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Antibiotic Resistance Patterns of Bacterial Isolates from Blood Cultures in Neonatal Sepsis Cases at Benghazi Medical Centre NICU

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Abstract

Neonatal sepsis is a major cause of morbidity and mortality in NICUs worldwide, complicated increasingly by antibiotic-resistant bacteria. Monitoring bacterial pathogens and their resistance patterns is crucial for informing effective treatment strategies. To identify bacterial isolates from positive blood cultures in neonates with sepsis admitted to the NICU at Benghazi Medical Centre and to assess their antibiotic resistance profiles. A retrospective review of 90 neonates with suspected sepsis was conducted; 67 with culture-confirmed sepsis were included. Clinical, laboratory, and microbiological data were analyzed. Antibiotic susceptibility was assessed, and data were analyzed using SPSS version 23. Gram-negative bacteria predominated (70.1%), with *Acinetobacter* (35.8%) and *Klebsiella* (31.3%) as the most common isolates. High resistance rates were observed to colistin (94.8%), amikacin (72.5%), and levofloxacin (67.2%). The overall mortality rate was 32.8%. Multidrug-resistant gram-negative bacteria, particularly *Acinetobacter* and *Klebsiella*, are the primary pathogens in neonatal sepsis at Benghazi Medical Centre. The high resistance rates highlight the urgent need for continuous surveillance and strict antibiotic stewardship to improve outcomes.

Keywords: Neonatal Sepsis, Antibiotic Resistance, Bacterial Isolates, Neonatal Intensive Care Unit (Nicu), Benghazi Medical Center, Multidrug Resistance

INTRODUCTION

Neonatal sepsis is a clinical syndrome occurring in infants within the first 28 days of life, characterized by systemic infection and bacteraemia, and remains a leading cause of morbidity and mortality in this age group (Kariniotaki et al., 2024; Singh et al., 2024; Vogel, 2017). It manifests as a systemic inflammatory response to infections caused by a variety of pathogens, including bacteria, viruses, and fungi, which can lead to multi-organ dysfunction, failure, and death (Kariniotaki et al., 2024; Singh et al., 2024). Despite advances in understanding sepsis pathophysiology and therapeutic approaches over the past two decades, neonatal mortality related to sepsis has not significantly decreased. Globally, neonatal sepsis accounts for approximately one million deaths annually (Vogel, 2017; WHO, 2025).

The risk of acquiring infections decreases with increasing gestational age, as neonates-especially preterm infants-have immature immune systems with reduced cell-mediated immunity. Key risk factors include premature rupture of membranes before labor, maternal infections or fever during

labor (including Group B Streptococcus colonization and intra-amniotic infection), preterm delivery, low birth weight, low Apgar scores or need for resuscitation, and male gender. Additionally, low socioeconomic status is associated with higher incidence and mortality from neonatal sepsis (Maurice, 2025).

Neonatal sepsis is classified based on timing of onset into early-onset sepsis (EOS), occurring within the first 7 days of life, and late-onset sepsis (LOS), occurring between 8 and 28 days (Kariniotaki et al., 2024; Singh et al., 2024; Wu et al., 2025). EOS often presents as a severe multi-system disease with respiratory failure, shock, and meningitis in approximately 30% of cases and is primarily caused by pathogens transmitted vertically from the mother during delivery. Common bacteria involved in EOS include Group B Streptococcus, *Escherichia coli*, *Listeria monocytogenes*, other streptococci, enterococci, and *Haemophilus influenza* (Kariniotaki et al., 2024; Singh et al., 2024). In contrast, LOS typically affects previously healthy full-term infants after discharge and presents with nonspecific symptoms such as lethargy, poor feeding, seizures, and fever. LOS is commonly caused by hospital-acquired pathogens, including coagulase-negative staphylococci, *Staphylococcus aureus*, *Escherichia coli*, enterococci, and various gram-negative bacteria such as *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Citrobacter*, and *Serratia* species, as well as *Candida* species. These organisms can cause focal infections like meningitis, osteomyelitis, arthritis, and urinary tract infections (Kariniotaki et al., 2024; Singh et al., 2024; Wu et al., 2025).

The World Health Organization recommends injectable gentamicin and ampicillin as first-line treatment for hospitalized neonates with sepsis, and recent reviews support continued use of this regimen (Fuchs et al., 2018). However, rising antimicrobial resistance and sepsis-related complications led many international guidelines to recommend broader-spectrum antibiotics—either alone or in combination—such as cephalosporins, imipenem, vancomycin, piperacillin/tazobactam, amikacin, metronidazole, and clindamycin (Fuchs et al., 2018; Gaze, 2025; WHO, 2025). This reflects current clinical practice, where first-line treatments may be supplemented or replaced to address resistant pathogens and complex infections. Understanding the bacterial pathogens involved in neonatal sepsis and their antibiotic resistance patterns is crucial for guiding effective treatment strategies at both national and local healthcare levels (Dudeja, 2020; Singh et al., 2018).

This study aims to identify the common bacterial pathogens causing neonatal sepsis in the Neonatal Intensive Care Unit (NICU) at Benghazi Medical Centre and to determine their antibiotic sensitivity patterns. The findings will guide effective antimicrobial therapy and improve infection control strategies within the NICU.

MATERIALS AND METHODS

Study Design and Setting

This retrospective study was conducted in the Neonatal Intensive Care Unit (NICU) at Benghazi Medical Centre, Libya. Medical records of neonates admitted to the NICU during the study were reviewed.

Study Population

Neonates who had blood cultures performed due to suspected sepsis were considered for inclusion. Neonates with negative blood culture results (no bacterial growth) were excluded. A total of 67 neonates with positive blood cultures were included in the final analysis.

Data Collection

Data on bacterial isolates and their antibiotic susceptibility were obtained from microbiology labor-

atory records. Clinical and demographic data were excluded to maintain focus on microbiological analysis, consistent with the study's primary aim.

Laboratory Procedures

Blood cultures were processed according to standard microbiological protocols at the Benghazi Medical Centre microbiology laboratory. Identification of bacterial isolates and antibiotic susceptibility testing were performed. Antibiotic susceptibility was interpreted following the Clinical and Laboratory Standards Institute guidelines.

Antibiotic Treatment Protocol

Empirical antibiotic therapy in the NICU followed a stepwise protocol. First-line treatment consisted of amikacin or gentamicin combined with amoxicillin-clavulanate. If clinical response was inadequate or cultures indicated resistant organisms, second-line antibiotics such as meropenem or ciprofloxacin were initiated. Third-line therapy was guided by antibiotic susceptibility results, with vancomycin and levofloxacin commonly used for resistant infections.

Statistical Analysis

Data were analyzed using SPSS version 23. Descriptive statistics summarized bacterial isolates and antibiotic resistance patterns. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as means ± standard deviations or medians with interquartile ranges, as appropriate.

RESULTS.

The study included 67 neonates with culture-confirmed sepsis admitted to the NICU at Benghazi Medical Centre. The majority were male (61.2%) and weighed ≥ 2500 grams (61.2%). Most neonates were born at term (≥ 37 weeks' gestational age, 67.2%). Late-onset sepsis was more common (55.2%) than early-onset sepsis (32.8%). Maternal risk factors such as urinary tract infection, premature rupture of membranes, and chorioamnionitis were present in 29.9% of cases. Nearly all neonates (98.5%) had a history of medical interventions, including umbilical line placement, chest tube insertion, or intubation. Abnormal Apgar scores were observed in 82.1% of neonates. Laboratory findings revealed normal white blood cell counts in 76.1% of cases, while thrombocytopenia was noted in 46.3%. C-reactive protein (CRP) levels were <20 mg/L in 46.3% and >60 mg/L in 35.8% of neonates All neonates received first-line antibiotic therapy; 91% required escalation to second-line antibiotics, and 37.3% progressed to third-line treatment. The overall mortality rate was 32.8% (Table 1).

Gram-negative bacteria predominated, accounting for 70.1% of isolates. The most common pathogens were *Acinetobacter* (35.8%) and *Klebsiella* (31.3%), followed by *Staphylococcus aureus* (17.9%). Less frequent isolates included *Staphylococcus haemolyticus*, *Enterococcus*, and *Moraxella*, comprising 9% and 3% of isolates, respectively (Figure 1).

High resistance rates were observed to colistin (94.8%), amikacin (72.5%), and levofloxacin (67.2%), indicating a significant prevalence of multidrug-resistant organisms. Gentamycin and ciprofloxacin showed moderate resistance rates (44.4% and 43.3%, respectively), while meronam (39.6%) and imipenem (33.8%) had relatively lower resistance. The lowest resistance was noted for ceftoxitin (22.5%) and amoxicillin-clavulanate (18.3%), suggesting these may be more effective options. Resistance percentages were calculated excluding untested samples, underscoring the need for targeted antibiotic stewardship. (Table 2).

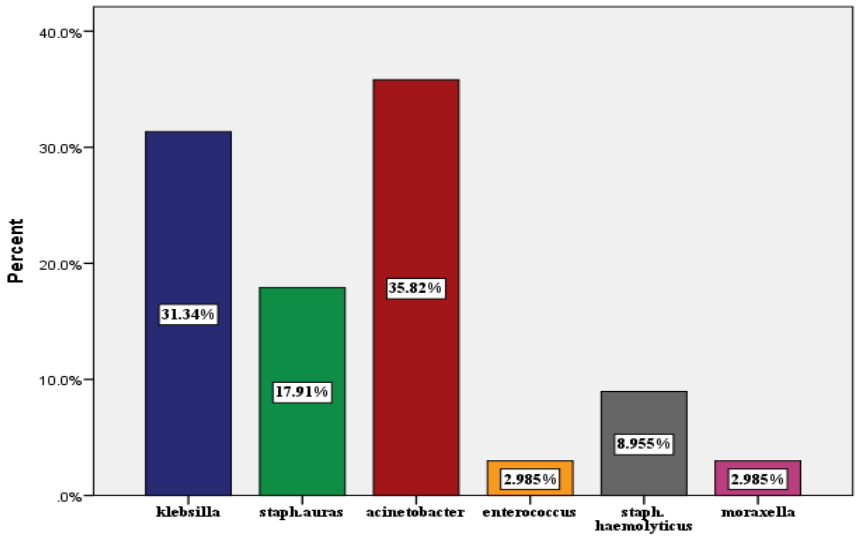

Figure: (1). illustrates the distribution of bacterial pathogens, with Acinetobacter being the most prevalent pathogen (35.8%), followed by Klebsiella (31.3%) and Staphylococcus aureus (17.9%). The lowest bacterial isolates were Staphylococcus haemolyticus, Enterococcus, and Moraxella, with 9% and 3%, respectively

Table:(1). General characteristics of study population

Characteristics of neonates		Number	Percent
Gender	Male	41	61.2%
	Female	26	38.8%
Weight	< 2500 g	41	61.2%
	≥ 2500 g	25	37.3%
Gestational age	< 37 weeks	45	67.2%
	≥ 37 weeks	22	32.8%
Onset of sepsis	Early onset	37	55.2%
	Late onset	30	44.8%
Type of isolated bacteria	Gram -ve	47	70.1%
	Gram +ve	20	29.9%
History of maternal risk factors	Yes	22	32.8%
	No	45	67.2%
History of intervention	Yes	66	98.5%
	No	1	1.5%
Apgar score	Normal	12	17.9%
	Abnormal	55	82.1%
Laboratory findings			
C. Reactive protein	< 20	31	46.3%
	20-60	12	17.9%
	> 60	24	35.8%
White blood cell	Normal	51	76.1%
	Leucopenia	15	22.4%
	Leukocytosis	1	1.5%
Platelets	Normal	36	53.7%
	Thrombocytopenia	31	46.3%
Type of antibiotic			
Second-line antibiotics	Yes	61	91%
	No	6	9%
Third-line antibiotics	Yes	25	37.3%
	No	42	62.7%
Outcome	Discharge	45	67.2%
	Die	22	32.8%

CRP = C-reactive protein, Apgar score = Assessment of newborn health at 1 and 5 minutes after birth,
Leucopenia = Low white blood cell count, Leukocytosis = High white blood cell count, Thrombocytopenia = Low platelet count

Table (2) Antibiotic susceptibility profile of bacterial isolates from neonatal sepsis cases (n=67).

Antibiotic	Resistant (R) n (%)	Intermediate (I) n (%)	Sensitive (S) n (%)	Total Tested (n)
Colistin	63 (94.8)	0 (0)	3 (4.5)	66
Amikacin	48 (72.5)	2 (3.0)	16 (24.2)	66
Levofloxacin	45 (67.2)	1 (1.5)	21 (31.3)	67
Gentamicin	28 (44.4)	3 (4.8)	32 (50.8)	63
Ciprofloxacin	29 (43.3)	0 (0)	38 (56.7)	67
Meropenem	25 (39.6)	0 (0)	38 (60.3)	63
Imipenem	22 (33.8)	0 (0)	43 (66.2)	65
Ceftoxitin	5 (7.5)	0 (0)	62 (92.5)	67
amoxicillin-clavulanate	4 (6.0)	0 (0)	63 (94.0)	67

Resistance (R), intermediate (I), and sensitivity (S) were determined according to standard microbiological criteria. Percentages are calculated as (number in category/total tested) × 100. The total number tested varies by antibiotic due to the availability of susceptibility data.
R = Resistant, **I** = Intermediate susceptibility. **S** = Sensitive, Total tested = Number of isolates tested for susceptibility to the antibiotic.

Among common isolates, *Acinetobacter* exhibited the highest resistance to colistin (100%), amikacin (82.6%), and levofloxacin (70.8%). *Klebsiella* showed high resistance to colistin (94.1%), amikacin (80.9%), and levofloxacin (66.6%), with moderate resistance to meropenem and imipenem. *Staphylococcus aureus* demonstrated the highest resistance to amikacin (55.5%), while *Staphylococcus haemolyticus* exhibited 80% resistance to levofloxacin. These findings underscore the prevalence of multidrug-resistant organisms and highlight the limited efficacy of commonly used antibiotics against these pathogens (Table 3).

Table (3) Antibiotic resistance rates among common bacterial isolates from neonatal sepsis cases at Benghazi Medical Centre NICU (n=67)

Antibiotic	The most common bacterial isolates in the study population															
	Acinetobacter				Klebsiella				Staph. auras				Staph. heamolyticus			
	R	S	UT	R%	R	S	UT	R%	R	S	UT	R%	R	S	UT	R%
Amikacin	19	4	1	82.6	17	4	0	80.9	5	4	3	55.5	3	3	0	50
Gentamycin	12	10	2	54.5	8	13	0	38	3	8	1	27.2	4	2	0	66.6
Imipenem	8	16	0	33.3	8	12	1	40	2	8	2	20	1	2	3	**
Meronam	10	14	0	41.6	9	11	1	45	1	5	6	**	0	2	4	0
Ceftoxitin	4	20	0	16.6	6	15	0	28.5	3	6	3	**	1	4	1	50
amoxicillin-clavulanate	0	24	0	0	7	13	1	33.3	3	7	2	30	1	1	4	**
Colistin	19	0	5	100	16	1	4	94.1	1	0	11	**	0	0	6	**
Levofloxacin	17	7	0	70.8	12	6	3	66.6	2	4	6	**	4	1	1	80
Ciprofloxacin	12	10	2	54.5	5	15	1	25	3	6	3	**	4	2	0	66.6

Note: Resistance percentages (%) are calculated based on the number of isolates tested for each antibiotic (excluding untested isolates). R = Resistant; S = Sensitive; UT

Significant difference in antibiotic resistance between gram-negative and gram-positive bacterial isolates for most tested antibiotics. Amikacin showed a significant disparity in resistance (R) and sensitivity (S) between gram-negative (R: 36, S: 10) and gram-positive (R: 9, S: 7), with a p-value of 0.013. Similarly, imipenem, meronam, Ceftoxitin, amoxicillin-clavulanate, colistin, and levofloxacin exhibited highly significant levels of resistance and sensitivity between the two groups. For instance, meronam resistance was markedly higher in gram-negative isolates (R:20) compared with gram-positive (R: 1), with a p-value of 0.000. In contrast, gentamycin and ciprofloxacin did not show a significant difference ($p = 0.690$ and $p = 0.692$, respectively), suggesting similar resistance and sensitivity patterns across gram-positive and gram-negative isolates for these

antibiotics. Overall, the findings highlight varying efficacy of antibiotics against two groups, with several antibiotics demonstrating statistically significant disparities in their effectiveness (Table 4).

Table (4) Comparison of antibiotic resistance and sensitivity between gram-negative and gram-positive bacterial isolates (n=67)

The given antibiotics		Type of bacterial isolates		Level of significance
		Gram -ve	Gram +ve	
Amikacin	R	36(80%)	9(20%)	0.013**
	S	10(58.8%)	7(41.2%)	
	U.T	1(20%)	4(80%)	
Gentamycin	R	20(71.4%)	8(28.6%)	0.690
	S	25(71.4%)	10(28.6%)	
	U.T	2(50%)	2(50%)	
Imipenem	R	17(85%)	3(15%)	0.001**
	S	29(74.4%)	10(25.5%)	
	U.T	1(12.5%)	7(87.5%)	
Meronam	R	20(95.2%)	1(4.8%)	0.000**
	S	25(78.1%)	7(21.9%)	
	U.T	2(14.3%)	12(85.7)	
Ceftoxitin	R	10(71.4%)	4(28.6%)	0.002**
	S	37(77.1%)	11(22.9%)	
	U.T	0(0%)	5(100%)	
amoxicillin-clavulanate	R	7(63.6%)	4(36.4%)	0.001**
	S	39(79.6%)	10(20.4%)	
	U.T	1(14.3%)	6(85.7%)	
Colistin	R	35(94.6%)	2(5.4%)	0.000**
	S	2(100%)	0(0%)	
	U.T	10(35.7%)	18(64.8%)	
Levofloxacin	R	30(81.1%)	7(18.9%)	0.008**
	S	13(72.2%)	5(27.8%)	
	U.T	4(33.3%)	8(66.7%)	
Ciprofloxacin	R	18(69.2%)	8(30.8%)	0.692
	S	25(73.5%)	9(26.5%)	
	U.T	4(57.1%)	3(42.9%)	

R: resistant, S: sensitive, U.T: untested. ** Statistical significance

Percentages (if included) are calculated based on the number of isolates tested (R + S).
Statistical significance (p-values) was determined using the Chi-square test.
A p-value < 0.05 indicates a statistically significant difference between gram-negative and gram-positive isolates.
Antibiotics tested include amikacin, gentamicin, imipenem, meropenem, ceftoxitin, colistin, levofloxacin, and ciprofloxacin.

The distribution of neonatal outcomes based on the causative agents of sepsis. Acinetobacter was the leading cause of neonatal death (40.9%), followed by Klebsiella (36.3%), Staphylococcus aureus (13.6%), and Staphylococcus haemolyticus (9%). In contrast, infections caused by Moraxella and Enterococcus were not associated with neonatal mortality. The small sample size in this study limited our ability to assess the relationship between antibiotic resistance and neonatal sepsis outcomes. Both survivors and non-survivors exhibited high resistance to the same antibiotics, making it difficult to establish a meaningful (Figure 2)

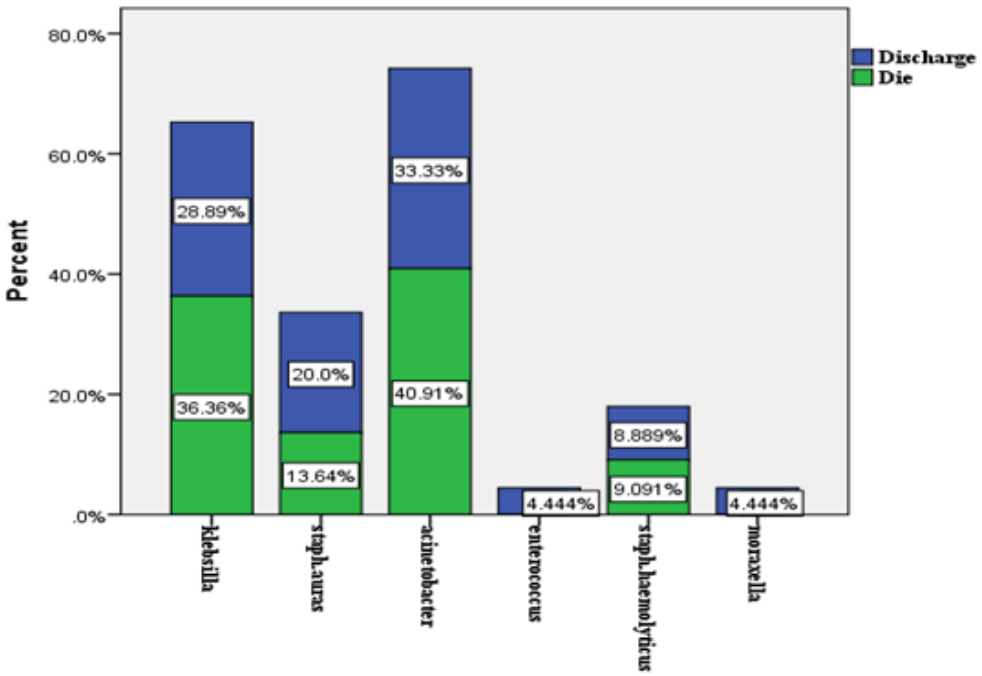

Figure (2). The outcome distributions—mortality versus discharge—among neonates with sepsis, stratified by different causative agents = Untested. Percentages are calculated as (Number resistant ÷ Number tested) × 100.

DISCUSSION

This study analyzed 67 neonates with culture-confirmed neonatal sepsis admitted to the NICU at Benghazi Medical Centre. The predominance of male neonates (61.2%) and term infants (≥37 weeks gestation, 67.2%) with birth weight ≥2500 grams (61.2%) is consistent with reports from low- and middle-income countries, where male sex and prematurity are recognized risk factors for neonatal sepsis and mortality (13,14,15). The higher incidence of late-onset sepsis (55.2%) compared to early-onset sepsis (32.8%) reflects the increasing burden of hospital-acquired infections in NICUs, often linked to invasive procedures and prolonged hospitalization (Abdalla & Amgitif, 2023; Fuchs et al., 2018; Shane et al., 2017).

Gram-negative bacteria accounted for 70.1% of isolates, with *Acinetobacter* (35.8%) and *Klebsiella* (31.3%) as the predominant pathogens, followed by *Staphylococcus aureus* (17.9%). The predominance of multidrug-resistant gram-negative organisms aligns with findings from similar settings and underscores their critical role in neonatal sepsis (Shane et al., 2017; Singh et al., 2020; Zaidi et al., 2005). The high resistance rates observed to colistin (94.8%) and amikacin (72.5%) raise significant concerns about the effectiveness of current empiric antibiotic regimens and highlight the urgent need for antimicrobial stewardship and infection control programs (Mwananyanda et al., 2021; Singh et al., 2020).

Despite the use of first-line antibiotics (amikacin or gentamicin combined with amoxicillin/clavulanate), 91% of neonates required escalation to second-line therapy, and 37.3% received third-line antibiotics such as vancomycin and levofloxacin, reflecting the challenge posed by multidrug-resistant pathogens. The overall mortality rate of 32.8% emphasizes the severity of neonatal sepsis in this setting and the impact of antimicrobial resistance on outcomes (Abera et al., 2021; Spss, 2015; Zaidi et al., 2005).

These findings highlight the urgent need for continuous surveillance of bacterial pathogens and their resistance patterns, tailored antibiotic protocols, and strengthened infection prevention measures in NICUs. Multicenter studies are warranted to better understand regional variations and inform effective interventions in Libya and similar resource-limited settings (Fuchs et al., 2018; Kariniotaki et al., 2024; Maurice, 2025).

CONCLUSION

Neonatal sepsis at Benghazi Medical Centre is mainly caused by multidrug-resistant gram-negative bacteria and is associated with high mortality. Maternal factors and medical interventions contribute to the spread of resistant pathogens, highlighting the urgent need for improved infection control and targeted antibiotic stewardship.

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Duality of interest: The authors declare that there are no conflicts of interest, financial or non-financial, related to this study.

Ethical consideration

Verbal approval was obtained under the care of the Neonatal Department in Benghazi Medical Center due to the retrospective nature of the current study.

Author contributions:

K.I.A. Shreef collected the data and developed the theoretical formalism with assistance from A.I. Kadwar. N.A.M. Eldarogi performed the data analysis. I.A. Alsaeiti assisted with reference collection and manuscript preparation. All authors contributed to the final version of the manuscript. K.I.A. Shreef supervised the project.

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