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RESEARCH

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Occurrence of *Pantoea agglomerans* bloodstream infection in neonatal intensive care unit at tertiary hospital in Tanzania: antibiotic susceptibility profile and clinical outcome

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Abstract

Background *Pantoea agglomerans (P. agglomerans)* is an environmental gram-negative bacterium that rarely infects humans. *P. agglomerans* infections have never been reported in Tanzania. We investigated the occurrence of *P. agglomerans* bloodstream infections among neonates in the Intensive Care Unit (NICU) and their subsequent clinical outcome that occurred in 2019.

Methodology Blood samples were collected from neonates with sepsis. A total of 19 *P. agglomerans* were isolated from 17 infected neonates; two of the neonates had *P. agglomerans* isolated twice. A total of 14 patient files were retrieved from medical records.

Results The mean age of the infected neonates were 3.75 ± 7.95 days. Isolated *P. agglomerans* showed high sensitivity to the antibiotics particularly chloramphenicol (94.7%), piperacillin-tazobactam (94.7%) and meropenem (94.7%). The mortality rate was 71.4% with 35.7% of infected neonates dying before Antibiotic Susceptibility Test results for appropriate management. The Infection Prevention and Control (IPC) team shut the NICU for thorough decontamination which helped to stop the *P. agglomerans* occurrence.

Conclusions *P. agglomerans* occurrence at the NICU was an uncommon aetiology pathogen for neonatal sepsis associated with high rates of mortality despite high sensitivity to multiple antibiotics. This calls for the strengthening of infection control measures and introduction of surveillance for environmental pathogens capable of causing human infections.

Keywords *Pantoea agglomerans*, Bloodstream infection, Antibiotic sensitivity, NICU, Outbreak, Tanzania

Background

Pantoea agglomerans is a gram-negative bacterium frequently isolated from environmental samples. The bacterium rarely causes human infections; in case of infection, *P. agglomerans* infects neonates and immunocompromised individuals (Büyükcam et al. 2018; Casale et al. 2023; Aljameely et al. 2024; Van Den Bosch et al. 2024). Neonatal sepsis of *P. agglomerans* presents similarly as other sepsis recorded in neonates including temperature liability, altered feeding behaviour,

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lethargy, jaundice, respiratory distress, and hypoglycaemia. Hospital-acquired *P. agglomerans* infection is associated with contaminated medical equipment such as intravenous catheters and contaminated intravenous fluids like anticoagulants, citrate dextrose solution, blood products and parenteral nutrition (Kaur et al. 2020; Van Den Bosch et al. 2024).

P. agglomerans outbreaks in neonates, paediatrics and cancer patients have been reported previously in Europe and North America (Kaur et al. 2020; Oo et al. 2021; Aljameely et al. 2024; Van Den Bosch et al. 2024). A study investigated a hospital-associated outbreak of *P. agglomerans* in an oncology clinic (Yablon et al. 2017). Twelve patient cases were identified due to poor preparation and handling of medications as a source of infection. Environmental samples revealed contamination in facility sinks, attributing the exposure of contaminated sinks. Concluding that improved medication preparation practices can control outbreaks (Yablon et al. 2017).

Due to the rare and opportunistic nature of *P. agglomerans,* resistance profiles of the bacteria have not been thoroughly evaluated. *P. agglomerans* isolated in a 12-year retrospective study in Hungary showed high levels of susceptibility to broad and narrow spectrum antibiotics (Gajdács 2019). The study in Hungary presented emerging of multi-drug resistance (MDR) stains of *P. agglomerans* with high resistance to amoxicillin-clavulanic acid (60%) and ampicillin (82.8%) similar to other gram-negative bacteria (Gajdács 2019).

Human infections with *P. agglomerans* have not been previously reported in Tanzania or Sub-Saharan Africa. Here, we report occurrence of *P. agglomerans*, clinical characteristics and outcomes of the patients in NICU at Muhimbili National Hospital (MNH).

Methods

Study design and setting

Our study took place between September and October of 2019. These data were retrieved and analysed for infected neonates admitted at the NICU with positive microbiological culture results that revealed *P. agglomerans* at Central Pathology Laboratory (CPL) at MNH. Patient files of positive blood culture samples were reviewed to collect relevant clinical data. MNH is the largest tertiary health care facility in Tanzania, with a 1500 bed capacity and serving as a university teaching hospital and a national referral hospital. Presently, the MNH attends to 1000–1200 outpatients per day with an estimated 1000–1200 admission per week. The MNH NICU on average admits about 10–20 neonates per day.

Study population

The population were neonates admitted at the NICU of MNH with a clinical diagnosis of neonatal sepsis categorised either as early onset neonatal sepsis (EONS) defined as signs of infection 0- to 72-h post-delivery or late onset neonatal sepsis (LONS) defined as signs of infection > 72-h post-delivery with a positive *P. agglomerans* blood culture sample.

Laboratory procedures

Anaerobic and aerobic blood samples were collected from NICU. Blood cultures were incubated in BD-BACTEC-FX40 until indication of presumptive presence of microorganism growth. Thereafter, primary gram-stain and inoculation of positive blood samples were performed on sheep blood agar base from HIMEDIA:M1301-500G, chocolate agar base HIMEDIA:M103-500G and MacConkey agar HIMEDIA:MHD81-500G incubated at 37 °C in ambient air for 18 to 24 h. Colony morphology, gram-stain and conventional biochemical tests were performed to identify the bacteria. Analytical Profile Index Test-20E (API-20E) was used to confirm the bacteria. Antibiotic susceptibility test (AST) for significant bacteria isolates were performed using Kirby-Bauer discs diffusion and categorized according to the Clinical and Laboratory Standards Institute (CLSI) (2019) antibiotic breakpoints for *Enterobacterales.*

Microbiological properties of *P. agglomerans*

Direct gram-stain from sample and culture revealed gram-negative bacilli. All isolated were wet, non-haemolytic colonies and produced yellow pigments on 5% sheep blood agar, while on MacConkey agar colonies were non-lactose fermenters with slight yellowish pigmentation. (Fig. 1). The isolates were found to be oxidase-negative, urease-negative, indole-negative, and non-motile and did not produce gas and H_2S on Kligler's iron agar. The conventional biochemical tests yield inconclusive results, and API-20E used identified the bacteria as *P. agglomerans.*

Ethical consideration

Ethical approval for the study was sought out from the Senate Research and Publications of Muhimbili University of Health and Allied Sciences (MUHAS): Reference Number: DA. 287/298/01A/. Waiver of informed consent was granted by Committee of MUHAS and permission to conduct the study at CPL was granted by the MNH administration: Reference Number: MNH/TRCU/IRB/permission/2019/117.

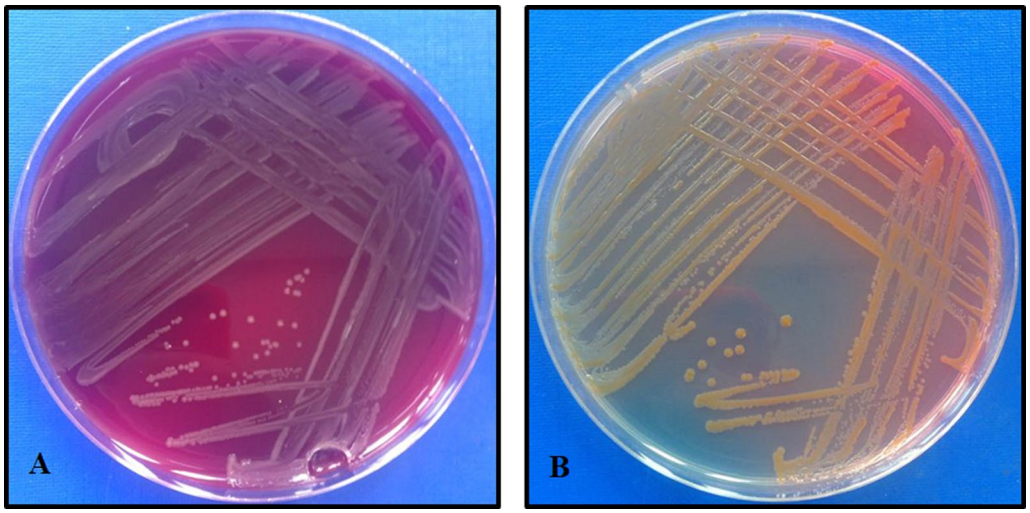

Fig. 1 Morphology of colonies on sheep blood agar and MacConkey agar. **A** shows the morphology of *P. agglomerans* colonies on 5% sheep blood agar. **B** shows the morphology of *P. agglomerans* colonies on MacConkey agar

Results

Patients' characteristics

A total of 19 *P. agglomerans* were isolated from 17 infected neonates as two infected neonates submitted samples to the laboratory twice. Out of the 17 neonates, only 14 patient files were retrieved. All the infected neonates affected by *P. agglomerans* BSI were pre-term: 78.6% of the neonates were delivered at MNH with the remaining referred to MNH for further treatment regarding prematurity and associated prematurity complications. The mean age of the patients were 3.75 ± 7.95 days old (IQR: 1 day to 19 days). The overall mortality was 71.4% as shown in Tables 1 and 2.

Antibiotic susceptibility profile of *P. agglomerans*, treatment and clinical outcome

The *P. agglomerans* isolates were highly sensitive to most of the tested antibiotics according to the CLSI 2019. Aztreonam (89.5%), chloramphenicol (94.7%), piperacillin-tazobactam (94.7%) and meropenem (94.7%) were the most effective drugs against *P. agglomerans*, while the lowest sensitivity was observed with ampicillin (68.4%), ciprofloxacin (68.4%), ceftazidime (68.4%), ceftriaxone (68.4%), cefepime (73.6%) and gentamycin (78.9%) as shown in Table 3.

Out of the 17 infected neonates, only 14 patient files of the infected neonates were retrieved. Table 1 illustrates that 64.3% (n = 9/14) of the cases received empirical antibiotics of which the *P. agglomerans* were susceptible to; however, 55.5% patients died despite appropriate initial antibiotics management. The duration of hospital stays for the patients with *P. agglomerans* infection was 16.2 days ± 3.75 days. A total of 28.6% of the infected neonates presented with MDR *P. agglomerans* infection, with one exhibiting carbapenem-resistant *P. agglomerans.*

Change of susceptibility pattern

We received two different samples from patients 12 and 16. AST was conducted on both samples submitted to the laboratory. Prior to the initiation of the initial antibiotic regimen showed that the isolates were sensitive to the antibiotics that have been tested. However, the AST results of blood samples collected five days after using the initial antibiotic regimen revealed that the isolates were resistant to the previously tested antibiotics particularly ampicillin, gentamycin, ciprofloxacin, ceftazidime and ceftriaxone.

Monthly trend of detection of *P. agglomerans*

The rise of *P. agglomerans* occurred in late September and October of 2019. The laboratory began noticing the abnormal trend of the isolated *P. agglomerans* in early October as the *P. agglomerans* isolation in the laboratory was uncommon and rare. The laboratory, therefore, notified the IPC team which decided to close the NICU for three days for thorough disinfection as other method of identifying the source of infection failed. After the ICU was reopened, active surveillance of *P. agglomerans* was continued for a period of four weeks without isolation of any *P. agglomerans*.

Discussion

The study aimed to describe the occurrence *P. agglomerans* transpired in the NICU at MNH between September and October 2019. *P. agglomerans* is a rare human

Table 1 Socio-demographic and clinical characteristics of infected neonates with *P. agglomerans* BSI

Characteristics	*N* (%)
Sex	
Male	10 (71.4)
Female	4 (28.6)
Age groups (days)	
0–3 days	8 (57.2)
4–7 days	3 (21.4)
> 7 days	3 (21.4)
Patient Status	
In-patient	11 (78.6)
Referral	3 (21.4)
Sample collection post admission (days)	
0–3 days	9 (64.3)
4–7 days	3 (21.4)
> 7 days	2 (14.3)
Mode of delivery	
Spontaneous vaginal delivery	14 (100)
Invasive procedure	
Central venous catheter	14 (100)
Total parenteral nutrition	14 (100)
Continuous positive airway pressure	5 (35.7)
Surgery	1 (7.1)
Primary diagnosis	
Prematurity with:	
Early onset neonatal sepsis (EONS)	6 (42.9)
a. EONS and respiratory distress syndrome	1 (7.1)
Late onset neonatal sepsis (LONS)	5 (35.7)
a. LONS and respiratory distress syndrome	1 (7.1)
b. LONS and imperforated anus	1 (7.1)
Initial antibiotic regimen	
Ampicillin–cloxacillin and gentamycin	10 (71.4)
Ciprofloxacin	3 (21.3)
Others	2 (14.2)
Sensitive to initial antibiotic regimen	
Yes	9 (64.3)
No	5 (35.7)
Clinical outcome	
Alive	4 (28.6)
Dead	10 (71.4)

Data of 14 infected neonates were retrieved; the remaining data of the three infected neonates could not be obtained from medical records

pathogen, and as per our knowledge, this is the first time to be reported in hospital setting in Tanzania. The increase isolation of this pathogen was alarming as the bacteria is not among common aetiology isolated for BSI in NICU at MNH. The known common pathogen includes *Klebsiella species, Escherichia coli, Coagulase-negative Staphylococci species, Staphylococcus aureus* and *Pseudomonas aeruginosa* (Hani et al. 2023; Pishori et al. 2023; Andrea et al. 2023). Unfortunately, the source of the infection in this occurrence failed to be identified. Generally, there is limited information regarding the origin of *P. agglomerans* in the healthcare setting. However, studies suggest the spread of *P. agglomerans* is through ingestion of plant products, the presence of normal hand flora, and the household environment (Yablon et al. 2017; Hani et al. 2023; Van Den Bosch et al. 2024).

In the description of this occurrence, there is a significant time gap between the first isolated *P. agglomerans* BSI to the second isolated *P. agglomerans* as shown in Fig. 2. This suggests that the progression of the uncommon aetiology was through hospital-acquired infection (HAI) with the probable mode of transmission to be cross-infection and other contributing factors such as use of contaminated or poorly disinfected devices such as mechanical ventilators and catheters (Archibald and Jarvis 2011; Yablon et al. 2017; Wang et al. 2019; Masoud et al. 2022). In this study, most of the neonates were subjected to invasive procedures which also suggest that the spread of *P. agglomerans* infection may have been acquired at the hospital. There was several neonatal sepsis which were categorized as EONS as shown in Table 2. All the neonatal sepsis were suspected 48-h post-admission thus considered for reclassification to the HAI; however, still considered as EONS as occurred within 72 h post-delivery (Simonsen et al. 2014; Giannoni et al. 2018).

Out the 17 patients, two patients' samples were submitted more than once to make a total sample received in the laboratory to be 19 and all were positive. The two blood samples were submitted approximately five days apart. One was at the initial diagnosis and another during the follow-up due to the deteriorating condition of the patient. Both the samples showed significant change of resistance pattern. The possible reasons to the change of resistance of the isolated bacteria could be a re-infection from other patients with different strains of the *P. agglomerans* or selection pressure after administration of the empirical antibiotics. A possible explanation for the bacterial acquisition of the resistance genes could also be exposure to antibiotic resistance genes (ARGs) in the NICU as *Pantoea agglomerans* is a common isolate in the environment (Mączyńska et al. 2023). A study examined the changes in development of resistance patterns among gram-negative *Enterobacterales* focusing particularly on *Klebsiella pneumonia* and *Escherichia coli*, over a period of five years from 2017 to 2021. Findings revealed effect of selection pressure reported on empirical broad-spectrum antibiotics used in guidelines according to the study setting (Mączyńska et al. 2023). Similarly, trends in the use of broad-spectrum antibiotics and its relationship to multidrug-resistant organisms (MROs) were

Table 2 Socio-demographic and clinical characteristics of individual infected neonates with *P. agglomerans* BSI

Case	Age (days)	Sex	Patient status	SCPA (days)	Primary diagnosis	Initial antibiotic regime	Sensitive initial antibiotic	Died before Results	AMR	DOHS (days)	Mortality
1	1	Male	In-Patient	1	EONS, prematurity	Ciprofloxacin	Yes	No	Non-MDR	5	Died
4	3	Male	In-Patient	3	EONS, prematurity	Ampicillin, gentamicin	Yes	No	Non-MDR	5	Died
5	20	Male	Referral	14	LONS, imperforate anus, prematurity	Ampicillin, gentamicin	No	No	Non-MDR	37	Died
7	3	Male	In-Patient	3	LONS, prematurity	Ampicillin, gentamicin	Yes	No	MDR	12	Recovered
8	6	Male	In-Patient	6	LONS, prematurity	Ceftriaxone	Yes	No	Non-MDR	29	Recovered
9	22	Male	Referral	2	LONS, prematurity	Ciprofloxacin, vancomycin	Yes	No	Non-MDR	6	Died
10	7	Male	In-Patient	7	LONS, prematurity, RDS	Ampicillin, gentamicin	Yes	No	Non-MDR	14	Died
11	13	Male	Referral	11	LONS, prematurity	Ampicillin, gentamicin	No	Yes	Non-MDR	11	Died
12#	7	Male	In-Patient	4	LONS, prematurity	Ampicillin, gentamicin	No	Yes	MDR	8	Died
13	3	Female	In-Patient	3	EONS, prematurity, RDS	Ampicillin, gentamicin	Yes	No	Non-MDR	62	Recovered
14	3	Female	In-Patient	2	EONS, prematurity	Ampicillin, gentamicin	No	Yes	Non-MDR	4	Died
15	1	Female	In-Patient	2	EONS, prematurity	Ciprofloxacin	Yes	No	MDR	11	Recovered
16#	3	Female	In-Patient	3	EONS, prematurity	Ampicillin, gentamicin	No	Yes	MDR	25	Died
17	2	Male	In-Patient	2	EONS, prematurity	Ampicillin, gentamicin	Yes	Yes	Non-MDR	2	Died

Data of 14 infected neonates were retrieved; the remaining data of the five infected neonates could not be obtained

SCPA sample collected post admission, *EONS* early onset neonatal sepsis, *LONS* late onset neonatal sepsis, *DOHS* duration of hospital stays, *RDS* respiratory distress syndrome, *Case# Pantoea* spp. yielded from multiple samples, *In-patient* neonates delivered at MNH

Table 3 Antibiotic susceptibility profile of 19 *P. agglomerans* isolated from 17 infected neonates admitted at NICU, MNH

Antibiotic tested	Sensitivity patterns *n* (%)
Ampicillin	13 (68.4)
Ceftriaxone	15 (78.9)
Ceftazidime	13 (68.4)
Ciprofloxacin	13 (68.4)
Gentamicin	15 (78.9)
Chloramphenicol	18 (94.7)
Sulfamethoxazole-Trimethoprim	16 (84.2)
Piperacillin-Tazobactam	18 (94.7)
Aztreonam	17 (89.5)
Cefepime	14 (73.6)
Meropenem	18 (94.7)

analysed in a three-year surveillance research carried out in a tertiary care hospital in Kuala Lumpur, Malaysia. The results showed that the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* spp., ESBL-producing *Escherichia coli*, and *Acinetobacter baumannii* was positively correlated with the consumption of fluoroquinolones and extended-spectrum cephalosporins (Tan et al. 2022). Several studies also report similar findings suggesting exogenous routes of acquired resistance of bacteria, including cross-infection, selection pressure or hospital-acquisition (Lynch et al. 2021; Abejew et al. 2024).

The mortality in this described occurrence was high in the *P. agglomerans* BSI infected neonates. Majority of the isolated showed in vitro sensitivity to the commonly used antibiotics in Tanzania as per the neonatal guideline. However, many infected neonates were premature, which increased their vulnerability to complications associated with prematurity and led to their deaths. Similarly reported in multiples case series describing *Pantoea* outbreaks in NICUs, all the neonates in the study were subjected to invasive procedures subsequently increasing their exposure to the *P. agglomerans* infection (Hani et al. 2023; Aljameely et al. 2024; Van Den Bosch et al. 2024).

Study limitation

The investigation failed to determine the source of *P. agglomerans* in the NICU. Initiating investigations to find the source of pathogen is necessary to prevent such occurrences in NICUs and other departments at MNH. No further isolation of *P. agglomerans* was identified post-fumigation and thorough disinfection of the NICU department for 3 days. The study also did not conduct any molecular characterization of the bacteria isolates which could have provided a useful insight on the development of MDR isolates.

The study did not include details of clinical information of patients which could further help to predict the source

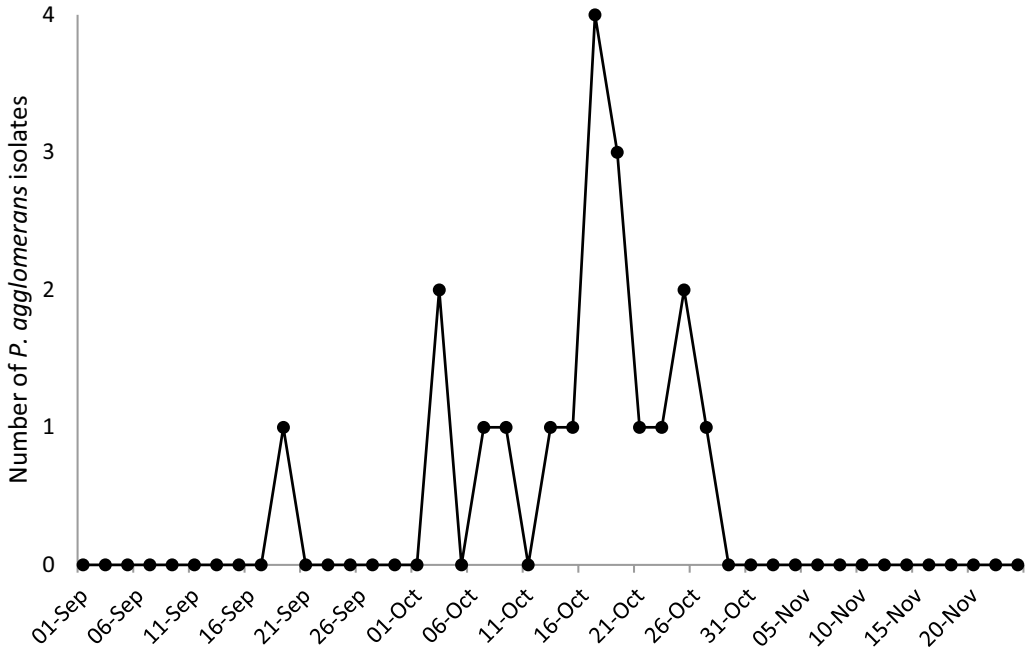

Fig. 2 Outbreak description of 19 *P. agglomerans* BSI isolated from 17 infected neonates admitted at the NICU, MNH

of the pathogen like invasive procedures which patient underwent such as parenteral nutrition, central venous access and surgical procedures. In addition, patient factors like gestational age, post menstrual age and mode of delivery were not included which could also play a role in patient outcome.

Conclusions

We demonstrated the presence of hospital-acquired *P. agglomerans* infections in the NICU at MNH. The findings showed that *P. agglomerans* BSI causes severe morbidity and mortality in the infected neonates. Further investigation is required to report the burden of HAI in NICU and other departments at MNH. The study suggests strengthening of the infection control measures in identifying pathogens, finding the source of origin, and surveillance for the environmental pathogens capable of causing human infections. The study further recommends thorough investigation of the underlying pathogenesis of *P. agglomerans* that have shown to cause high mortality in infected neonates.

Abbreviations

BSI	Bloodstream infection
NICU	Neonatal Intensive Care Unit
MDR	Multi-drug resistance
MNH	Muhimbili National Hospital
CPL	Central Pathology Laboratory
EONS	Early onset neonatal sepsis
LONS	Late onset neonatal sepsis
AST	Antibiotic susceptibility test
CLSI	Clinical and Laboratory Standards Institute
MUHAS	Muhimbili University of Health and Allied Sciences
IPC	Infection and prevention control
HAI	Hospital-acquired infection
ARGs	Antibiotic resistance genes
ESBL	Extended-spectrum β-lactamase *Enterobacterales*

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Significance and impact of the study

To our knowledge, this is the first-time multiple cases of *Pantoea agglomerans* causing neonatal sepsis have been reported in the Sub-Saharan Africa. Affected neonates were followed up until outcome of interest: survive or death.

Author contributions

S.S.M. contributed to conceptualization, data curation, formal analysis, investigation, methodology, writing original draft and review and editing. M.M. contributed data curation, methodology, supervision, writing review and editing. R.R.G. contribute to conceptualization, data curation, formal analysis, investigation, methodology, writing original draft and review and editing. H.N. contributed to investigation, writing review and editing. A.N. and M.K. contributed to methodology and writing review and editing. F.M. and D.K. contributed to formal analysis and writing review and editing. J.M. contributed to formal analysis, writing review and editing and supervision. All authors have read and approved the manuscript.

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Availability of data and materials

All data analysed during the current study are included in this published article.

Declarations

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Ethical approval for the study was sought out from the Senate Research and Publications of Muhimbili University of Health and Allied Sciences (MUHAS): Reference Number: DA. 287/298/01A/. Waiver of informed consent was granted by Committee of MUHAS and permission to conduct the study at CPL was granted by the MNH administration: Reference Number: MNH/TRCU/IRB/permission/2019/117.

Consent for publication

Not applicable.

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