter Baumannii*. Based on the antimicrobial resistance and sensitivity patterns, all were classified as multi-drug resistant pathogens, highly resistant to the current empirical therapy (ceftriaxone), leading to outbreaks of sepsis and increased mortality rates. A combination of carbapenem (imipenem, meropenem and doripenem) and aminoglycosides (amikacin) is the selected empirical therapy. This empirical therapy needs to be conducted in conjunction with strict infection control measures. However, further monitoring of the organisms and antimicrobial susceptibility patterns needs to be performed to find the most appropriate therapy strategies for the RSCM burn unit in the future.

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