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ORIGINAL ARTICLE

Bacteraemia in Haematopoietic Stem Cell Transplant Recipients in a Single Tertiary Referral Centre

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ABSTRAK

Bakteremia merupakan salah satu komplikasi yang kerap dan serius dalam transplantasi sel stem hematopoietik (HSCT). Sehingga kini, tiada data yang diterbitkan mengenai kerentanan antibiotik dan kesudahan klinikal di kalangan penerima HSCT di Malaysia. Matlamat kajian ini adalah untuk menganalisis kekerapan, kerentanan antibiotik dan kesudahan klinikal akibat bakteremia yang dihidapi oleh penerima HSCT dalam tempoh 100 hari selepas transplantasi. Kami secara retrospektif menganalisa kadar kekerapan, pola kerentanan antibiotik dan kadar kematian dikalangan penerima HSCT di satu pusat perubatan selama tempoh 5 tahun (2013-2017). Tiga puluh daripada 85 penerima HSCT menghidapi bakteremia dengan 40 kultur positif, menghasilkan kadar kekerapan bakteremia sebanyak 47% (40/85). Gram negatif bakteria (GNB) menyumbang kepada 60.5% daripada jumlah pencilan. Enterobacteriaceae dan Coagulase negatif Staphylococcus (CoNS) adalah patogen yang paling kerap ditemui. GNB menunjukkan kadar kerentanan antibiotik sangat tinggi terhadap ciprofloxacin. Antibiotik empirikal pilihan pertama hanya berkesan terhadap 30% penerima yang menghidap demam neutropenia (DN). Kadar kematian disebabkan oleh bakteremia adalah 13.3% (4/30), di mana 50% disebabkan oleh kerentanan pelbagai antibiotik (MDR) Acinetobacter dan 25% extended spectrum beta-lactamase (ESBL) Enterobacteriaceae. Bakteremia merupakan komplikasi awal yang kerap dan mengancam nyawa di kalangan penerima HSCT di mana MDR GNB merupakan penyebab utama kematian. Kadar kerentanan yang tinggi kepada ciprofloxacin dan kegagalan antibiotik empirikal pilihan pertama untuk merawat DN memerlukan penilaian menyeluruh kepada protokol antibiotik pencegahan dan rawatan empirikal yang sedia ada. Penemuan

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ini mempunyai implikasi klinikal yang penting mengenai penggunaan dan pemilihan kedua-dua rejimen antibiotik profilaktik dan empirik untuk merawat DN.

Kata kunci: bakteremia, kadar kematian, kerentanan antibiotik, transplantasi sel stem hematopoietik

ABSTRACT

Bacteraemia is a common and one of the serious complications in haematopoietic stem cell transplantation (HSCT). To date, there are no published data on antibiotic resistance and clinical outcome among HSCT recipients in Malaysia. The aim of the present study was to analyse the prevalence, antibiotic resistance and clinical outcome of bacteraemia in HSCT recipients, within 100 days following transplantation. We retrospectively analysed the prevalence, antibiotic resistance pattern and mortality rate of early bacteraemia among HSCT recipients in a single centre over a 5-year period (2013-2017). Thirty patients of 85 HSCT recipients developed bacteraemia with 40 positive cultures resulting in prevalence of 47% (40/85). Gram negative bacteria (GNB) accounted for 60.5% of total isolates. *Enterobacteriaceae* and Coagulase negative *Staphylococcus (*CoNS) were the commonest pathogens isolated. GNB showed a high resistance rate to ciprofloxacin. Only 30% of recipients responded to first line empirical antibiotics for febrile neutropenia (FN). The mortality rate was 13.3% (4/30), of which 50% was attributed to multi-drug resistance (MDR) *Acinetobacter* and 25% to extended spectrum beta-lactamase (ESBL) *Enterobacteriaceae*. Bacteraemia is a frequent and life-threatening early complication among HSCT recipients with MDR GNB being the commonest cause of mortality. The high rate of resistance to ciprofloxacin and failure of the first line empirical antibiotics to treat FN calls for a thorough evaluation of the current antibiotic prophylaxis and empirical treatment protocols. These findings have important clinical implications regarding the use and selection of both prophylactic and empiric antibiotic regimens to treat FN.

Keywords: antibiotic resistance, bacteraemia, haematopoietic stem cell transplantation, mortality rate

INTRODUCTION

Bacteraemia is a common and one of the serious complications among haematopoietic stem cell transplantation (HSCT) recipients. It has significant transplant related morbidity and mortality worldwide with an estimated incidence of 40-42% (Bock et al. 2013). The most common risk factors for bacteraemia include prolonged neutropenia and profound

impairment of T and B cell functions resulting from transplant conditioning, chemo-radiation and graft-versus-host disease (GvHD) prophylaxis. Disruption of anatomical barriers by mucositis and indwelling catheters also increases the risk of bacteria inoculation into the bloodstream. Other contributing factors towards bacteraemia in HSCT recipients are age >18 years, underlying disease, late stage of underlying disease, presence of other comorbidities, severe GvHD and steroid use (Rafeah & Fadilah 2009). Following transplantation, immune reconstitution may take months to years to recover depending on transplant types (autologous versus allogeneic), source of stem cells (bone marrow versus peripheral blood), conditioning regimen (myeloablative conditioning (MAC) versus reduced intensity conditioning (RIC)), degree of histocompatibility in allogeneic HSCT (matched sibling, haplo-identical or unrelated), GvHD prophylaxis (calcineurin inhibitors, *in vivo* T-cell depletion) and the presence and grade of GvHD and subsequent anti-GvHD therapy.

In HSCT, three distinguished periods can be identified with the predominance of specific pathogens/ bacteria in each phase (Rafeah & Fadilah 2009). The present study focused on bacterial infections during phases I and II only, which period commensurate with in-patient hospitalisations and frequent out-patient day-care visits during the first 100 days.

During pre-engraftment stage (Phase 1; Day 0 to +30), bacterial infections are mainly attributed to neutropenia, mucositis and vascular access. Functional asplenia and depressed cellular/ humoral immunity are also relevant. The principal pathogens are skin and intestinal flora including Gram-positive (e.g. *Staphylococcus*, *Streptococcus, Enterococcus* species and Gram-negative bacteria (e.g. *Bacillus* species), although Gram-negative organisms which are often associated with high mortality rates may also occur late following transplantation (Mitchell et al. 2004).

Following neutrophil recovery and marrow engraftment (Phase II; Day +30 to +100), the risks of infection persisted via vascular access, immunodeficiency and functional asplenia which may be worsened by GvHD and its immunosuppressive therapy. Although this favours viral and fungal infections, risks of Gram-negative and encapsulated organisms persevered (Mitchell et al. 2004). Over the last three decades, several studies have demonstrated a shift in the aetiology of bacteraemia infections from the predominance of Gram-negative rods to Gram-positive cocci (Poutsiaka et al. 2007). Prior to the availability of methicillin, penicillin-resistant *Staphylococcus aureus* was the main threat to neutropenic patients and its mortality rate exceeded 50%. By the end of the 1980s and lately during 1990s, Gram-positive microbes re-emerged (Viscoli et al. 1994). The commonest culprit was coagulase-negative *Staphylococci*, mainly *Staphylococcus epidermidis.*

Compared to the contemporaneous hospital population, HSCT recipients

develop more frequent antibiotics resistance which has important clinical implications on the use and selection of both prophylactic and empiric antibiotic regimens. According to recent Bone Marrow Transplant (BMT) guidelines, fluoroquinolone prophylaxis should be considered for HSCT patients with anticipated neutropenic periods of more than 7 days. Antibacterial prophylaxis is generally started at the time of haematopoietic cell infusion and continued until recovery from neutropenia or initiation of empirical antibacterial therapy for fever.

Despite an improvement in nursing care, supportive measures, use of RIC regimens and prophylactic strategies, there is still a need for improvement in the antibiotic stewardship programme. Common antibiotic prophylaxis e.g. quinolones, broad spectrum B lactams or other broad spectrum antibiotics have been found to reduce the rate of Gram negative infections, including *Pseudomonas aeruginosa* (Mitchell et al. 2004). However, there is a growing concern of antibiotic resistance following use of prophylaxis and empirical antibiotic therapy during neutropenic sepsis. Other than that, a previous study found that empirical antibiotic with piperacillin - tazobactam was independently associated with treatment success at all time points for high risk febrile neutropenia patient with haematological malignancies (Bow et al. 2006)

A dedicated antimicrobial stewardship program for HSCT with multidisciplinary input will be valuable in improving infection outcomes and transplant mortality, de-escalating broad spectrum empirical therapy, reducing side effects and inappropriate antibiotic usage with an overall benefit of reducing treatment costs and resistant strains. Thus, we conducted a retrospective study aiming to analyse the prevalence, antibiotic resistance and clinical outcome of bacteraemia in HSCT recipients within 100 days following transplantation.

MATERIALS AND METHODS

Data Collection

For a total of 5-year period from January 2013-December 2017, all HSCT recipients aged 13 years and above with febrile episodes and culture positive bacteraemia within the first 100 days of transplantation, were retrospectively identified at Pusat Terapi Sel (PTS), Universiti Kebangsaan Malaysia Medical Centre (UKMMC). Data were extracted from HSCT registry, ward census, medical records, clinical charts and computer system databases. All bacteraemia that occurred within the first 100 days of HSCT were evaluated. All information obtained were manually recorded on data worksheets and transferred to SPSS software program for analysis.

Bacteraemia was defined as isolation of bacterial pathogen from at least one blood culture from peripheral source or central source. For Coagulase negative *Staphylococci* (CoNS), *Corynebacterium* and other common skin contaminants, positive results from 2 consecutive blood cultures were required (Bock et al. 2013). Prevalence of bacteraemia

was determined by the total number of bacteraemia or positive cultures (regardless of the number of individual bacteria isolates from a given positive culture) within the first 100 days of HSCT divided by total HSCT performed, between January 2013 and December 2017. Bacteraemia was considered polymicrobial if two or more pathogens isolated in a single blood culture or in separate blood cultures obtained 48 hours apart (Bock et al. 2013). Antibiotic resistance was considered if the pathogens presented with intermediate susceptibility or resistance (Frère et al. 2006). Gram-negative bacteria (GNB) were defined as extended spectrum beta-lactamase (ESBL) producers if resistant to most beta-lactam antibiotics, including penicillins, cephalosporins and the monobactam aztreonam (Munoz-Price et al. 2012). They were considered multidrug-resistant (MDR) if resistant to three or more antibiotic classes; carbapenem; penicillins; cephalosporins; monobactams; aminoglycosides and fluoroquinolones (Dandoy et al. 2017). Febrile neutropenia (FN) was defined as an oral temperature >38.5˚C or two consecutive readings of >38.0˚C for 2 hours and an absolute neutrophil count <0.5 x 10^9/l, or expected to fall below 0.5 x 10^9/l (de Naurois et al. 2010). Antibiotic success rate was defined by the resolution of fever and associated symptoms or signs with no modifications to the initial antibiotic regimen and with no recurrence within 7 days (Jacobsohn & Vogelsang 2007). Mortality rate was determined by the total number of mortality among HSCT recipients with bacteraemia within 100 days of HSCT divided by the total HSCT recipients with bacteraemia over 5-year period x 100 (Gordis 2000; Ortega et al. 2005).

Transplantation Procedures and Management of Infections

Transplantation was performed according to institutional protocols (Abdul Wahid et al. 2014). Cultures were taken from central lines and peripherally upon admission for all HSCT patient. Later, conditioning with MAC (myeloablative conditioning) included either total body irradiation (TBI) and cyclophosphamide or thiotepa based regimen or RIC was administered before transplantation. Furthermore, prophylaxis consisted of oral ciprofloxacin, antiviral and antifungal were commenced prior to stem cell infusion. The day of stem cell infusion was considered as Day 0. Later, blood cultures and cultures of other clinically relevant sites tests were obtained if the patient developed a clinical suspicion of systemic infection. Other than that, an imaging study of the suspected sites of infection were performed to identify the source and sites of infection. In addition, the initial prophylactic antibiotics were discontinued and broad-spectrum antibiotics (piperacillin - tazobactam with or without amikacin/gentamicin) were given empirically. Finally, the antibiotics would be modified according to the clinical response and sensitivity of organisms isolated.

Statistical Analysis

Statistical comparisons were performed using Statistical Software Statistical Product and Services (SPSS) software package using appropriate statistical tests at a significance level of 95%. Demographic and baseline characteristic variables were analysed using descriptive analysis. Mean comparison between variable was analysed using independent T-test. Categorical variables were analysed using Pearson Chi-square or Fisher exact test. A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Prevalence and Timing of Bacteraemia

During the 5-year observation period, 85 patients underwent HSCT at our institution. Amongst the 85 HSCT recipients, 30 developed bacteraemia that resulted in a total of 40 episodes of positive cultures. The prevalence of bacteraemia was 47% (40/85). A total of 20 recipients developed single bacteraemia episode and another 10 recipients had at least 2 episodes of bacteraemia during the study period. The median time to the development of bacteraemia was 6 days and median day of neutrophil engraftment among our HSCT recipients with bacteraemia were 14 days after stem cell infusion.

Patients' Characteristics

Out of 47 male recipients, 18 developed bacteraemia (38.3%). The majority of HSCT procedures were matched sibling transplants (51/85), amongst which 18 recipients developed bacteraemia (35.3%). 67 HSCT used RIC, of which 23 recipients developed bacteraemia (34.3%). The remaining HSCT procedures used MAC with seven of its recipients developed bacteraemia (38.9%). The commonest diagnosis amongst HSCT recipients with bacteraemia was lymphoproliferative disorder (12/33, 36.4%) followed by acute leukaemias (11/37, 29.7%) (Table 1).

Factors Associated with Bacteraemia

There was no significant association between development of bacteraemia with the age of recipients, source of stem cells (autologous or matched sibling HSCT), the intensity of conditioning regimens and underlying diagnosis (Table 1). Similarly, there was no significant relationship between developments of bacteraemia with day of engraftment.

Frequency of Bacteraemia Over the 5-year Period

Throughout the 5-year period, the highest number of bacteraemia recorded was in 2013 (8/16, 50%) and the lowest was recorded in 2016 (5/19, 26.3%) (Figure 1). A 15.4% increment of bacteraemia episodes occurred in 2017 (5/12, 41.7%) from 2016 (p= 0.142). There was no significant difference in the frequency of bacteraemia episodes between the transplanted years ($p=0.59$). Similarly, there was no significant difference between recipients with bacteraemia

Table 1: Characteristics of HSCT recipients and risk factors association for development of BBSI (N= 85)

	Recipients with BBSI, N (%)	Recipients without BBSI, N (%)	p value
All (N=85)	30 (35.3)	55 (64.7)	
Gender			0.52
Male	18 (38.3)	29 (61.7)	
Female	12 (33.3)	26 (66.7)	
Recipient age			0.84*
Mean ± SD	33.8 ± 14.48	33.3 ± 13.07	
Source of stem cells			0.07 Ω
Matched sibling	18 (35.3)	33 (64.7)	
Autologous	9 (29.1)	22 (70.9)	
Haploidentical	3 (100.0)	0 (0.0)	
Conditioning regimen			0.72
Reduced Intensity	23 (34.3)	44 (65.7)	
Myeloablative	7 (38.9)	11 (61.1)	
Underlying diagnosis			0.63
Acute leukaemias	11 (29.7)	26 (70.3)	
Lymphoproliferative disorder	12 (36.4)	21 (63.6)	
Myeloproliferative disorder	3 (37.5)	4 (57.1)	
Bone marrow failure syndromes	4 (57.1)	3 (42.9)	
HLH	0 (0)	1 (100.0)	
Days to ANC engraftment			
Mean ± SD	14.90 ± 3.29	14.9 ± 32.50	0.98*

Univariate analysis performed by chi square test
*Analysis performed by independent T-test
ΩAnalysis performed by fisher exact test

and no bacteraemia in each particular year (p>0.05).

Aetiology of Bacteraemia

Among all of 40 bacteraemia episodes, a total of 43 bacterial pathogens were isolated over the 5-year period. GNB was the commonest pathogen identified with 26 isolates (60.5%). Interestingly, there was a noticeable change of pattern of bacteraemia during the 5-year period. As shown in Figure 2, GPB isolates were identified 50% in 2013 and decreased by almost half in 2014 before disappeared completely in 2015. They were detected again in 2016 and 2017. The number of GNB increased significantly during the first 3 years and remained between 42.9% to 50% in 2016 and 2017. In addition, there were four polymicrobial bacteraemia episodes with a total of nine pathogens, of which 5 were GNB and 4 were GPB. The majority of polymicrobial bacteraemia involved

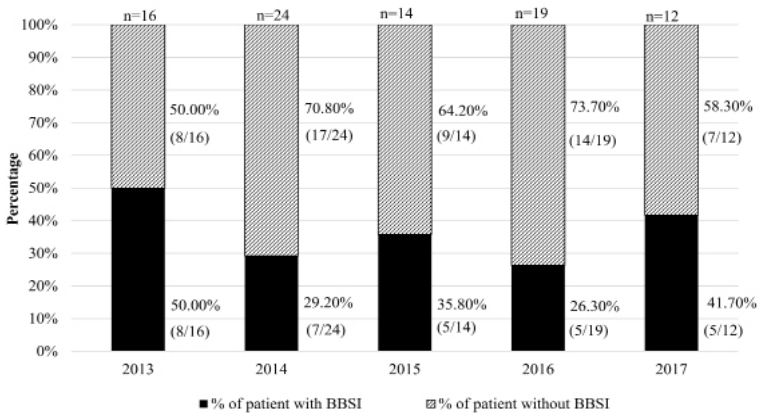

Figure 1: Frequency and percentage of BBSI of each corresponding year (N=85)

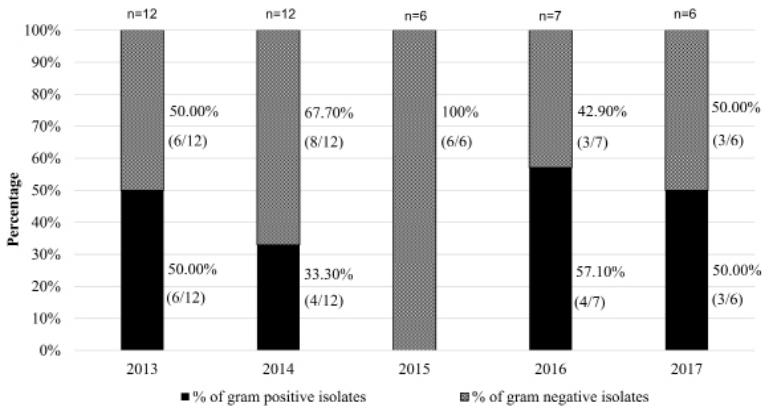

Figure 2: Frequency and percentage of GNB and GPB (N = 43) of each corresponding year.

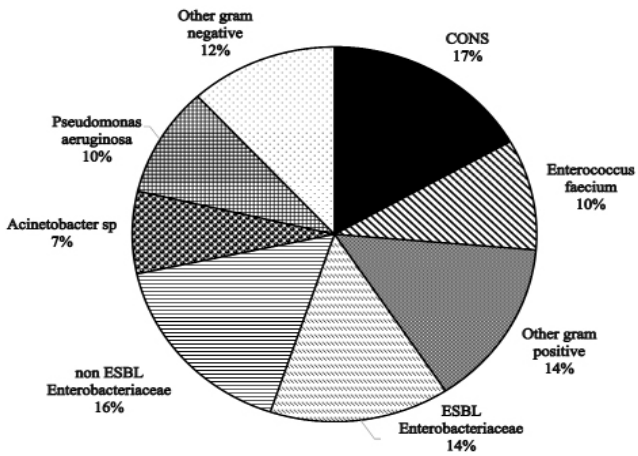

Figure 3: Pie chart showing types of pathogen isolated (N=43)

Table 2: Frequency of different types of pathogen from 2013 – 2017

Organisms	Number of isolates, (%)					
	2013	2014	2015	2016	2017	Total (N = 43)
Gram positive (total)	**6**	**4**	**0**	**4**	**3**	**17 (39.5)**
CoNS	2	3	0	1	1	7 (16.3)
Enterococcus faecium	1	0	0	2	1	4 (9.3)
Others (e.g Streptococcus viridans, Bacillus sp, Diptheroids sp)	3	1	0	1	1	6 (14.0)
Gram negative (total)	**6**	**8**	**6**	**3**	**3**	**26 (60.5)**
ESBL *Enterobacteriaceae*	2	1	3	0	0	6 (14.0)
Non-ESBL *Enterobacteriaceae*	0	3	1	2	1	7 (16.3)
Acinetobacter sp	1	0	1	1	0	3 (6.9)
Pseudomonas aeruginosa	1	1	1	0	1	4 (9.2)
Others (*Flavobacterium sp, Sternotrophomonas maltophilia etc*)	2	3	0	0	1	6 (14.0)

were CoNS*, Enterococcus faecium, Escherichia coli* and *Pseudomonas aeruginosa*.

Types of Pathogen Isolated

Among all of 43 isolates, the most frequently detected were CoNS (7/43) and non-ESBL *Enterobacteriaceae* (7/43), followed by ESBL *Enterobacteriaceae* (6/43). No *Methicillin resistant Staphylococcus aureus* (MRSA) were isolated during the 5-year study period. There were 7% *Acinetobacter* sp. identified from the total isolates (Figure 3). Frequency of each isolates was low with one to three isolates were detected annually (Table 2).

Antibiotic Resistance

Table 3: An antibiogram of GPB isolates and the pattern of antibiotic resistance

Isolated pathogen (Gram positive)	Total isolates	Number of isolates (% resistance)							
		Penilicillins			amino-glycosides	quinolone	cephalo-sporin	Others	
		PEN	CLOXA	AMP	GEN	CIP	CRO	VANC	ERY
CoNS	7	4 (57)	4 (57)	NT	4 (57)	4 (57)	NT	NT	2 (29)
Streptococci viridans	1	0 (0)	NT	NT	NT	NT	0 (0)	NT	1 (100)
Enterococcus faecium	4	3 (75)	NT	3 (75)	1 (25)	NT	NT	0 (0)	NT
Bacillus sp	4	3 (75)	NT	NT	NT	NT	NT	0 (0)	NT

NT = not tested; PEN = Penicillin; CLOXA = Cloxacillin; AMP = Ampicillin; CIP = Ciprofloxacin; CRO=Ceftriaxone; GEN=Gentamicin; VANC = Vancomycin; ERY= Erythromycin

Table 4: An antibiogram of GNB isolates and the pattern of antibiotic resistance

Isolated pathogen (Gram positive)	Total isolates	Number of isolates (% resistance)												
		Beta Lactams				amino-glycosides		quinolone		cephalosporin			Others	
		AMP	IMI	MERO	TZP	GEN	AMI	CIP	FEP	CXM	CAZ	CTX	POLY B	SCF	AUG
ESBL Enterobacteriaceae	6	6 (100)	0(0)	0(0)	1 (17)	NT	1 (17)	5 (83)	5 (83)	5 (83)	NT	5 (83)	NT	NT	3 (50)
Non-ESBL Enterobacteriaceae	7	5(71)	0(0)	0(0)	0 (0)	NT	0 (0)	4 (57)	0 (0)	4 (57)	NT	1 (14)	NT	NT	3 (43)
Acinetobacter sp	3	NT	2 (67)	2 (67)	2 (67)	1 (33)	1 (33)	2 (67)	NT	NT	2 (67)	NT	0 (0)	2 (67)	NT
Pseudomonas aeruginosa	4	NT	2 (50)	2 (50)	1 (25)	1 (25)	1 (25)	1 (25)	2 (50)	NT	2 (50)	NT	0 (0)	NT	NT

NT = not tested; AMP: Ampicillin; IMI: Imipenem; MERO: Meropenem; TZP: Piperacillin-Tazobactam; GEN: Gentamicin; AMI: Amikacin; FEP: Cefepime; CXM: Cefuroxime; CAZ: Ceftazidime; CTX: Cefotaxime; Poly B: Polymicin B; SCF: Sulperazone

For GPB, 4/7 isolates with CoNS were resistant to penicillin, cloxacillin, gentamicin and ciprofloxacin. All *Enterococcus faecium* and *Bacillus* sp. isolates were fully sensitive to vancomycin. However, 3 of 4 *Enterococcus faecium* isolates were resistance to penicillin and ampicillin (Table 3).

For GNB, all ESBL *Enterobacteriaceae* isolates demonstrated 100% resistance against Ampicillin and 70% sensitivity to piperacillin-tazobactam, 80% sensitivity to amikacin and 100% sensitivity to carbapenem. Furthermore, Non ESBL *Enterobacteriaceae* were 100% sensitive to piperacillin-tazobactam and amikacin (Table 4). *Acinetobacter sp* were fully sensitive to polymyxin B.

Overall, 16 out of 24 isolates (GPB and GNB) that were tested for ciprofloxacin were noted to be resistant (66.7%). There were a total of nine (20.9%) MDR sp. out of 43 isolates recorded in the study (5 out of 6 ESBL *Enterobacteriaceae*, 2 out of 3 *Acinetobacter sp*, 1 out of 4 *Pseudomonas aeruginosa* and 1 *Sternotrophomonas maltophilia*).

Clinical Outcome

i) Antibiotic success rate

The standard first line empirical antibiotics for FN was piperacillin - tazobactam with or without amikacin. The antibiotic success rate was significantly lower in patients with bacteraemia compared to those without bacteraemia ($p = 0.01$). Only 9 patients with bacteraemia (30%) responded to

Table 5: Antibiotic success rate among HSCT recipients with BBSI (N=30)

Success to 1st line antibiotics	Recipients with BBSI (%)	Recipients without BBSI (%)	p value
			0.01
Yes	9 (30.0)	32 (58.1)	
No	21 (70.0)	23 (41.9)	
Total	30 (100.0)	55 (100.0)	

Analysis performed by chi square test

this first line antibiotic regime without antibiotic modification throughout the FN episode. The remaining 21 patients (70%) required a change to second line antibiotic regimen which included carbapenems, vancomycin, cefepime or sulperazone (Table 5). Among all recipients with bacteraemia that were treated and tested with tazocin and amikacin, 4 patients (4/20, 20%) had isolates that showed resistance to (Piperacillin/Tazobactam) and 3 patients to amikacin (3/20, 15%).

ii) Mortality rate

Over the 5-year study period, the 100-day mortality rate among all HSCT recipients was 13.3% (4 deaths out of 30 bacteraemia patients). Early mortality rates due to bacteraemia at D0 until D+14 and at D+15 until D+30 were similar (1/4 recipients, 25%). Late mortality rate at D+31 until D+100 was 2/4 (50%). Two mortality occurred in 2015 and one each in 2016 and 2017. *Acinetobacter* MDR were associated with the early (D0 until D+14) and late mortality (D+31-100) while *Enterococcus faecium,* ESBL *enterobacteriaceae* and *Sternotrophomonas maltophilia* were associated with D+15 until D+100 mortality periods, respectively.

DISCUSSION

The present study reported a total of 47% bacteraemia occurring among the HSCT recipients, particularly during the pre-engraftment phase. The median time to the development of bacteraemia was 6 days and median day of engraftment among our HSCT recipients with bacteraemia were 14 days following stem cell infusion.

Our observation concurred with findings from other HSCT centres reporting that bacteraemia occurs in approximately 13-60% of the HSCT recipients (Engelhard et al. 1996; Liu et al. 2011; Ferreira 2018: Ustun 2018; Mikulska 2018; Mikulska et al. 2009; Wang et al. 2015; Weisser et al. 2017; Williamson et al. 1999), particularly during the pre-engraftment phase (Almyroudis et al. 2005; Ustun 2018). This is mainly due to neutropenia with a breakdown of mucosal barriers and presence of central venous catheter during the pre-engraftment phase (Sahin et al. 2016; Tomblyn et al. 2009). We observed that clinical risk factors such as the source of stem cells were not associated with the development of bacteraemia post HSCT. In contrast to other studies, we did not find matched sibling transplantation to be associated with a higher risk of

bacteraemia compared to autologous transplantation (Frère et al. 2006; Gudiol et al. 2014; Mitchell et al. 2004). This may be explained by the small sample size between groups and the similar days of engraftment between patients receiving allogenic or autologous HSCT. We also did not observe any significant association between the day of engraftment and development of bacteraemia. The use of granulocyte colony stimulating factor (G-CSF) especially in our matched siblings HSCT recipients, may have contributed to the lower rate of febrile neutropenia episodes by shortening the duration of neutropenia (Kelly & Wheatley 2009; Mossad et al. 1996; To et al. 1992). In our centre, all HSCT recipients received G- CSF on Day +3 HSCT until neutrophil engraftment. In contrast to other studies, our study did not demonstrate a significant association between development of bacteraemia and patient's age, underlying diagnosis and conditioning regimen (Dandoy et al. 2017; Mitchell et al. 2004). The differences in the findings may be attributed to the small sample size in our study and the difference in conditioning regimens and G-CSF usage between transplant centres. We adopted RIC regimen more frequently compared to MAC regimen in our HSCT recipients which may have attributed to the lower risk of bacteraemia.

Our study showed the highest number of recipients with bacteraemia occurred in 2013 (8/16, 50%) and the lowest in 2016 (5/19, 26.3%). This was probably attributed to stem cell ward renovations and instalment of new facilities in 2014.

We identified a total of 43 bacterial pathogens isolates out of 40 bacteraemia episodes over the 5-year period. GNB (ESBL and non-ESBL *Enterobacteriaceae)* accounted for the highest isolated pathogens (13 of 43 isolates). A literature review from 49 manuscripts on bacteraemia in cancer patients considered papers published between January 1st 2005 and July 6th 2011, showed that Gram positive cocci organisms were the most common pathogens found (60%) (Mikulska et al. 2014). Among HSCT recipients, the sources of most bacteraemia were the skin, oral mucosa and gastrointestinal tract (GIT). It is likely that oral mucosa or GIT has replaced the skin as a portal of entry of for bacteraemia, causing high GNB infection in our study (Mikulska et al. 2009; Ferreira 2018). Apart from that, the absence of GPB isolates in 2015 probably due to enforcement of strict adherence to hand hygiene and proper infection control measures following renovation and implementation of antibiotic stewardship program in our HSCT ward in 2014.

Amongst GPB, the common isolation of CoNS was consistent with other studies (Chen et al. 2010; Engelhard et al. 1996; Gudiol et al. 2014; Ninin et al. 2001; Weisser et al. 2017; Ferreira 2018; Mikulska 2018). The high frequency of CoNS in our study during the 5-year period was likely due to contamination rather than true bacteraemia because rigorous definition criteria of bacteraemia was applied. It is more likely that the high frequency of CoNS was due to

several factors including the presence of central venous catheter and the universal use of prophylaxis with floroquinolones as pre-transplantation antibiotic prophylaxis in all recipients. Apart from that, our study also showed no MRSA infection over the 5-year study period. Another study also reported no incidence rate of MRSA among HSCT recipients (Wang et al. 2015). These findings might be due to a strict infection control measures such as proper hand hygiene, nasal screening, universal or selective decolonization and improvement in central line management guidelines (Liu et al. 2011).

The development of antibiotic resistance is of a great concern as MDR bacteraemia is one of the causes of early mortality among HSCT recipients. Our study reported a high resistance to ciprofloxacin (66.7%), which has been used as a prophylactic antibiotic for all HSCT recipients in our centre. Other study identified that there were more than 25% increased rate for ciprofloxacin resistance over 4 years among the 834 HSCT recipients (Freifeld et al. 2011). Another retrospective case control study reported 61% ciprofloxacin resistance among adults HSCT recipients and 31% among paediatric recipients (Mitchell et al. 2004). The results may suggest long term colonization with resistant organisms as most of our patients would have been exposed to multiple antibiotics during their primary treatment with various chemotherapy regimens prior to HSCT. The high usage of fluoroquinolones in oncology patients were also associated with fluoroquinolone resistant *Escherichia coli* and *Clostridium difficile* enterocolitis as reported in other study (Freifeld et al. 2011). Therefore, the implementation of prophylactic protocols should be carefully evaluated in future.

We found a total of nine (20.9%) MDR GNB (5 out of 6 ESBL *Enterobacteriaceae*, 2 out of 3 *Acinetobacter* sp., 1 out of 4 *Pseudomonas aeruginosa* and 1 *Sternotrophomonas maltophilia*) out of the 43 isolates. Mikulska and Wang et al. found an MDR incidence of 30-35 % for the whole GNB population (Mikulska et al. 2009; Wang et al. 2015) while another study from Spain found 11% MDR of all GNB (Gudiol et al. 2013; Gudiol et al. 2010). Our MDR GNB incidence was still not very high as compared to other study but remained a major concern, due to lack of effective antibiotics or controlled trials to guide the therapeutic choices. The choices of antibiotic were guided by local epidemiology and history of previous colonization (Balletto & Mikulska 2015).

Our study showed 70% of the HSCT recipients with bacteraemia was labelled as failed to response to first line empirical regimen of piperacillin-tazobactam with or without amikacin for the treatment of FN and required an upgrade to second line antibiotic regimens. However, the microbiological resistance was low with only 4 patients (20%) grew isolates with resistance to piperacillin-tazobactam and 3 patients (10%) to amikacin. This resistance rate was lower than the rate reported by a previous study, where 42% of

all bacteraemia isolates in 200 HSCT recipients were resistant to first line empirical piperacillin-tazobactam and/or gentamicin (Mitchell et al. 2004). Clearly, it is very important to modify empirical antibiotic regimes based on subsequent blood culture finding and sensitivity. The need to consider an early change in the antibiotic for those who clinically failed to respond to first line empirical antibiotic therapy has been a standard practice in management of chemotherapy induced FN (Mitchell et al. 2004).

The observed low success rate of FN in our recipients towards first line empirical piperacillin-tazobactam with/without amikacin may be explained by a number of factors. Firstly, transplant physicians usually have a low clinical threshold of upgrading to second line antibiotics as guided by early clinical signs or symptoms associated with catheter related sepsis or skin / soft tissue infection. Secondly, plasma antibiotic levels may be subtherapeutic due to renal functions and body weight. Thirdly, broad spectrum antibiotics such as carbapenem take precedence over piperacillin-tazobactam especially in high risk groups such as patients in intensive care unit (ICU), severe pneumonia, known ESBL colonizers or patients with multiple medical co-morbidities. However, in lower risk patients, a 3-5 days interval is usually reserved for piperacillin-tazobactam (with or without amikacin) to exert full clinical effects and avoid resistance before switching to second line agents.

GNB has been associated with highly morbid sequelae, such as severe pneumonia and septic shock, contributed in part by greater inflammatory response triggered by endotoxaemia (Joo et al. 2011; Mikulska et al. 2009; Mitchell et al. 2004). MDR *Acinetobacter* and ESBL *Enterobacteriaceae* were associated with both early and late transplant mortality, with a high crude death rate ranging between 17% and 52% (Eliopoulos et al. 2008). In our HSCT recipients, GNB was the highest causative pathogen contributed to mortality (13.3%) which was considerably similar with other report (59% in 106 recipients with bacteraemia) (Poutsiaka et al. 2007; Wang et al. 2015) Our recipients with MDR *Acinetobacter* bacteremia died despite treatment with polymyxin B because of overwhelming septic shock and multi-organ failure with concomitant GvHD and severe neutropenia.

This study was conducted retrospectively which unfortunately may have led to inferior level of evidence compared to a prospective study. Any missing data or incomplete record keeping would act as confounding factor in the data interpretation. The small number of bacterial isolates reflected our small sample size, hence limiting us from drawing any definitive conclusion on the changes of antibiotic resistance observed over the 5-year study period.

CONCLUSION

In conclusion, bacteraemia continues to be a significant early complication among HSCT recipients. GNB was

associated with a high rate of antibiotic resistance and also the commonest cause of mortality in recipients with bacteraemia. Notably, the success rate to first line empirical antibiotics piperacillin-tazobactam with or without amikacin for FN was very low. The high rate of resistance to ciprofloxacin and failure of the first line empirical antibiotics to treat FN calls for a thorough evaluation of the current antibiotic prophylaxis and empirical treatment protocols. The findings of this preliminary study may need to be confirmed in a large prospective interventional study before corrective measures can be implemented effectively.

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