Antimicrobial Susceptibility Status of Bloodstream Infections in Children Presenting with Acute Lymphoblastic Leukemia at Moi Teaching and Referral Hospital in Kenya

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Abstract

Acute Lymphoblastic Leukaemia occurs mainly in children leading to immuno-compromised patients susceptible to bloodstream infections. Choice of antimicrobial treatment requires the epidemiological knowledge of pathogens, which constantly changes, necessitating periodic review. Knowledge of antimicrobial susceptibility profiles of pathogens would not only help in formulating an antibiotic policy, but also planning for the larger infection prevention and control measures. The aim of this study was to determine the antimicrobial profiles of bloodstream pathogens against commonly prescribed antibiotics in children presenting with ALL at the Moi Teaching and Referral Hospital. A cross-sectional study was used to investigate 80 children with ALL in MTRH with approval from MTRH Ethical Review Committee. Blood samples were collected into Bactec™ bottles for culture. Positive cultures were sub-cultured and characterized to identify the pathogens. Antimicrobial susceptibility was performed by disc diffusion method against Azithromycin, Cefuroxime, Ceftriaxone, Clindamycin, Co-trimoxazole, Cefepime, Gentamycin, Imipenem, Meropenem, Levofloxacin, Nitrofurantoin, Piperacillin/Tazobactam. The diameter of the clear zones was measured and interpreted as Susceptible, Intermediate or Resistant to a particular antimicrobial as per CLSI guidelines (CLSI, 2022). E. coli ATCC 25922 and S. aureus ATCC 1026 were used as positive controls for gram-negative and gram-positive isolates respectively. All experiments were conducted in triplicates. The analysis determined the Susceptible, Intermediate or Resistant pathogens to particular antimicrobials. A total of 24 bacteria were isolated. Coagulase negative Staphylococcus species was the most isolated at 66.67% with Acinetobacter species and Staphylococcus aureus accounting for 8.33% and 25% respectively. All isolates were 100% sensitive to Cefepime, Meropenem and Piperacillin/Tazobactam. The other antibiotics had at least one instance of intermediate sensitivity and/or antibiotic resistance recorded. The study established a statistically significant relationship between bacterial isolates associated with bloodstream infections in children presenting with acute lymphoblastic leukaemia at MTRH and their susceptibility to commonly prescribed antibiotics (p-value =0.0000; $P \leq 0.05$). This study concludes that the bacterial isolates obtained from children presenting with ALL at MTRH have high sensitivity to Cefepime, Meropenem and Piperacillin/Tazobactam but minimal sensitivity to Azithromycin and Cefuroxime. Continued surveillance and periodical monitoring to determine the susceptibility profile of the most commonly isolated bloodstream pathogens in particular a population to enhance clinical approach and antibiotics treatment among children with ALL is recommended. Re-evaluation of treatment options particularly the use of Azithromycin and Cefuroxime in routine treatments should also be taken into consideration to curb antibiotic resistance.

Keywords: Acute lymphoblastic leukaemia, Bloodstream infections, Antimicrobial, Sensitivity

INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is the most common type of cancer and leukaemia affecting children worldwide, accounting for approximately 25% of all childhood cancers and 75% of paediatric leukaemia cases (Khazaei *et al.,* 2019). In Western Kenya, where Moi Teaching and Referral Hospital (MTRH) is located, ALL accounts for 15% of all childhood cancer, and is ranked second after non-Hodgkin lymphoma (Mostert *et al.,* 2012).

Bloodstream infections (BSIs) are defined as a positive isolate in a blood culture that is associated with clinical findings and cause severe complications in children with ALL during chemotherapy (Yao *et al.,* 2016). BSIs consequent to immunosuppressive therapy have become a major cause of morbidity and mortality in this population; not only increasing the duration of hospital stay, but also significantly increasing the cost of treatment (Cerceo *et al.,* 2016). Children with cancer account for up to 18% of severe sepsis in children, with Intensive Care Unit mortality rates as high as 64% in high-risk children (Hartman *et al.,* 2013).

Antimicrobial treatment is defined as adequate when an active antimicrobial agent is prescribed at appropriate doses, at appropriate dosing intervals, and by the correct route and with the capacity to achieve adequate concentrations in the infectious focus (Abushaheen *et al.,* 2020). The choice of antimicrobial treatment requires the knowledge of the epidemiology of common pathogens in the given setting, which constantly changes, necessitating periodic review (Kaye & Pogue, 2015). Furthermore, knowledge of contemporary epidemiological and drug sensitivity profiles of bacterial pathogens would not only help in formulating antibiotic policy, but also in planning for the larger infection prevention and control measures. This study aimed at identifying and determining the antimicrobial susceptibility profiles of bacteria associated with blood stream infections in children presenting with acute lymphoblastic leukaemia at MTRH.

MATERIALS AND METHODS

A cross-sectional study among children aged 15 years and below with confirmed clinical diagnosis of acute lymphoblastic leukaemia was conducted at Moi Teaching and Referral Hospital. The institution has a designated cancer centre for outpatient services known as Chandaria Cancer and Chronic Diseases Centre and an inpatient facility known as Shoe4Africa children's hospital. Children presenting with acute lymphoblastic leukaemia (ALL) are attended to in these two facilities at Moi Teaching and Referral Hospital. The study targeted children (aged 15 years and below) presenting with fever or suspected to have a blood stream infection by their attending clinician at Chandaria cancer and chronic diseases centre and Shoe4Africa Children's hospital within Moi Teaching and Referral Hospital (MTRH).

A parental/guardian informed consenting process and a paediatric assent procedure (if the child was seven years or older) were first conducted where the study objectives, methods, potential risks and benefits, confidentiality and participant autonomy were clearly explained. If the parent/guardian and/or the child agreed to participate in the study, then sample collection was subsequently conducted. Ethical approval to conduct this research was sought and granted by the Moi Teaching and Referral Hospital

(MTRH)/ Moi University (MU) Institutional Research Ethics Review Committee (IREC) under approval no. **0003171**

The children who met eligibility requirements were sampled consecutively until the desired sample size (80) was achieved. From every participant, a sample for blood culture was drawn after cleansing the skin site using 70% isopropyl alcohol for 30 seconds followed by 1-2% tincture iodine and isopropyl alcohol. Approximately 2 ml blood sample was drawn from a peripheral vein and inoculated into two BactT/Alert (Paed Plus) bottles (Aerobic and Anaerobic) at a volume of approximately 1 ml each. Samples were transported from Chandaria Cancer Centre and Shoe 4 Africa Children's hospital to the microbiology laboratory in a cool-box. The bottles were then incubated in a Bactec incubator for 7 days at 37°C. Each set of blood culture consisted of two bottles, one for Standard Aerobic/F culture and the other for Standard Anaerobic/F culture. Positive cultures were Gram-stained and sub-cultured onto sheep blood agar, MacConkey agar and chocolate agar. Isolates of bacteria were identified by a combination of morphological characteristics, gram staining and a variety of biochemical techniques to identify the bacteria responsible for the bloodstream infections.

Antimicrobial susceptibility testing of isolated pathogens to clinically used antimicrobials was performed by disc diffusion method and interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2022). A suspension of bacteria pure colony was spread over the surface of Muller-Hinton agar plate and paper discs containing each antibiotic (Azithromycin, Cefuroxime, Ceftriaxone, Clindamycin, Co-trimoxazole, Cefepime, Gentamycin, Imipenem, Meropenem, Levofloxacin, Nitrofurantoin, Piperacillin/Tazobactam) were placed onto the inoculated surfaces. After overnight incubation at 37^0 Celsius, the diameter of the clear zones (Figure 1) produced by antimicrobial inhibition of bacterial growth was measured and interpreted as Susceptible, Intermediate or Resistant to a particular antimicrobial as per CLSI guidelines (CLSI, 2022). American Type Culture Collection (ATCC) *E. coli* ATCC 25922 and *S. aureus* ATCC 1026 bacteria were used as positive controls for gram-negative and gram-positive isolates. All the experiments were conducted in triplicates**.** The results were shared with the attending clinicians and a copy given to the child's parent/guardian. ANOVA was used to determine inferential statistical significance between data sets (test isolates and the controls) at 95% confidence level ($p \leq 0.05$) and considered to be statistically significant if the difference in antimicrobial performance between the tested isolates and the controls had p values of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

A total of twenty-four (24) bacterial pathogens were isolated in this study. Gram negative organisms accounted for 8.33% (2) while gram positive bacteria accounted for 91.67% (22) of the total isolates. Coagulase negative *Staphylococcus* species (CoNS) was the most isolated bacterial group at 66.67% with *Acinetobacter* species and *Staphylococcus aureus* accounting for 8.33% and 25% respectively as indicated in Table 1 below.

In this study, a total of twelve (12) commonly prescribed antibiotics of different classes/families were tested against the isolated bacteria. The tested antibiotics were Azithromycin, Cefuroxime, Ceftriaxone, Clindamycin, Co-trimoxazole, Cefepime, Gentamycin, Imipenem, Meropenem, Levofloxacin, Nitrofurantoin and Piperacillin/Tazobactam.

Table 1: Distribution of isolates associated with Blood stream infections by gram reaction type

Type of Organism	ISOLATE	No. of isolates	% of Isolates
Gram negative Cocci	*Acinetobacter species*	2	8.33
Gram positive Cocci	*Staphylococcus aureus*	6	25
Gram positive Cocci	Coagulase negative *Staphylococcus* species	16	66.67

The isolated bacteria showed varying degrees of susceptibility to the antibiotics they were subjected to. All the isolates in this study showed 100% sensitivity to Cefepime, Meropenem and Piperacillin/Tazobactam. These findings concur with the findings of Chen *et al.* (2019) who documented that the sensitivity of the bacteria they isolated to Piperacillin/Tazobactam, Imipenem, Meropenem, and Amikacin were high. Fawad (2022) also documented that all the bacteria they isolated showed high sensitivity to Meropenem, Clindamycin and Piperacillin/Tazobactam similar to the findings of the current study. Yao *et al.* (2016) reported that most commonly isolated bacteria from bloodstreams of children with Acute lymphoblastic Leukaemia (ALL) are resistant to Carbapenem antibiotics (Imipenem and Meropenem) but highly sensitive to Amikacin. Irfan *et al.* (2008) however reported no resistance to Meropenem.

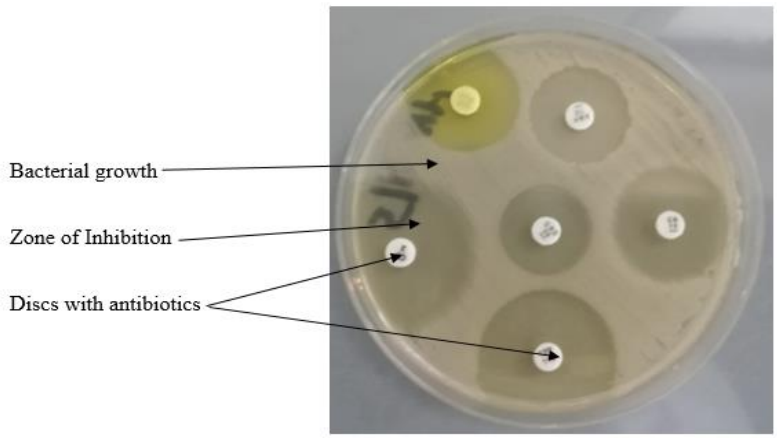

Figure 1: Antimicrobial susceptibility test results showing clear zones of inhibited bacterial growth

Acinetobacter species were 100% susceptible to Cefepime, Ceftriaxone, Clindamycin, Gentamycin, Imipenem, Meropenem and Piperacillin/Tazobactam while 50% were susceptible to Azithromycin, Cefuroxime, Co-trimoxazole, Levofloxacin and Nitrofurantoin. The findings of this study concur with the study findings of Pokhrel *et al.*, (2018) that *Acinetobacter* species isolated from the bloodstream have good susceptibility to commonly prescribed first- and second-line antibiotics particularly Cephalosporins, Macrolides and Tetracyclines.

Staphylococcus aureus isolated in this study were 100% resistant to Ceftriaxone and Imipenem, 50% resistant to Cefuroxime but 100% susceptible to Azithromycin, Clindamycin, Co-trimoxazole, Cefepime, Gentamycin, Meropenem, Levofloxacin, Nitrofurantoin and Piperacillin/Tazobactam. This concurs with the findings of Bhat *et*

al., (2011) who documented that *S. aureus* presented better susceptibility to commonly used antibiotics like Azithromycin, Ceftriaxone and Clindamycin.

Isolated Coagulase negative *Staphylococcus* species (CoNS) in the current study showed varying susceptibility to antibiotics at 100% to Cefepime, Imipenem, Meropenem and Piperacillin/Tazobactam, 93.75% to Gentamycin, 81.25% to Clindamycin, Levofloxacin and Nitrofurantoin, 62.5% to Co-trimoxazole, 56.25% to Ceftriaxone, 25% to Azithromycin and 18.75% to Cefuroxime as determined by their growth inhibition (Figure 2). These findings differ with Chen *et al.* (2019) findings as the author documents that CoNS isolated in their study were often resistant to a variety of antibiotics, including Penicillin, Amoxicillin, Erythromycin, Azithromycin and Cotrimoxazole, but were sensitive to Gentamicin, Rifampicin and Levofloxacin.

ANOVA determined a statistically significant p-value of 0.0000 at 95% confidence level ($P \leq 0.05$) (Table 2). The findings indicate that bacterial isolates associated with bloodstream infections in children presenting with acute lymphoblastic leukaemia at Moi Teaching and Referral Hospital (MTRH) are susceptible to commonly prescribed antibiotics.

Table 2: ANOVA table

Differences in performance	p-value	Inference
Between antibiotics on isolates	0.0000	Significant
On controls		

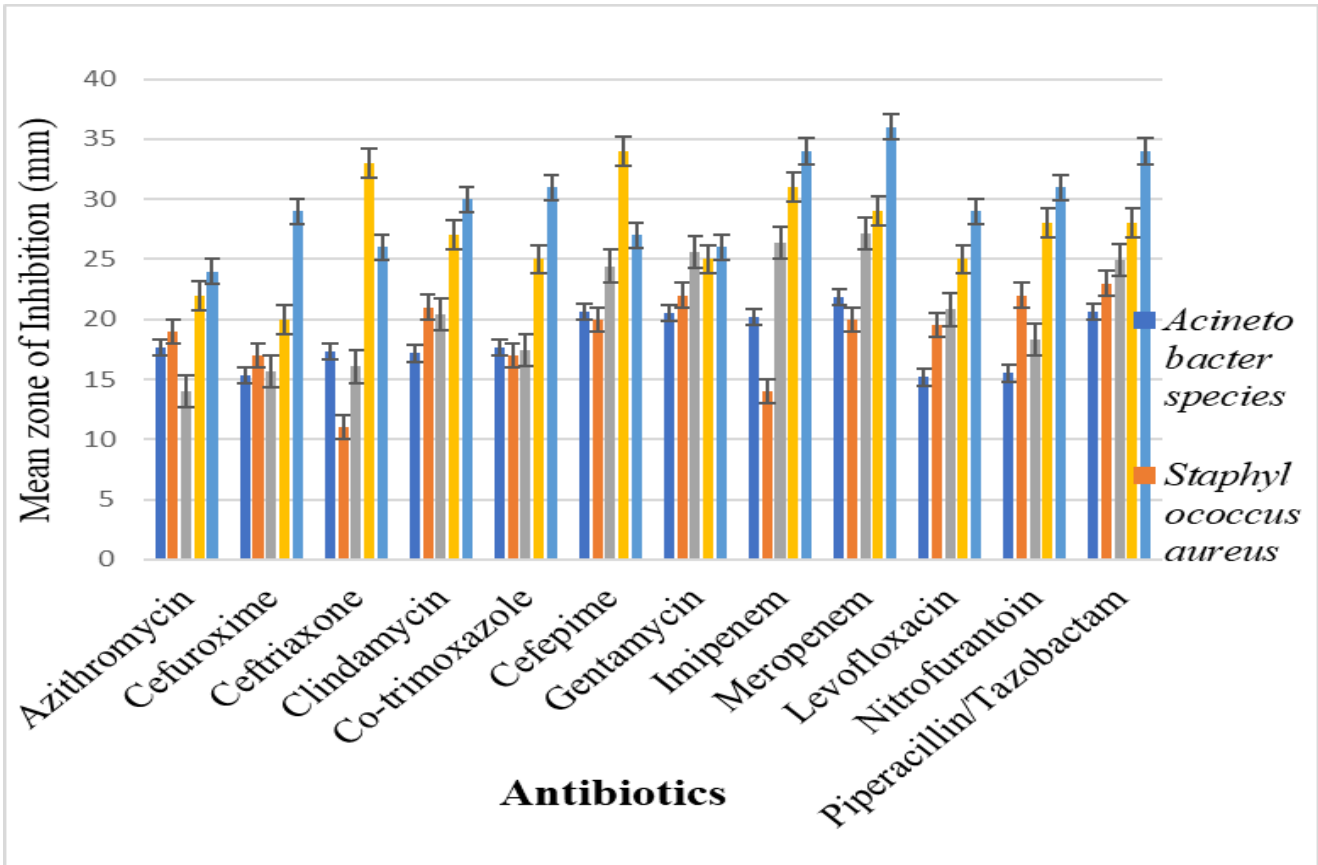

Figure 2: Antibacterial Susceptibility profiles of bloodstream isolates obtained from children presenting with ALL at MTRH.

CONCLUSIONS AND RECOMMENDATIONS

In this study, twenty-four (24) bacteria groups were isolated and identified to be Coagulase negative *Staphylococcus* species (CoNS), *Acinetobacter* species and *Staphylococcus aureus*. Coagulase negative *Staphylococcus* species (CoNS) was the most commonly isolated bacterial group at 66.67% with *Acinetobacter* species and *Staphylococcus aureus* accounting for 8.33% and 25% respectively. These isolates showed maximum sensitivity towards Cefepime, Meropenem and Piperacillin/Tazobactam. The other antibiotics had at least one instance of intermediate sensitivity and/or antibiotic resistance recorded. The bacterial isolates obtained from children presenting with Acute Lymphoblastic Leukemia at Moi Teaching and Referral Hospital have high sensitivity to Cefepime, Meropenem and Piperacillin/Tazobactam but minimal sensitivity to Azithromycin and Cefuroxime ($p = 0.0000$; $P \leq 0.05$).

The current study recommends prescription of the effective antibiotics and continued surveillance and periodical monitoring to determine the susceptibility profile of the most commonly isolated bloodstream infections microorganisms in a particular population to enhance the clinical approach and antibiotics treatment among children with ALL. Re-evaluation of treatment options particularly the use of Azithromycin and Cefuroxime in routine treatments should also be taken into consideration to prevent widespread antibiotic resistance.

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