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Multi-drug Resistant Organisms in Stool Culture and Its Relationship with Morbidity and Mortality in Pediatric Patients with Acute Leukemia

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ABSTRACT

Background: Enteric colonization with multidrug-resistant organisms (MDRO) in acute leukemic pediatric patients may constitute a major risk for serious infections as bloodstream infections (BSI), particularly during periods of neutropenia, and contribute significantly to increased morbidity and mortality.

Objective: To assess the frequency of MDRO isolation from stool cultures of pediatric patients with acute leukemia and its relationship with blood culture positivity and mortality.

Patients and Methods: The study involved analysis of pediatric patients with newly diagnosed acute leukemia less than 18 years of age from January 2019 to June 2019 in Zagazig University Hospital, a total of 60 patients were included in this study. Stool cultures were sent within 48 hours of hospital admission and blood cultures were sent when clinically indicated. Isolates were identified by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) analysis. Antimicrobial susceptibility was tested using VITEK-2. The survival rate for all patients was followed up for 60 days.

Results: Blood culture results were positive in a significantly higher ratio of patients with positive stool culture (36.1%) compared to those with negative stool culture (12.5%) (P=0.043). *Enterococcus faecalis* and *Escherichia coli (E. coli)* were the most frequently isolated MDRO from positive stool cultures (40% and 31%, respectively), while *E. coli* was the most frequent MDRO isolated from blood (50%). MDRO were isolated from stool cultures of 32 patients with a frequency of (53.8 %). Among patients with positive stool and blood cultures, mortality was significantly higher (P < 0.029) among those with MDRO isolated from both cultures compared to those isolating non-MDRO (85.7% vs 16.7%).

Conclusion: Newly diagnosed children with acute leukemia have a high frequency of enteric colonization by MDRO, which is significantly associated with increased positivity of blood cultures and mortality.

Keywords: Multi-drug resistance, Stool culture, Acute leukemia.

INTRODUCTION

Acute leukemia is the most frequent hematological malignancy in children, accounting for around 35% of all malignancies in childhood[(1)].

Bone marrow suppression can cause periodic episodes of leukopenia (especially neutropenia) during therapy, which raises the risk of infections, particularly bloodstream infections (BSI), which are the most common infectious consequences during neutropenia. Sepsis is a leading cause of mortality in these individuals due to an insufficient immunological response [(2)].

The management of febrile neutropenia has grown even more difficult since the number of infections caused by multidrug resistant organisms (MDRO) has increased dramatically in the community and hospitals [(3)]. This is especially concerning because no new antibiotic classes have been identified in the previous two decades, making the battle against drug-resistant bacteria much more difficult [(4-5)].

Prompt administration of empirical antibiotic therapy has been adopted as a mandatory step in managing leukemic patients as it may lower morbidity and mortality of febrile patients[(6-8)]. Understanding the risk factors of BSI may additionally help improve survival in leukemic patients[(9)].

Prior colonization with MDRO has been identified as a significant risk factor for developing MDRO infections[(10, 11)], as it has been discovered that the source of infection in immunocompromised children is frequently endogenous, with most MDRO colonising their skin and mucosal surfaces on a regular basis, causing infections when the host's physical and/or immunological defences are breached by chemotherapy and/or disease [(12)].

In our facility, studies on the relationship between enteric colonization and infection, particularly the risk of BSI are still limited. Therefore, the present study has been designed to assess the frequency of MDRO isolation from stool culture of pediatric patients with acute leukemia (either lymphoblastic, ALL, or myeloid, AML) and its relationship with blood culture positivity and mortality of those patients.

PATIENTS AND METHODS

Along the period from January 2019 to June 2019, a cross-sectional study has been carried out in the Pediatric Department and Clinical Pathology Department, Zagazig University, Egypt. The study included 60 newly diagnosed pediatric patients (mean age 8.6±2.36) with acute leukemia (ALL and AML) for whom induction chemotherapy was undertaken. Stool and blood specimens were obtained from each patient within 48 hours of admission.

Ethical consideration:

The study was approved by the Institutional Review Board (IRB) of Zagazig University and was carried out in consistence with the Declaration of

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Helsinki. Informed consent was obtained from each patient-caregiver before sampling.

Sampling procedure:

For blood culture, 4 ml of blood were withdrawn from central and peripheral lines under complete aseptic measures and inoculated in aerobic "BacT/ALERT" culture bottles (BactAlert; bioMerieux, Marcy-l'Étoile, France). Standard precautions were applied all over the process. Positive blood culture bottles were subcultured on blood agar, chocolate agar, and MacConkey agar (Oxoid, England).

Stool samples were all cultivated for 24 to 48 hours on cysteine lactose electrolyte deficient (CLED) medium. Negative stool cultures included those with no bacterial growth, polymicrobial growth, or typical intestinal bacterial flora such coliforms. Antibiotic sensitivity testing was done on stool cultures that showed a single kind of bacterial growth [(13)].

Preparation of isolates for mass spectrometric identification:

MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight) was used to identify isolates (VITEKMS; bioMerieux).

A single colony was directly applied to a disposable target slide (bioMérieux, Marcy l'Etoile, France) consisting of a polypropylene carrier with a stainless-steel layer, using 1micro Litre loop (Sarstedt, Newton, NC), and then lysed by adding 0.5 micro litre formic acid (25 percent [vol/vol]; bioMérieux) to the isolate and left to dry at room temperature for mass spectrometric analysis. After that, 1 micro litre of matrix solution (bioMérieux, 3.1 percent [wt/vol] -cyano-4-hydroxycinnamic acid) was added and left to dry at room temperature [(14)].

The Vitek MS MALDI-TOF mass spectrometer was used to examine the samples in linear positive-ion mode throughout a mass-to-charge ratio range of 2000–20,000 Da. 500 laser blasts at 50 Hz were fired at each point. To calibrate the target plates before and after data collecting, Escherichia coli (E. coli) ATCC 8739 was employed as a control.

Data analysis:

The Vitek MS identification method allows for a comparison of the generated spectra's features to the Vitek MS v2.0 database. Because of its uniqueness, each peak for each species is given a weight. The programme compares the resulting spectra to peak weights for each species. The confidence value and quantitative values are computed. The degree of resemblance between the tested organism and each of the database's organisms or groups of organisms may then be stated.

Antibiotic susceptibility testing:

VITEK 2 was used to evaluate antibiotic susceptibility in accordance with Clinical Laboratory Standard Institute (CLSI) guidelines[(15)]. Non-susceptibility (i.e., resistant or intermediate) to at least one antimicrobial agent in at least three antimicrobial classes was characterised as MDR bacteria[(16)].

Statistical analysis

The SPSS program (Statistical Package for the Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA) was used. Age was expressed as mean ± standard deviation. Qualitative data were represented as frequencies and relative percentages and were compared by Chi square test (χ^2) or Fisher's exact test. P value ≤ 0.05 was considered significant.

RESULTS

Among a total of 60 patients, 60 % showed positive stool culture. Blood culture results were positive in a significantly higher ratio of patients with positive stool culture compared to those with negative stool culture (Table 1).

Table (1): Frequency of stool culture positivity and blood culture positivity among the studied group

		Positive stool culture	**Negative stool culture**	**P-value**
		No. = 36	**No. = 24**	
Blood culture	Positive	13 (36.1%)	3 (12.5%)	**0.043**
	Negative	23 (63.9%)	21 (87.5%)	

The types and antibiotic susceptibility patterns of the obtained isolates are demonstrated in table 2. As for MDRO isolates, gram-positive cocci presented by *E. faecalis* was the most frequently isolated MDRO from positive stool cultures, followed by *E. coli*. However, gram-negative bacilli presented by *E. coli* was the most frequently isolated MDRO from blood cultures.

Table (2): Microbiological profile and antibiotic sensitivity pattern of MDRO

Organism Number (%)	Carbapenem	Aminoglycoside	Tigecycline	Ampicillin	Vancomycin/ teicoplanin/ Linezolid	Colistin
Stool MDR Gram +ve (stool MDR+ve = 32 patients)						
Enterococcus faecalis **(13)**	**R - 13/13 (100%)**	**R - 13/13 (100%)**	**S - 13/13 (100%)**	**R - 13/13 (100%)**	**R - 13/13 (100%)**	-
Stool MDR Gram –ve (stool MDR+ve = 32 patients)						
Escherichia coli **(10)**	**S - 1 /10 (10 %)**	**R-10/10 (100%)**	**S-10/10 (100%)**	-	-	**S-10/10 (100%)**
Klebsiella oxytoca **(5)**	**R- 5/5 (100%)**	**R- 5/5 (100%)**	**S- 5/5 (100%)**	-	-	**S- 5/5 (100%)**
Klebsiella pneumoniae **(4)**	**R- 4/4 (100%)**	**R- 4/4 (100%)**	**S- 4/4 (100%)**	-	-	**S- 4/4 (100%)**
Stool non- MDR Gram +ve (Stool non- MDR = 4 patients)						
Enterococcus faecalis **(2)**	**S- 1/2 (50%)**	-	**S- 2/2 (100%)**	**S- 1/2 (50%)**	**S- 2/2 (100%)**	-
Yeast candida tropicalis **(2)**	S-Fluconazole/voriconazole/amphotericin-B					
Blood MDR Gram –ve (blood MDR +ve = 6 patients)						
Escherichia coli **(3)**	**R- 3/3 (100%)**	**S- 2/3 (67 %)**	**S- 3/3 (100%)**	-	-	**S- 3/3 (100%)**
Klebsiella pneumoniae **(2)**	**R- 2/2 (100%)**	**R- 2/2 (100%)**	**S- 2/2 (100%)**	-	-	**S- 2/2 (100%)**
Klebsiella oxytoca **(1)**	**R- 1/1 (100%)**	**R- 1/1 (100%)**	**S- 1/1 (100%)**	-	-	**S- 1/1 (100%)**
Blood non MDR Gram +ve (blood Non-MDR = 4 patients)						
Staphylococcus aureus**(2)**	**S- 2/2 (100%)**	**S- 2/2 (100%)**	**S- 2/2 (100%)**	**R - 1/2 (50 %)**	**S- 2/2 (100%)**	-
Blood non MDR Gram –ve						
Klebsiella pneumoniae**(1)**	**S- 1/1 (100%)**	**S- 1/1 (100%)**	**S- 1/1 (100%)**	-	-	**S- 1/1 (100%)**
Escherichia coli **(1)**	**S- 1/1 (100%)**	**S- 1/1 (100%)**	**S- 1/1 (100%)**	-	-	**S- 1/1 (100%)**

MDR, multi-drug resistant; S, sensitive; R, resistant.

MDRO were isolated from 89% (32/36) of positive stool cultures constituting 53.3% (32/60) of the total cohort of patients. Among MDRO stool cultures positive patients (n=32), 36.1% (n=13) had positive blood cultures where 53.8% of them (n=7) had MDRO and 46.1% (n=6) had non-MDRO (Table 3).

Table (3): Stool culture results, blood culture results, and outcome of studied patients (n=60)

Positive stool culture 36 (60%)	MDRO +ve 32 (89%)	Blood +ve 13 (40.6%)	MDRO +ve 7 (53.8%)	Deaths 6 (85.7%)
			Non-MDRO 6 (46.2%)	Deaths 1 (16.7%)
		Blood -ve 19 (59.4%)		
	Non-MDRO 4 (11%)			
Negative stool culture 24 (40%)		Blood +ve 3 (12.5%)	MDRO +ve 1 (33%)	Deaths 1 (100%)
			Non-MDRO 2 (67%)	Deaths 0 (0.0%)
		Blood -ve 21 (87.5%)		

MDRO, multidrug-resistant organisms

The death rate (within 60 days follow-up) was significantly higher among those with MDRO isolated from both stool and blood cultures compared to those with non-MDRO (Table 4). However, among negative stool and positive blood culture patients, a non-significant difference was detected in the death rate between patients yielding MDRO in their blood culture and those yielding non-MDRO (Table 5).

When stool and blood culture results were compared in those isolating MDRO from both cultures, 85.7% of them showed concordant results with the same organism isolated from both cultures. The death rate was 85.7% among them. One patient had different organisms in both cultures and died during the follow-up period (Table 4).

Table (4): Comparison between positive blood and positive stool cultures as regard MDRO mortality rate

Positive blood and stool		MDRO	Non MDRO	P-value
		No. = 7	No. = 6	
Mortality	Died	6 (85.7%)	1 (16.7%)	**0.029**
	Alive	1 (14.3%)	5 (73.3%)	

MDRO, multidrug-resistant organisms

Table (5): Comparison between positive blood and negative stools cultures as regard MDRO mortality rate

Positive blood and negative stool		MDRO	Non MDRO	P-value
		No. = 1	No. = 2	
Mortality	Died	1 (100.0%)	0 (0.0%)	0.333
	Alive	0 (0.0%)	2 (100.0%)	

MDRO, multidrug-resistant organisms

DISCUSSION

Infections are a leading source of morbidity and death in children with hematologic malignancies, particularly during neutropenia episodes. The cause of infection is frequently endogenous, since most MDRO colonize their skin and mucosal surfaces on a regular basis, producing infections when the host's physical and/or immunological defences are compromised by chemotherapy and/or illness [3, 12]. The rising prevalence of MDRO infection in leukemia patients has made febrile neutropenia therapy difficult and costly [17,18].

This study has shown that 53.8% of newly diagnosed acute leukemia patients had grown MDRO in their stool cultures upon admission. This reflects the widespread of MDRO in community settings. Previous community-based studies recorded a prevalence of MDRO in stool cultures that ranged from 24% to 38%[19-21]. The higher ratio recorded in the current study could be attributed to several factors. The easy availability of antibiotics without prescription, overuse of antibiotics in agriculture and animals, which exceeds human use, in addition to the use of antibiotics in the household products such as antibacterial soaps or gels as well as triclosan in plastics, and probably the lack of proper antibiotic stewardship, all can contribute to this finding[13, 17, 18]. Furthermore, most of the study patients had received empiric antibiotics prior to admission or within 72 hour of admission. This is because fever is the most common presenting symptom of acute leukemia. This additionally, may have contributed to the finding of the high prevalence of MDRO in those patients.

In this study, patients who had positive stool cultures had considerably greater blood culture positivity and mortality than those who had negative stool cultures. There was also a high concordance between the MDRO in the stool and blood, confirming that it is the patients' endogenous gut flora that is responsible for the bacteremia. This comes consistent with previous reports proposing mucositis caused by anthracyclines during AML and ALL induction as an event that could be associated with an increased translocation of bacteria from the gut into the bloodstream[12-14]. However, the obtained isolated were identified and compared at the phenotypic level only in this study, not the genotypic one.

The finding of this study comes consistent with **Shankar and colleagues**[17] who reported a high prevalence of MDRO in newly diagnosed children with acute leukemia (87%) and showed that colonization with MDRO in stools is associated with increased positivity of blood cultures and mortality. Furthermore, they reported a high concordance between the MDRO in the stool and blood (86%)[13].

The most frequent MDRO isolated from stool cultures in this study was *E. faecalis* (40 %) followed by *E. coli* (31 %). It was surprising that gram-positive bacteria were the most common MDRO in the stool. However, this comes consistent with what was reported by **Shankar *et al.*** [17]**.** On the other hand, gram-negative bacilli (*E. coli* and *Klebsiella* species) were the most frequently MDRO isolated in the blood. Gram-negative organisms were four times more frequent (81%) than gram-positive organisms (19 %) in the present study. This has been observed in previous reports from other studies[3, 12].

The findings of this study highlight the high prevalence of enteric colonization by MDRO in newly diagnosed leukemic pediatric patients and its significant impact on bacteremia and patient mortality. This requires the implementation of more strict infection prevention control strategies to limit further spread of these organisms in the oncology unit and the adoption of a judicious antibiotic approach for the treatment of febrile neutropenia[3]. However, novel effective medicines such as fecal duodenal infusion for the elimination of resistant infections and immune-based or target host inflammatory therapies should be considered before initiating rigorous chemotherapy in children with cancer [22-23].

CONCLUSION

Newly diagnosed children with acute leukemia have a high frequency of enteric colonization by MDRO, which is significantly associated with increased positivity of blood cultures and mortality.

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