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Original Article

Prevalence of CRE Colonization in Hematopoietic Stem Cell Transplant Recipients and Its Impact on Clinical Outcomes: Experience from a Developing Country

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Abstract

Objectives: To assess impact of CRE colonization on infectious complications and transplant-related outcomes.

Methodology: This single-center prospective study, conducted at the Clinical Hematology and Bone Marrow Transplant Department, National University of Medical Sciences, Rawalpindi, Pakistan, included 173 patients who received HSCT between January 2023 and May 2025. Stool samples were analyzed for CRE colonization before start of conditioning protocol. Statistical analysis was done using SPSS version 27.

Results: Median age at time of transplant was 30 (range: 1-69) years. Of 173 patients included in the study, 114 (65.9%) were CRE-positive, while 59 (34.1%) were negative. CRE-positive patients had higher incidence of febrile neutropenia (93% vs. 84.7%, $p=0.08$). CRE-targeted antibiotics were required in 38.6% CRE-positive patients compared to 25.4% CRE-negative patients ($p=0.08$). After starting CRE-targeted antibiotics, fever resolved in median 2 days in CRE-positive group compared to median 3 days in CRE-negative group ($p=0.59$). CRE non-colonized patients had better OS of 91.5% with mean survival 728 days (95% CI: 668-718 days) compared to CRE colonized patients who had OS of 81.4% with mean survival of 642 days (95% CI: 580-704 days, $p=0.12$).

Conclusion: Our single-center study demonstrates that patients with CRE-colonization before hematopoietic stem cell transplant have a trend towards inferior transplant outcomes and increased complications compared to CRE non-colonized patients.

Keywords: Hematopoietic stem cell transplantation; Carbapenem-Resistant Enterobacteriaceae; Bloodstream infection; Carbapenem-Resistant Enterobacteriaceae-associated bloodstream infection; Neutropenic fever.

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Introduction

Hematopoietic stem cell transplantation (HSCT) is a curative treatment option in many malignant and non-malignant hematological disorders.¹ However, Infectious complications are the third most common cause (20%) of 100-day mortality among Allo-HSCT recipients, preceded by disease relapse (27%) and organ failure (25%).² Risk factors include myeloablative conditioning, prolonged neutropenia, graft-versus-host disease (GVHD), mucositis, central venous catheters, and immunosuppressive therapy.³ Due to low culture positivity rate (20-30%), management of infections in neutropenic patients is difficult, and selection of antimicrobial therapy almost always follows an empirical approach.⁴ Surveillance is the

best way to determine the agents of colonization with resistant microorganisms in targeted units. With respect to the source of infections in neutropenic^{5, 6} patients, the main source is the patient's own skin bacterial flora, followed by gut microbial flora. The emergence of Carbapenem-Resistant Enterobacteriaceae (CRE) is a major threat to global health, and the World Health Organization (WHO) has listed them as critical priority pathogens requiring new antibiotic development.^{7, 8} Patients undergoing HSCT are at high risk of CRE colonization and infection.^{9, 10} Correlation between CRE colonization and CRE-associated bloodstream infection (CRE-BSI) has been documented.¹¹⁻¹⁴ CRE-BSI are associated with a higher mortality rate (45.5%) compared to patients infected with Carbapenem-sensitive *Enterobacteriaceae*.¹⁵ CRE-BSI in Allo-HSCT patients has a 30-day mortality rate of up to 50% and early screening for CRE colonization can be an early indicator and warning sign for developing CRE-BSI later during treatment. Early multi-drug therapy in such

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patients showed a slightly lower mortality rate.¹⁶ CRE colonized patients undergoing Allo-HSCT had higher rates of BSI and higher non-relapse mortality (NRM) in comparison to non-CRE colonized patients.¹⁷

Our study aims to assess CRE colonization rates in HSCT patients and their impact on infectious complications and transplant-related outcomes in a developing resource-limited country.

Methodology

This single-center prospective cohort study was conducted at the Clinical Hematology and Bone Marrow Transplant Department of the National University of Medical Sciences, Rawalpindi, Pakistan. The study was approved by the hospital ethics committee and adhered to the principles of the Declaration of Helsinki (IRB number: IRB-018/AFBMT/Approval/2023). Written consent was taken from all the patients included in the study. Patients admitted for Allogeneic and Autologous HSCT between January 2023 and May 2025 who had no signs and symptoms of infection were screened for CRE colonization. A stool sample was taken for CRE colonization before initiating the conditioning regimen. Based on stool culture results, patients were divided into 2 groups: CRE-colonized and CRE non-colonized. CRE-colonized was defined as patients with positive stool culture and having no signs and symptoms of active infection. CRE non-colonized was defined as patients with a negative stool culture without evidence of active infection.

All patients were treated in an isolated single room with contact isolation, HEPA filters, and a neutropenic diet. All patients received antibacterial prophylaxis (cefixime/moxifloxacin), acyclovir as antiviral prophylaxis, and (fluconazole/itraconazole) as an antifungal prophylaxis as per institutional protocol.

Demographic and transplant details were recorded for all patients. Stool surveillance samples were sent for CRE culture on the day of admission. Patients were followed from the start of the conditioning regimen to the last follow-up. Total days of hospitalization, Neutrophil and platelet engraftment, febrile neutropenia, Culture positivity, Mucositis, transplant complications, and overall survival (OS) were recorded.

Statistical analysis of data was done using SPSS version 27. Percentages and frequencies of qualitative variables were documented, including mean and standard deviations of quantitative variables. Kaplan Meier survival analysis was done for the calculation of OS and DFS.

Results

A total of 173 patients were included in our study who underwent HSCT during the study period. 77 (44.5%) patients received autologous HSCT, 84 (48.55%) patients HLA-matched allogeneic HSCT, and 12 (6.94%) patients underwent haploidentical HSCT. Median age at time of transplant was 30 (range: 1-69) years, 113 (65.3%) males and 60 (34.7%) females. Adults (age ≥ 16 years) were 124 (71.7%), while 49 (28.3%) were pediatric (age < 16 years). Median duration from diagnosis to HSCT was 381 (range 61-4773) days. Mean pre-transplant ferritin was 1142.07 (range 9-6293) ng. Among the Beta thalassemia major, 6 (50%) had class II and 6 (50%) had class III disease as per Lucarelli classification. The majority of patients, n=98 (97.9%), underwent their first HSCT, while 2 (2.1%) patients received their second HSCT. Most patients received myeloablative conditioning (n=139, 80.34%), followed by non-myeloablative conditioning (n=24, 13.87%) and reduced intensity conditioning (n=10, 5.78%). Detailed demographics and baseline characteristics are demonstrated in Table 1. Diseases for which patients received HSCT are shown in Figures 1 and 2. Conditioning regimens used are listed in Table III.

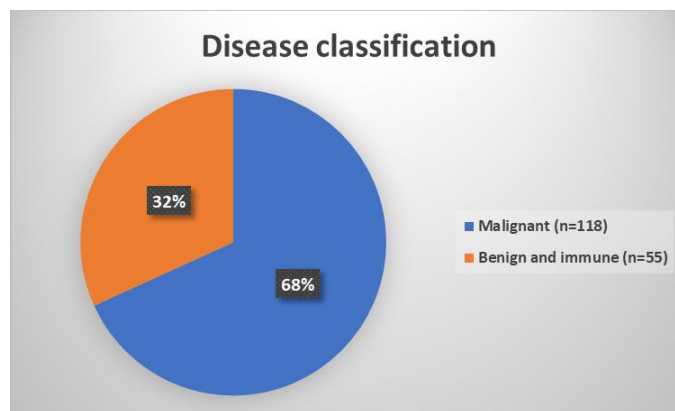


Figure 1. Disease classification.

Of the 173 patients included, 114 (65.9%) were CRE-colonized on surveillance stool culture, while 59 (34.1%) were CRE non-colonized. Details are provided in Tables 1-3.

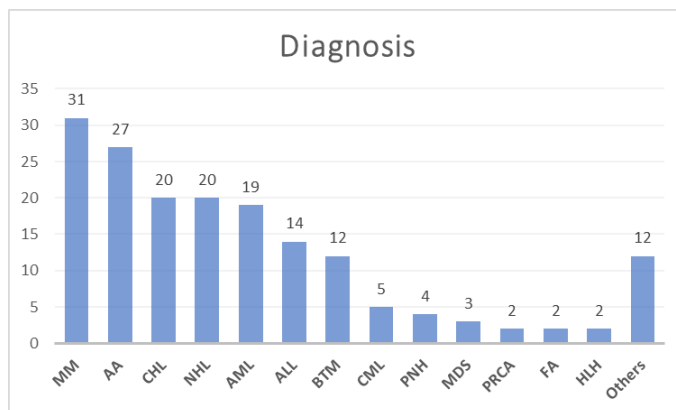


Figure 2. Diagnosis of patients.

CRE-positive patients had a higher incidence of febrile neutropenia (93% vs. 84.7%) compared to CRE-negative patients ($p=0.08$); however, the difference was not statistically significant. Culture positivity was similar in both groups (20.3%) in CRE-positive patients and 20.2% in CRE-negative patients).

The mean time from the start of conditioning protocol to the start of fever and the median days of febrile neutropenia were also similar in both groups.

There was no significant difference in the requirement of inotropic support due to septic shock.

CRE targeted antibiotics (Colistin, Tigecycline, Ceftazidime-Avibactam) were required in 38.6% ($n=44$) CRE-positive patients compared to 25.4% ($n=15$) in CRE-negative patients ($p=0.08$). Median time from start of febrile neutropenia to commencing CRE antibiotics was similar in both groups (4 days, $p=0.16$).

After starting CRE-targeted antibiotics, the fever resolved in a median of 2 days in the CRE-positive group compared to 3 days in the CRE-negative group ($p=0.59$).

The mean number of days for which CRE antibiotics were used was higher in the CRE-negative cohort (7.71 ± 2.57) compared to the CRE-positive cohort (6.77 ± 2.94 , $p=0.38$), but this difference was not statistically significant.

CRE-negative patients had early neutrophil (12.05 ± 1.83 vs. 13.04 ± 4.19 days, $p=0.12$) and platelet engraftment (17.72 ± 10.26 vs. 19.72 ± 10.82 days, $p=0.19$).

CRE-positive patients had longer hospital stays from the day of admission (32.37 ± 13.1 vs. 30.42 ± 7.4 days, $p=0.01$) and from the day of stem cell infusion compared to CRE-

negative patients (18.92 ± 8.3 vs. 17.05 ± 5.1 days, $p=0.01$).

Table I: Demographics.

		n	%
Gender	Male	113	65.3
	Female	60	34.7
Age group	Pediatric (≤ 16 years)	49	28.3
	Adult (>16 years)	124	71.7
Type of HSCT	Allogeneic	96	55.5
	Autologous	77	44.5
Allogeneic HSCT	HLA matched	84	87.5
	Haploidentical	12	12.5
The donor's relation with the patient	Brother	54	56.2
	Sister	40	41.7
	Mother	2	2.1
Disease status	CR1	69	39.9
	CR2	31	17.9
	CR3	3	1.7
	Partial Remission	8	4.6
	Not	62	35.8
ABO Mismatch	No ABO Mismatch	65	37.6
	Major ABO	16	9.2
	Minor ABO	13	7.5
	Bi-Directional ABO	2	1.2
	Auto HSCT	77	44.5
Patient CMV	IgG Positive	167	96.5
	IgG Negative	6	3.5
Donor CMV	IgG Positive	91	52.6
	IgG Negative	5	2.9
	Auto HSCT	77	44.5
ATG/TG in conditioning	Yes	86	49.7
	No	87	50.3
Conditioning intensity	Myeloablative	146	84.4
	Non Myeloablative	26	15
	Reduced intensity	1	0.6
Source of stem cells	BMH	69	39.9
	PBSC	82	47.4
	BMH+PBSC	22	12.7
GVHD Prophylaxis	Auto HSCT	77	44.5
	CSA+MTX	54	31.2
	CSA	21	12.1
	PTCy+CSA+MMF	12	6.9
	CSA+MMF	9	5.2
Stool culture for	Positive	114	65.9
	Negative	59	34.1

Higher rates of aGVHD were noted in CRE-colonized patients with no statistical significance (14% vs. 11.9%, $p=0.91$). (Table II)

CRE-colonized patients showed increased rates of hepatotoxicity and nephrotoxicity without statistical significance (hepatotoxicity: 35.1% vs. 22.0%, $p=0.07$, Nephrotoxicity: 13.2% vs. 5.1%, $p=0.09$). (Table II)

Overall survival (OS) was 85% with a mean survival of 671 days (95% CI: 623-718 days) (Figure #3).

CRE non-colonized patients had a better OS of 91.5%

with a mean survival of 728 days (95% CI: 668-718 days) compared to CRE-colonized patients who had an OS of 81.4% with a mean survival of 642 days (95% CI: 580-704 days). However, this difference in OS was not statistically significant ($p=0.12$). (Figure#4)

Patients who did not develop febrile neutropenia had an OS of 87.5% with a mean survival of 689 days (95% CI:

Table II: Outcomes		Stool CRE				
		Positive		Negative		p value
		n	%	n	%	
Graft Failure	Primary	3	2.6%	1	1.7%	0.927
	Secondary	2	1.8%	1	1.7%	
	No Graft Failure	109	95.6%	57	96.6%	
Relapse post HCT	Yes	10	8.8%	2	3.4%	0.187
	No	104	91.2%	57	96.6%	
Engraftment Syndrome	Yes	1	0.9%	0	0.0%	0.471
	No	113	99.1%	59	100.0%	
VOD/SOS	Yes	2	1.8%	3	5.1%	0.215
	No	112	98.2%	56	94.9%	
aGVHD	Yes	16	14.0%	7	11.9%	0.918
	No	48	42.1%	25	42.4%	
	Auto HSCT	50	43.9%	27	45.8%	
cGVHD	Yes	9	7.9%	5	8.5%	0.952
	No	55	48.2%	27	45.8%	
	Auto HSCT	50	43.9%	27	45.8%	
CMV Reactivation (PCR: ≥ 2000 copies)	Yes	48	42.1%	21	35.6%	0.407
	No	66	57.9%	38	64.4%	
Gut Toxicity	Yes	54	47.4%	31	52.5%	0.519
	No	60	52.6%	28	47.5%	
Hepatotoxicity	Yes	40	35.1%	13	22.0%	0.077
	No	74	64.9%	46	78.0%	
TA-TMA	Yes	8	7.0%	1	1.7%	0.135
	No	106	93.0%	58	98.3%	
Renal Complications	Yes	15	13.2%	3	5.1%	0.099
	No	99	86.8%	56	94.9%	
Neurological Complications	GTC Seizures	5	4.4%	2	3.4%	0.753
	Nil	109	95.6%	57	96.6%	
Mucositis	Yes	65	57.0%	38	64.4%	0.348
	No	49	43.0%	21	35.6%	
Mucositis Grade	No Mucositis	49	43.0%	21	35.6%	0.589
	Grade I	23	20.2%	17	28.8%	
	Grade II	21	18.4%	11	18.6%	
	Grade III	19	16.7%	10	16.9%	
	Grade IV	2	1.8%	0	0.0%	

Table III: Details of infections		CRE Positive	CRE Negative	P Value
Total days of FN	Median**	5	5	0.1
Day of fever from the start of conditioning	Mean $\pm$ st.d*	11.03 $\pm$ 5.05	11.3 $\pm$ 5.75	0.15
Antibiotics used	Median**	4	4	0.38
Days from fever to start of CRE Antibiotic	Median**	4	4	0.16
Time from start of CRE abx to fever resolution (Days)	Median**	2	3	0.59
Number of days of ABX against CRE	Mean $\pm$ st.d*	6.77 $\pm$ 2.57	7.71 $\pm$ 2.94	0.84
Note: chi-square test was applied, * independent t-test, and ** Mann-Whitney Test was applied				

550-829 days), compared to patients who developed febrile neutropenia had an OS of 84.6% with a mean survival of 689 days (95% CI: 550-829 days). However, this difference in OS was not statistically significant ($p=0.73$). (Figure#5)

No statistically significant correlation was seen with other transplant-related outcomes.

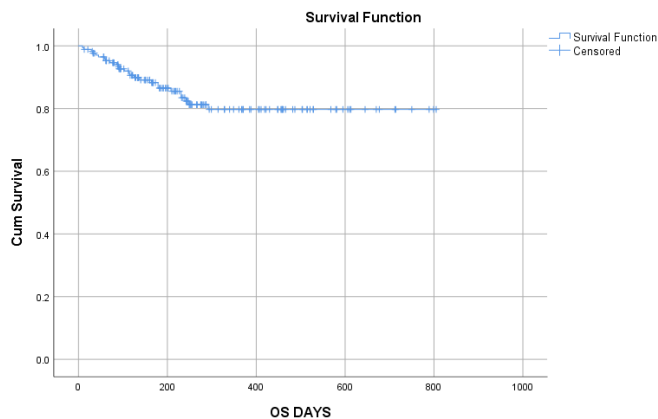


Figure 3. Overall survival.

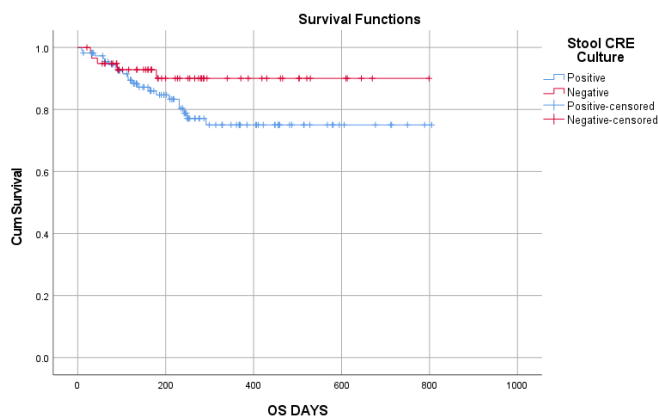


Figure 4. OS in CRE positive vs CRE negative patients.

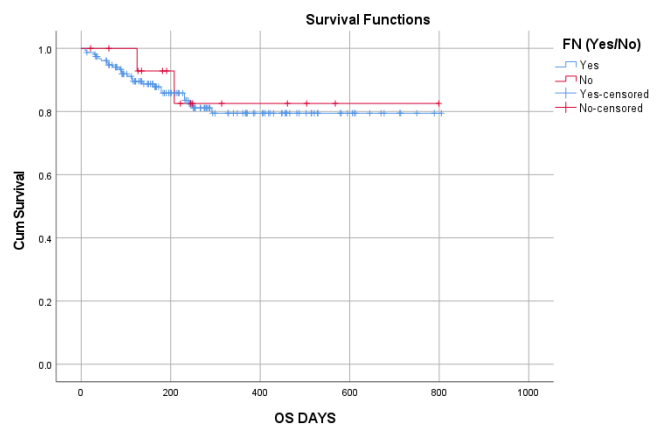


Figure 5. Febrile neutropenia in CRE positive vs CRE negative patients.

Discussion

Bacterial infections are one of the major causes of mortality in bone marrow transplant patients. These infections can occur during conditioning, neutropenia, or after neutrophil engraftment. Gram-negative organisms cause up to 75% of infections among documented bacterial infections during HSCT in Asia and the Indian subcontinent.¹⁸⁻²⁰

Gram-negative infections have been increasing, especially drug-resistant strains of Enterobacteriaceae in Western countries over the past two decades.^{21, 22}

A recent study showed that HSCT recipients having pre-transplant Levofloxacin-resistant ESBL colonization had higher rates of infections from the colonized strain compared to non-colonized patients while receiving Levofloxacin prophylaxis during neutropenia.²³ This study suggests a role for pre-transplant fecal surveillance and empirical antibiotic therapy in colonized HSCT recipients.

Table IV: Outcomes.

	Stool CRE	n	Mean	Std.	CI 95%		P-value
					Lower bound	Upper bound	
Neutrophil Engraftment	Positive	112	13.04	4.19	-0.147	2.133	0.122
	Negative	58	12.05	1.83	0.078	1.907	
Platelet Engraftment	Positive	107	19.72	10.828	-1.444	5.445	0.195
	Negative	57	17.72	10.266	-1.397	5.398	
Days in hospital from Day zero	Positive	114	18.92	8.343	-0.474	4.214	0.01
	Negative	59	17.05	5.097	-0.154	3.894	
Total admission days	Positive	114	32.37	13.072	-1.686	5.575	0.01
	Negative	59	30.42	7.405	-1.132	5.021	

In our study, 114 (65.9%) patients had CRE colonization, which is higher compared to transplant patients in India and the United States.^{24, 25}

CRE-colonized patients had higher rates of febrile neutropenia in our cohort; however, no difference in blood culture growth was observed between the two groups, which may suggest a different source of infection. Multiple studies show contradicting data regarding this finding; some studies show similar findings,^{24, 25} while other studies show higher rates of bloodstream infections in colonized patients.²⁶⁻²⁹

CRE-colonized patients in our study had delayed neutrophil and platelet engraftment. J Bilinski et al observed similar findings of delayed neutrophil engraftment in CRE colonized patients, but no difference was observed in platelet engraftment.²⁸ Wen-Qi Wu et al. observed delayed platelet engraftment in CRE-colonized patients.¹⁷

We observed a higher rate of acute GVHD in CRE-colonized patients; similar findings were also reported by J Bilinski et al.²⁸

CRE-colonized patients had increased duration of hospital stay as compared to CRE non-colonized patients; similar findings have been reported previously in multiple studies.³⁰⁻³³

CRE colonized patients had better OS as previously documented by Chen et al.³⁴

Conclusion

Our single-center study demonstrates that patients with CRE-colonization of the gut before hematopoietic stem cell transplant have a trend towards inferior transplant outcomes and increased complications compared to CRE non-colonized patients.

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