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Original Research Article

# Stool Surveillance and Its Correlation with Pathogens Isolated From Blood among Hematology-Oncology Patients in a Tertiary Care Hospital

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**Conflict of interest: Nil**

**Abstract**

**Background:** Hematology-oncology patients are at increased risk of febrile neutropenia and bloodstream infection, particularly with antimicrobial-resistant organisms. Gastrointestinal colonization may serve as a potential reservoir for subsequent infection.

**Methods:** This descriptive observational study included 140 patients with newly diagnosed acute lymphoblastic leukemia or acute myeloid leukemia undergoing chemotherapy and patients undergoing autologous or allogeneic hematopoietic stem-cell transplantation for any indication. Stool samples were subjected to culture, antimicrobial susceptibility testing and phenotypic detection of β-lactamase mechanisms. Patients were followed for 30 days, and blood cultures were obtained from those developing febrile neutropenia. Agreement between stool and blood isolates was assessed using Cohen's kappa.

**Results:** The mean age was 37.27±21.30 years, and 63.57% were males. Febrile neutropenia developed in 86 (61.43%) patients. Escherichia coli was the predominant stool isolate (88.57%). Among the 131 Gram-negative stool isolates, AmpC+MBL was the most frequently identified reported resistance phenotype (57/131, 43.51%), followed by AmpC+ESBL (21/131, 16.03%) and ESBL alone (16/131, 12.21%). Among E. coli, resistance to ampicillin was 89.52%, while resistance to imipenem and meropenem was 43.55% each. Most blood cultures showed no growth. Stool–blood culture concordance was only 5.81%, with poor agreement ($\kappa$=−0.007, p=0.334).

**Conclusion:** Resistant Gram-negative intestinal colonization was common among hematology-oncology patients, but stool surveillance showed poor concordance with subsequent blood-culture isolates. Stool surveillance may therefore be more useful for monitoring colonization and resistance patterns than for independently predicting bloodstream pathogens.

**Keywords:** Stool Surveillance; Febrile Neutropenia; Hematological Malignancy; Bloodstream Infection; Escherichia Coli; Antimicrobial Resistance; ESBL; AmpC; MBL.

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## Introduction

Patients with hematological malignancies are particularly vulnerable to severe bacterial infections because of disease-related immune dysfunction, intensive chemotherapy, mucosal barrier injury, and prolonged neutropenia. Alteration of the intestinal microbiota during treatment may result in domination by potentially pathogenic organisms, which can subsequently translocate across the damaged intestinal mucosa and cause bloodstream infection (BSI) [1].

Colonization with multidrug-resistant (MDR) Gram-negative bacteria is therefore of particular concern in patients receiving intensive chemotherapy or hematopoietic stem cell transplantation (HSCT), as colonizing organisms may subsequently be responsible for bacteremia [2].

Febrile neutropenia remains one of the most important infectious complications encountered during the management of hematological malignancies and requires prompt empirical antimicrobial therapy [3]. Increasing colonization with MDR organisms further complicates treatment because resistance to commonly used empirical antibiotics may lead to inappropriate initial therapy and adverse outcomes. Surveillance for MDR organisms among high-risk hematology patients may therefore provide useful information regarding colonization patterns and antimicrobial resistance [4].

Patients undergoing HSCT are particularly susceptible to intestinal colonization with resistant organisms because of repeated hospitalization, antibiotic exposure, chemotherapy-induced mucosal injury, and profound immunosuppression [5]. Similar concerns have been reported from Indian transplant centres, where MDR Gram-negative organisms constitute an important challenge in the management of transplant recipients [6]. Intestinal colonization with carbapenem-resistant Enterobacterales has also been associated with subsequent infection among patients with hematological malignancies [7]. Furthermore, Gram-negative bloodstream infections caused by resistant organisms continue to represent a major clinical problem in hematological patients [8].

Although intestinal colonization with antimicrobial-resistant organisms has been increasingly recognized as an important concern in hematology-oncology patients, limited data are available regarding the relationship between organisms recovered from surveillance stool cultures and subsequent bloodstream isolates in this patient population.

In particular, there is a paucity of data evaluating organism-level concordance together with phenotypic β-lactamase-mediated resistance mechanisms among patients with acute leukemia and those undergoing hematopoietic stem-cell transplantation. Furthermore, the clinical utility of routine stool surveillance in identifying organisms subsequently responsible for bloodstream infection remains uncertain. Therefore, prospective evaluation of stool colonization, antimicrobial susceptibility patterns, resistance phenotypes, and their concordance with subsequent blood culture isolates is warranted. The present study was undertaken to determine the pattern and antimicrobial susceptibility of organisms isolated by surveillance stool culture, characterize phenotypic β-lactamase-mediated resistance mechanisms, and assess the concordance between surveillance stool isolates and organisms subsequently isolated from blood among hematology-oncology patients.

**Aims and Objectives:** The aim of the present study was to evaluate intestinal bacterial colonization and antimicrobial-resistance patterns in hematology-oncology patients and to assess their concordance with subsequent bloodstream isolates.

The objectives were to determine the bacterial profile and antimicrobial susceptibility pattern of organisms isolated from surveillance stool cultures; to assess the concordance between surveillance stool culture findings and subsequent blood culture findings, including culture positivity and organism-level concordance; and to characterize the phenotypic β-lactamase-mediated resistance mechanisms, including ESBL, AmpC and MBL production, among bacterial isolates recovered from surveillance stool and blood cultures.

## Materials and Methods

**Study Design and Setting:** This single-centre descriptive observational study was conducted in the Department of Microbiology, Christian Medical College and Hospital, Ludhiana, in collaboration with the Department of Hematology-Oncology. The study was conducted from 1 November 2019 to 31 December 2021.

**Study Population:** A total of 140 newly diagnosed patients with hematological malignancies who were receiving chemotherapy or undergoing HSCT were included. The study population comprised patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and patients undergoing autologous or allogeneic HSCT for any indication. Stool and blood samples from patients admitted to the Clinical Hematology wards and Bone Marrow Transplant Unit were processed according to standard microbiological protocols.

**Eligibility Criteria:** Patients with newly diagnosed acute lymphoblastic leukemia or acute myeloid leukemia undergoing chemotherapy and patients undergoing autologous or allogeneic hematopoietic stem-cell transplantation for any indication were eligible for inclusion. Patients with hematological malignancies other than acute lymphoblastic leukemia or acute myeloid leukemia who were not undergoing HSCT, patients not undergoing chemotherapy at Christian Medical College and Hospital, patients with solid-organ malignancies, and patients who did not provide informed consent were excluded.

**Stool Sample Collection and Processing:** Stool specimens were collected in sterile universal containers and transported to the microbiology laboratory for processing. Gross examination was performed to assess consistency, colour, presence of blood, and visible parasites. A portion of the sample was examined microscopically using normal saline and iodine preparations, followed by formol-ether sedimentation concentration for detection of ova, cysts, and trophozoites.

For bacterial culture, the second portion of the stool specimen was inoculated onto blood agar, MacConkey agar, and deoxycholate citrate agar (DCA). Approximately 5 mL of the specimen was additionally inoculated into Selenite-F enrichment broth, incubated at 37°C for 18–24 hours, and subsequently subcultured onto DCA. Primary culture plates were incubated at 37°C and examined for bacterial growth. Stool cultures showing no growth after the prescribed incubation period were considered culture negative.

In patients undergoing HSCT, one stool sample was collected before initiation of conditioning therapy, whereas in patients with AML or ALL, one stool sample was collected before initiation of induction therapy.

**Identification of Bacterial Isolates:** Bacterial colonies were initially assessed according to colony morphology and Gram-staining characteristics. Isolates were identified using conventional biochemical tests, including catalase, oxidase, sugar fermentation, oxidation-fermentation, triple sugar iron, indole, methyl red, Voges-Proskauer, citrate utilization, urease, nitrate reduction, gelatin liquefaction, bile esculin, amino-acid decarboxylase, phenylalanine deaminase, motility, and coagulase tests, as applicable to the organism isolated.

**Surveillance for Febrile Neutropenia and Blood Culture:** All enrolled patients were monitored for 30 days. Patients who developed febrile neutropenia during this period underwent blood culture according to the institutional hematology protocol. Blood specimens were collected both from a peripheral vein (poke culture) and from the central venous catheter (line culture), preferably during an episode of fever >38°C.

Blood cultures were processed using the BD BACTEC semiautomated blood culture system. Positive bottles flagged by the system were subcultured onto blood agar and MacConkey agar and incubated at 37°C for 24–48 hours. Organisms recovered from positive cultures were identified using Gram staining and appropriate biochemical tests.

**Antimicrobial Susceptibility Testing:** Antimicrobial susceptibility testing of bacterial isolates obtained from both stool and blood cultures was performed using the Kirby-Bauer disc diffusion method according to the applicable Clinical and Laboratory Standards Institute (CLSI) recommendations. A bacterial suspension equivalent to a 0.5 McFarland standard was inoculated uniformly onto Mueller-Hinton agar, appropriate antimicrobial discs were applied, and plates were incubated at 37°C for 16–18 hours. Zones of inhibition were measured and isolates were categorized as susceptible, intermediate, or resistant according to the recommended interpretive criteria.

**Detection of β-Lactamase Production:** Phenotypic testing for important β-lactamase-mediated resistance mechanisms was performed among relevant isolates.

**Extended-spectrum β-lactamase (ESBL):** ESBL production was detected using cefotaxime alone and cefotaxime-clavulanic acid combination discs. An increase of ≥5 mm in the inhibition zone around the combination disc compared with cefotaxime alone was considered indicative of ESBL production.

**AmpC β-lactamase:** AmpC production was detected using the cefoxitin-cloxacillin disc diffusion test. Cefoxitin and cefoxitin supplemented with cloxacillin were compared, and an increase of >4 mm in the inhibition zone with the combination disc was considered positive for AmpC production.

**Metallo-β-lactamase (MBL):** MBL production was assessed using the imipenem-EDTA combination disc test. An increase of ≥7 mm in the zone of inhibition with imipenem-EDTA compared with imipenem alone was considered indicative of MBL production.

**Assessment of Concordance:** For patients who developed febrile neutropenia and had blood cultures performed, organisms isolated from blood were compared with organisms identified in their preceding surveillance stool cultures. Concordance between stool and blood culture findings was assessed according to the identity of the organisms isolated and their resistance characteristics.

**Statistical Analysis:** Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 21.0. Categorical variables were expressed as frequencies and percentages. Quantitative variables were presented as mean ± standard deviation or median with interquartile range (25th–75th percentile), as appropriate. Inter-rater kappa agreement was used to assess the strength of agreement between stool and blood culture findings. A p-value <0.05 was considered statistically significant.

## Results

A total of 140 hematology-oncology patients were included in the study. The mean age was 37.27 ± 21.30 years, with a median age of 35 years (IQR: 18–54.25) and a range of 1.5–78 years. Males constituted 63.57% of the study population.

Acute lymphoblastic leukemia (ALL) was the most frequent diagnosis (45.0%), followed by acute myeloid leukemia (AML) (37.86%). During the 30-day surveillance period, 86 (61.43%) patients developed febrile neutropenia.

**Table 1: Demographic and clinical characteristics of the study population (n=140)**

| Characteristic | n (%) / Value |
|---|---|
| **Age (years), mean ± SD** | **37.27 ± 21.30** |
| Median (IQR) | 35 (18–54.25) |
| Range | 1.5–78 |
| **Gender** | |
| Male | 89 (63.57) |
| Female | 51 (36.43) |
| **Underlying diagnosis** | |
| Acute lymphoblastic leukemia | 63 (45.00) |
| Acute myeloid leukemia | 53 (37.86) |
| Beta-thalassemia major undergoing HSCT | 8 (5.71) |
| Multiple myeloma undergoing HSCT | 8 (5.71) |
| Hodgkin lymphoma undergoing HSCT | 4 (2.86) |
| Aplastic anemia undergoing HSCT | 2 (1.43) |
| NSGCT yolk sac tumor undergoing HSCT | 1 (0.71) |
| Peripheral T-cell lymphoma undergoing HSCT | 1 (0.71) |
| **Febrile neutropenia** | |
| Present | **86 (61.43)** |
| Absent | 54 (38.57) |

Stool culture demonstrated marked predominance of Gram-negative bacilli. Escherichia coli was isolated from 124 (88.57%) patients, whereas Klebsiella pneumoniae and Enterobacter cloacae were each isolated from 3 (2.14%). Nine (6.43%) patients had no growth or suppression of normal flora.

**Table 2: Distribution of stool culture isolates and β-lactamase resistance mechanisms**

| Parameter | n (%) |
|---|---|
| **Organisms isolated from stool (n=140)*** | |
| Escherichia coli | **124 (88.57)** |
| Klebsiella pneumoniae | 3 (2.14) |
| Enterobacter cloacae | 3 (2.14) |
| Enterococcus faecalis | 2 (1.43) |
| Shigella flexneri | 1 (0.71) |
| Enterococcus faecium | 1 (0.71) |
| No growth/normal flora suppressed | 9 (6.43) |
| **β-lactamase phenotype among Gram-negative isolates (n=131)** | |
| AmpC alone | 5 (3.81) |
| ESBL alone | 16 (12.21) |
| AmpC + ESBL | 21 (16.03) |
| **AmpC + MBL** | **57 (43.51)** |

***More than one organism could be recovered from an individual stool specimen; therefore, isolate counts are not mutually exclusive.**

Among the 131 Gram-negative stool isolates, AmpC + MBL co-production was the predominant resistance phenotype (43.51%), followed by AmpC + ESBL (16.03%) and ESBL alone (12.21%). Because E. coli constituted the overwhelming majority of stool isolates, its antimicrobial susceptibility profile was evaluated separately. High resistance was observed to ampicillin (89.52%), ciprofloxacin (73.39%), and several cephalosporins (72.58%). Resistance to imipenem and meropenem was observed in 43.55% of isolates. In contrast, relatively high susceptibility was retained to chloramphenicol (79.84%), gentamicin (70.16%), and amikacin (66.94%).

**Table 3: Antimicrobial susceptibility pattern of Escherichia coli isolated from stool (n=124)**

| Antimicrobial | Resistant n (%) | Susceptible n (%) |
|---|---|---|
| Ampicillin | **111 (89.52)** | 13 (10.48) |
| Cefazolin | 90 (72.58) | 34 (27.42) |
| Gentamicin | 37 (29.84) | **87 (70.16)** |
| Amikacin | 41 (33.06) | **83 (66.94)** |
| Amoxicillin-clavulanate | 90 (72.58) | 34 (27.42) |
| Piperacillin-tazobactam | 59 (47.58) | 65 (52.42) |
| Cefepime | 90 (72.58) | 34 (27.42) |
| Cefoxitin | 79 (63.71) | 45 (36.29) |
| Cefotaxime | 90 (72.58) | 34 (27.42) |
| Ceftriaxone | 90 (72.58) | 34 (27.42) |
| Ciprofloxacin | **91 (73.39)** | 33 (26.61) |
| Imipenem | 54 (43.55) | 70 (56.45) |
| Meropenem | 54 (43.55) | 70 (56.45) |
| Trimethoprim-sulfamethoxazole | 61 (49.19) | 63 (50.81) |
| Ceftazidime | 90 (72.58) | 34 (27.42) |
| Chloramphenicol | 25 (20.16) | **99 (79.84)** |

Among the 86 patients with febrile neutropenia, peripheral (poke) blood cultures showed no growth in 82 (95.35%), while line cultures showed no growth in 83 (96.51%). Acinetobacter baumannii was recovered from both a poke and line culture in one patient. Pseudomonas aeruginosa was recovered from one line culture. Other positive cultures included coagulase-negative staphylococci and one diphtheroid contaminant.

**Table 4: Blood culture findings, resistance phenotype, and concordance with stool surveillance among patients with febrile neutropenia (n=86)**

| Parameter | n (%) |
|---|---|
| **Peripheral/poke blood culture** | |
| No growth | **82 (95.35)** |
| Acinetobacter baumannii | 1 (1.16) |
| Diphtheroids (contamination) | 1 (1.16) |
| Methicillin-resistant CoNS | 1 (1.16) |
| Methicillin-susceptible CoNS | 1 (1.16) |
| **Central-line blood culture** | |
| No growth | **83 (96.51)** |
| Acinetobacter baumannii | 1 (1.16) |
| Methicillin-resistant CoNS | 1 (1.16) |
| Pseudomonas aeruginosa | 1 (1.16) |
| **Resistance phenotype among Gram-negative blood isolates (n=3)** | |
| AmpC + ESBL | 1 (33.33) |
| AmpC + MBL | **2 (66.67)** |
| **Stool–blood organism concordance** | |
| Overall concordance | **5.81%** |
| Overall discordance | **94.18%** |
| Cohen's kappa | **−0.007** |
| p-value | **0.334** |

Agreement between stool culture positivity and blood culture positivity was also low, with an overall concordance rate of 11.62% and poor kappa agreement ($\kappa = -0.016$, $p = 0.334$). Among the three Gram-negative bacilli recovered from blood, one (33.33%) demonstrated AmpC + ESBL production and two (66.67%) demonstrated AmpC + MBL production. However, organism-level agreement between surveillance stool culture and subsequent blood culture was poor ($\kappa = -0.007$, $p = 0.334$). The overall concordance rate was only 5.81%, whereas the discordance rate was 94.18%. Thus, despite a high burden of resistant intestinal colonization, the specific organism isolated from surveillance stool culture did not significantly predict the organism subsequently recovered from blood in this cohort.

## Discussion

Patients with hematological malignancies are particularly vulnerable to infection because of the underlying disease, chemotherapy-induced mucosal

injury, prolonged neutropenia, and repeated exposure to broad-spectrum antimicrobial agents. In the present study, 61.43% of the 140 patients developed febrile neutropenia during the 30-day surveillance period. This high frequency emphasizes the infectious risk associated with intensive treatment of hematological malignancies and stem-cell transplantation. Similar concerns regarding infections and antimicrobial-resistant organisms in immunocompromised hematology patients have been emphasized in previous studies [9,10]. Stool surveillance demonstrated a marked predominance of Escherichia coli, which was isolated in 88.57% of patients. Other organisms included Klebsiella pneumoniae, Enterobacter cloacae, Enterococcus faecalis, Shigella flexneri, and Enterococcus faecium. The predominance of Enterobacterales is clinically relevant because the gastrointestinal tract represents an important reservoir of potentially pathogenic Gram-negative organisms in neutropenic patients. Previous studies have similarly demonstrated gastrointestinal colonization by resistant Gram-negative bacteria among patients with hematological malignancies and have investigated its possible relationship with subsequent bloodstream infection [11,12].

An important observation was the high burden of antimicrobial resistance among stool isolates. Among Gram-negative bacilli, AmpC plus MBL production was the most frequent resistance phenotype (43.51%), followed by AmpC plus ESBL (16.03%) and ESBL alone (12.21%). The emergence of multiple β-lactamase mechanisms among intestinal organisms is concerning because colonization with multidrug-resistant bacteria may complicate empirical antimicrobial selection during episodes of febrile neutropenia [13]. This is particularly important in patients undergoing hematopoietic stem-cell transplantation, in whom profound immunosuppression and prolonged antimicrobial exposure increase susceptibility to serious infections [14].

The antimicrobial susceptibility profile of E. coli further demonstrated the magnitude of resistance. Resistance was highest to ampicillin (89.52%), while approximately 72–73% of isolates were resistant to several cephalosporins and ciprofloxacin. Carbapenem resistance was also notable, with 43.55% of isolates resistant to both imipenem and meropenem. In contrast, comparatively greater susceptibility was observed to chloramphenicol, gentamicin and amikacin. These findings reinforce the importance of local antimicrobial surveillance because resistance patterns may substantially influence the effectiveness of empirical therapy. Infections in patients receiving chemotherapy can progress rapidly, making early recognition and appropriate antimicrobial treatment particularly important [15].

Blood-culture positivity, however, was relatively low. Among 86 patients who developed febrile neutropenia, 95.35% of poke cultures and 96.51% of line cultures showed no growth. Gram-negative blood isolates included Acinetobacter baumannii and Pseudomonas aeruginosa. Among the three Gram-negative isolates subjected to β-lactamase characterization, two demonstrated AmpC plus MBL and one demonstrated AmpC plus ESBL production. The occurrence of multiple β-lactamase mechanisms is important because ESBL, AmpC and carbapenemase-mediated resistance may substantially restrict therapeutic options and can complicate laboratory detection and antimicrobial selection [16].

Despite the high prevalence of resistant organisms in stool, organism-level concordance between surveillance stool isolates and subsequent blood culture isolates was poor. The overall organism-level concordance was 5.81%, with a discordance rate of 94.18%; Cohen's kappa was −0.007 ($p = 0.334$). Thus, in this cohort, the organism identified on routine stool surveillance generally did not correspond to the organism subsequently isolated from blood. The small number of positive blood cultures should, however, be considered when interpreting this association. These findings suggest that stool surveillance provides useful information regarding gastrointestinal colonization and the local reservoir of antimicrobial resistance but, when used alone, may have limited ability to predict the specific pathogen responsible for subsequent bloodstream infection. The small number of positive blood cultures should, however, be considered when interpreting this association.

**Limitations of study:** This study was conducted at a single tertiary-care centre and included a relatively small sample. The number of patients with positive blood cultures was low, limiting the ability to establish a reliable association between surveillance stool isolates and subsequent bloodstream pathogens. Further multicentre studies with larger sample sizes are required to validate the predictive value of stool surveillance.

**Future Recommendations:** Future studies should include larger, multicentre cohorts to better evaluate the relationship between intestinal colonization and subsequent bloodstream infections in hematology-oncology patients. Serial stool surveillance at predefined time points during chemotherapy and hematopoietic stem-cell transplantation may help determine changes in intestinal colonization and antimicrobial-resistance patterns over time.

Molecular characterization of resistance determinants may also be considered to assess the genetic relatedness between stool and blood isolates. Further studies should evaluate whether

incorporating stool surveillance results into antimicrobial or infection-control strategies can improve early recognition of patients at risk of bloodstream infection and guide targeted antimicrobial therapy.

## Conclusion

The present study demonstrated a high burden of intestinal colonization with antimicrobial-resistant Gram-negative organisms among hematology-oncology patients, with E. coli being the predominant stool isolate. Febrile neutropenia occurred in 61.43% of patients, while blood-culture positivity remained low.

AmpC, ESBL and MBL-mediated resistance was frequently detected, including substantial carbapenem resistance among stool E. coli.

However, stool and blood isolates showed poor agreement ($\kappa$=−0.007, p=0.334), indicating that routine stool surveillance alone had limited value for predicting the organism responsible for subsequent bloodstream infection.

Nevertheless, surveillance may remain useful for monitoring colonization and institutional antimicrobial-resistance patterns.

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