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Perioperative mortality in children in a tertiary teaching hospital in Nigeria: a prospective study

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ABSTRACT

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Dr Ademola Olusegun Talabi; ademolatalabi1608@gmail.com **Background** Perioperative mortality is one of the tools that can help to assess the adequacy of healthcare delivery in a nation. This audit was conducted to determine the 24-hour, 7-day and 30-day perioperative mortality rates and the predictors of mortality in a pediatric surgical cohort.

Methods This was a prospective study of children whose ages ranged from a few hours to 15 years and who were operated on between May 2019 and April 2020. The primary outcome was to determine the incidence of inhospital perioperative mortality.

Results A total of 530 procedures were done in 502 children. Their ages ranged from a few hours to 15 years with a median of 36 months. The 24-hour, 7-day and 30day mortality rates were 113.2 per 10000 procedures [95% confidence interval (Cl) =40 to 210], 207.6 per 10000 procedures (95% CI=110 to 320) and 320.8 per 10000 procedures (95% CI=190 to 470), respectively. Congenital anomalies complicated by postoperative sepsis contributed to death in the majority of cases. The predictors of mortality were neonatal age group [adjusted odds ratio (AOR)=19.92, 95% CI=2.32 to 170.37, p=0.006], higher American Society of Anesthesiologists Physical Status III and above (AOR=21.6, 95% CI=3.05 to 152.91, p=0.002), emergency surgery (AOR=17.1, 95% CI=5.21 to 60.27, p=0.001), congenital anomalies (AOR=12.7, 95% CI=3.37 to 47.52, p=0.001), and multiple surgical procedures (AOR=9.7, 95% CI=2.79 to 33.54, p=0.001).

Conclusion Perioperative mortality remains high in our institution.

INTRODUCTION

An increasing number of pediatric patients present for surgical and interventional procedures under general anesthesia with approximately 5 billion of the world's population lacking access to safe surgery and anesthesia. The situation is even worse in low and middle-income countries (LMICs) where surgical care falls short of global standard. It is estimated that children constitute about 40%–50% of the population in LMICs and that approximately 80% of these children will require one form of operation or another before their 15th birthday. The obstacles to

Key messages

What is already known about this subject

- Perioperative mortality is high in low and middleincome countries (LMICs) compared with highincome nations.
- Data are few and retrospective in nature.
- Sepsis contributes significantly to mortality rate in LMICs.
- Most studies are confined to neonatal/emergency surgeries.

What are the new findings

- The current study is one of the few prospective studies and provides data on perioperative death in a low-income setting.
- Complex or multiple congenital anomalies contribute significantly to perioperative mortality rate.
- Current study provides insight into neurosurgical and cardiac death in a poor setting.
- Perioperative death is uncommon in ophthalmic, ear, nose and throat, maxillofacial and plastic surgeries.

receiving safe and efficient care in LMICs are even more threatening in the pediatric population, especially those located in rural communities where 75% of the population resides while most major pediatric surgical facilities are situated in urban centers. Therefore, pediatric patients in these regions may experience high morbidity and mortality.^{1–5}

A variety of factors have been identified as mitigating against surgical safety and access to surgical care in LMICs especially in sub-Saharan Africa: lack of infrastructure, dearth of manpower (nurses, pediatric surgeons and anesthetists), non-availability of pediatric intensive care units, defective health insurance scheme, and deficit in monitoring equipment.^{4 6-8}

Perioperative mortality rate (POMR) is a tool to assess adequacy of surgical care and safety. It also aids in judicious allocation of scarce resources in terms of manpower and equipment.⁹ The World Health Organization (WHO) defined POMR as death

occurring after a surgical and anesthesia procedure at two time periods: the first period is on the day of surgery (including death in operating theater), and the second period either before discharge from hospital or within 30 days of operation.^{1 10–12}

During the last 30 years, POMR has steady declined in children and adults in the USA, whereas in LMICs, avoidable morbidity and mortality remain high.^{13–16} Newton *et al*⁴ observed that mortality rate in LMICs was more than 100 times higher than that in high-income countries (HICs). This higher mortality rate is associated with American Society of Anesthesiologists Physical Status (ASA PS) III and above, with emergency and multiple surgeries, and with non-adherence to surgical checklist. Other factors include advanced diseases, late presentation, malnutrition and sepsis.^{16–20}

There are few case series on pediatric POMR in LMICs that reach publication levels in peer-reviewed journals, and most of these studies are retrospective, despite reported high mortality rate in children.^{416 21} More data on POMR in children are needed in LMICs in order to understand the various clinical factors associated with death after surgery and to improve quality and access to care. Identification of risk factors for perioperative death may guide planning, clinical decision-making, intervention, and allocation of resource needs at the health system level for national surgical programs so as to reduce perioperative death.

The primary objective of the present study was to provide prospective benchmark data for the incidence of in-hospital perioperative death. The secondary objective was to identify factors associated with perioperative mortality in a tertiary surgical center in Nigeria.

METHODS

Study design and settings

We conducted a hospital-based prospective study during a period of 1 year between May 2019 and April 2020 among children whose ages ranged from a few hours to 15 years. Our institution is located in a semiurban area of southwestern Nigeria, with a catchment population of about two million people who are predominantly farmers, artisans and civil servants. There is no community-based health insurance scheme in our catchment area. The bulk of the patients pay out of pocket for health services. Our hospital lacks neonatal and pediatric intensive care units. The available intensive care unit is a six-bedded facility with a nurse to bed ratio of 1:2. The hospital have two pediatric ventilators in the intensive care unit.

Data collection

Patients were recruited consecutively from all pediatric surgical subspecialties in the hospital. The inclusion criteria were: patients whose ages were ≤ 15 years while their parents gave consent to participate in the study. Participants were given full right to withdraw from the study at any time. The demographic characteristics

of all children who had anesthesia and surgical exposures were documented in a spreadsheet. Other clinical data collected included duration of symptoms (especially those who had emergency operations), diagnosis, number of surgeries within 30 days, ASA PS, urgency of surgical procedure (elective or emergency procedure), whether congenital or acquired pathology, anesthesia technique, and the type of operation (major or minor procedures). The outcome in terms of survival or death was noted.

Children were classified into five groups according to their ages: neonates up to 30 days of age, infants from 31 days to 12 months of age, preschool children of 1–5 years of age, children aged 6–10 years and 11–15 years. The clinical records of children who died within 30 days of the last anesthesia were examined by two consultant surgeons and two consultant anesthesiologists. Patients were discussed and surgery and anesthesia-related death were reached by the panel. The entire team agreed to classify the principal causes of death according to a previous study.²² The main causes of death were examined and tabulated into the following four major categories.

- 1. Attributable to preoperative condition or disease (when comorbidities were the only or the major contributory factors).
- 2. Attributable to a preoperative trauma event (with subsequent surgery).
- 3. Anesthesia either fully or partially contributed to the death (when the child's disease or condition was the primary factor but anesthesia-related problems represented additional factors).
- 4. The surgical procedure either fully or partially contributed to the death (when the child's disease or condition was the primary factor but surgery-related death represented an additional factor).

Categories 1 and 2 include all deaths in which a panel of surgeons and an anesthetist agree that neither surgery nor the anesthetic procedure contributes to death. Death attributable to anesthesia is defined using the definition by van der Griend et al.²³ Patients for which the panel agree that anesthesia or factor under the control of the anesthetist contributes to death constitute category 3. The same panel-based assessment will be applied to 'the category surgery related death'. The panel was not blinded to the rank of surgeons and anesthetists who were involved in all the procedures. The anesthesia was performed by consultants and senior residents in anesthesia and all pediatric surgical subspecialties in the hospitals. All patients in the study had general anesthesia with occasional caudal block to minimize postoperative pain especially in those who had lower abdominal surgeries.

We defined major surgery as an invasive surgical procedure where there was extensive tissue dissection and/ or resection or where a body cavity was entered. Minor surgery was defined as one in which neither a body cavity was entered nor extensive tissue dissection or resection was performed.

Statistical methods

Mortality rate was expressed per 10000 procedures with 95% confidence interval (CI). Data collected were analyzed for frequencies and percentages using SPSS software V.21 (IBM). The results were presented as tables. A prior decision was made to include candidate variables potentially associated with 30-day perioperative mortality in the univariate analysis (χ^2 analysis or Fisher's exact test). Variables with p value <0.2 were included in a multivariable logistic regression model to identify risk factors associated with perioperative mortality. A p value <0.05 was considered as significant.

RESULTS

A total of 530 procedures were performed in 502 patients. There were 388 males and 142 females with a male to female ratio of 2.7:1. Their ages ranged from a

few hours to 15 years with a median of 36 months and an interquartile range (IQR) of 9.75-94.00 months. In-hospital mortality rates within 24 hours, 7 days and 30 days were 113.2 per 10000 procedures (95% CI=40 to 210), 207.6 per 10000 procedures (95% CI=110 to 320) and 320.8 per 10000 procedures (95% CI=190 to 470). These incidence-based rates were equivalent to in-hospital POMRs of 1.132% (95% CI=0.4% to 2.10%), 2.076% (95% CI=10% to 3.20%), and 3.208% (95% CI=1.9% to 4.7%), respectively.

The majority (n=10, 58.8%) of deaths were recorded among neonates with a mortality rate of 2439.0 per 10000 procedures, p<0.05 (table 1). Most (n=28, 68.3%) of the neonates presented with features of sepsis from delivery centers. Congenital anomalies, ASA PS, urgency of surgical procedure, number of operations and severity of surgical condition influenced mortality (table 1).

	Procedures (n)	Death ≤30 d, n (%)	Mortality rate/ 10000 procedure	χ^2	P value
Total	530	17	320.8		
Gender				0.061	1
Male	388	12 (70.6)	309.3		
Female	142	5 (29.4)	352.1		
Age (mon)				34.735	0.001
<1	41	10 (58.8)	2439.0		
1.1–12	113	4 (23.5)	353.9		
12.1–60	198	3 (17.7)	151.5		
60.1–120	104	0 (0)	0		
120.1–180	74	0 (0)	0		
Type of pathology				4.399	0.047
Congenital	242	12 (70.6)	495.8		
Acquired	288	5 (29.4)	173.6		
Urgency of surgical procedure				18.101	0.001
Emergency	138	12 (70.6)	869.5		
Elective	392	5 (29.4)	127.5		
ASA status				48.532	0.001
I	298	0 (0)	0		
II	143	3 (17.6)	209.7		
III	78	10 (58.8)	1282.1		
IV	9	3 (17.6)	3333.0		
V	2	1 (5.9)	5000.0		
Severity of surgery				9.109	0.003
Minor	181	0 (0)	0		
Major	349	17 (100)	487.1		
Time of death					
≤24h		6 (35.3)	113.2		
>24h to 7 d		5 (29.4)	94.4		
>7 to 30 d		6 (35.3)	113.2		

 χ 2, Chi square; ASA, American Society of Anesthesiologists.

1

Table 2 Association betw

veen mo	ortality rate and numb	per of procedures per patie	nt		
			Mortality rate/10 000		
Pa	atients (n)	Deaths <30 d (n)	procedures	P value	

Procedures per patient (multiple operations), n 481 10 207.9 2-5 21 7 3333.3

Mortality rate was higher among those who had multiple surgeries, p<0.001 (table 2).

Of the cohort of patients who had emergency surgery, three (10%) deaths were recorded among those who presented within 24 hours of earliest symptom compared with nine (8.3%) deaths among those who presented after 24 hours of earliest symptom. The difference was not statistically significant (p=1.000).

Multivariable logistic regression analysis identified neonatal age group, ASA PS III and above, emergency operations, multiple surgeries (reoperations) and congenital anomalies as predictors of mortality (table 3).

Mortality was recorded in three pediatric surgical specialties as shown in table 4.

Sepsis contributed to death in a substantial number of patients. The clinical pathology, type of surgery and causes of death are as shown in online supplemental table 1. Most children died from their preoperative

Table 3 Multivariable logistic regression analyses of variables that predict mortality					
Variable	OR	95% CI	P value		
Age of patients (months)					
<1 (neonates)	19.92	2.32 to 170.37	0.006		
1.1–12	2.37	0.25 to 23.03	0.455		
12.1–60	1.94	0.21 to 17.95	0.558		
60.1–120	1.28	0.10 to 16.15	0.845		
120.1–180 (ref)	1				
Type of pathology					
Acquired (ref)	1				
Congenital	12.7	3.37 to 47.52	0.001		
Urgency of surgical procedure					
Elective (ref)	1				
Emergency	17.1	5.21 to 60.27	0.001		
ASA status					
1 (ref)	1				
II	1.69	0.42 to 6.96	0.461		
111	7.01	1.98 to 24.73	0.002		
IV–V	21.6	3.05 to 152.91	0.002		
Multiple operations, n					
1 (ref)	1				
2–4	9.7	2.79 to 33.54	0.001		

P values in bold are considered to be statistically significant. ASA, American Society of Anesthesiologists; CI, confidence interval; OR, odds ratio.

conditions, comorbidities or postoperative complications. There were no mortalities among those who had preoperative trauma. Surgery-related factors contributed to death in one (18.9 per 10000 procedures) patient while anesthesia-related factors also contributed to death in one (18.9 per 10000 procedures) patient. In none of these patients could death be attributed to surgery or anesthesia alone; comorbidities play a substantial role in the cause of death of these patients.

0.001

Surgery or factors under the control of the surgeons (or in combination with preoperative condition of the patient) contributed to death of a 19-day-old patient who presented with a diagnosis of esophageal atresia with distal tracheoesophageal fistula as well as aspiration pneumonitis. The patient was resuscitated and had cervical esophagostomy, distal esophageal banding and feeding gastrostomy. The child had failed distal esophageal banding plus leakage from gastrostomy site that necessitated repeated surgeries. However, the patient died within 7 days of the third anesthetic exposure. The parents were financially handicapped from the outset and could not afford medications. This child could have benefited from supplemental parenteral nutrition and intensive care monitoring, if available.

The patient in which anesthesia or factors under the control of the anesthetists (in combination with preoperative conditions of the patient) contributed to death was a 12-month-old child who had congenital diaphragmatic hernia and ventricular septa defect with underlying malnutrition. Anesthesia contributed to death because of intraoperative hypoxia and cardiac arrest. The patient died on the operating table. The cause of progressive

Table 4 Mortality rate recorded in each pediatric surgical specialty				
Pediatric surgical specialties	Procedures (n)	Mortality rate/10 000 procedures		
General pediatric surgery/ urology	357	12 (336.1)		
Cardiothoracic	54	2 (370.4)		
Neurosurgery	15	3 (2000.0)		
Maxillofacial surgery	20	-		
Plastic surgery	15	-		
Orthopedic surgery/trauma	20	-		
Ophthalmic surgery	14	-		
Ear, nose and throat	35	-		

 Table 5
 Perioperative mortality rates for surgery for certain pathologies

pathologies				
Pathology	Cases treated (n)	Deaths (n)	Mortality rate (%)	
Gastroduodenal perforation secondary to herbal concoction	1	1	100	
Gastroschisis	3	2	66.7	
Esophageal atresia with distal tracheoesophageal fistula	6	3	50	
Congenital diaphragmatic hernia	2	1	50	
Necrotizing enterocolitis	4	2	50	
Jejunoileal atresia	6	2	33.3	
Congenital hydrocephalus	6	2	33.3	
Cranial tumors	3	1	33.3	
Congenital cyanotic heart defects	8	2	25	
Biliary atresia	5	1	20	

desaturation could only be explained by the associated pulmonary hypoplasia of the patient. The parents declined autopsy requested by the attending surgeon.

The POMRs for surgery for certain pathologies treated during the study period are highlighted in table 5.

Of the 138 patients who had emergency operations, 26 cases were operated by consultant surgeons with a mortality rate of 7.69% compared with 112 operations performed by senior residents with a mortality rate of 8.93%. The difference was not statistically significant (p=1.000).

DISCUSSION

The most important finding in this study was that in-hospital POMR within 30 days was 320 per 10000 procedures and that higher ASA PS (III and above), emergency surgery, congenital anomalies, repeated (multiple) surgeries, and neonatal age groups were significant predictors of unexpected perioperative death. In addition, the majority of patients died from preoperative conditions complicated by sepsis.

Perioperative mortality has been reported to vary in LMICs from 2 to 10 times higher of that in HICs and can be 100-fold to 200-fold higher in certain low-income countries (LICs).² In the present study, POMRs within 24 hours, 7 days, and 30 days were 113.2 per 10000 procedures, 207.6 per 10000 procedures, and 320.8 per 10000 procedures, respectively. These figures are higher than those reported in HICs.^{13 14 24} In a GlobalSurg Collaborative study,¹⁰ 4 to 7 fold higher 30-day mortality was observed in LMICs compared with HICs. In this same study, the 24-hour mortality rates for LICs, medium-income countries and HICs were 2.6%, 0.7% and 0.3%, respectively, while the 30-day mortality rates were 8.3%,

2.9% and 0.9%, respectively. In a multicenter study in an LIC like ours, Newton *et al*⁴ found 24-hour and 7-day mortality rates of 0.8% and 1.7%, respectively, that were comparable to our study. However, Torborg et al,⁵ in South Africa (a middle-income country), reported a much lower 30-day mortality rate of 1.1%. A much lower 30-day mortality rate of 0.7% was reported by Bonasso et *al* in the USA.¹⁴ These studies^{5 14 24} suggest that 30-day mortality rate in our center is at least 4 to 5 times higher than reports from middle and high-income countries(MHICs). The potential reasons for this difference in mortality rates could be multifactorial and speculative. These include late presentation, sepsis, inadequate health personnel, grossly inadequate physiological monitoring facilities on the wards, and a dearth of a dedicated neonatal intensive care unit. We also noted that the inclusion of all types of surgeries especially cardiac, neurosurgeries and patients with poorer ASA status certainly influenced our mortality rates.

Gender played no role in perioperative death in our series and this was consistent with most reports.¹⁰ ²⁴ However, in a mixed population study, males had a two fold higher risk of death than females.³

As in our previous study and in other literature reports,^{5 16 25} neonates are more susceptible to perioperative death than older children. The risk of neonatal death in the present series is 20 times higher than that of their older counterparts. We noted that 58.8% of deaths recorded in the present study were neonates. The high mortality rate of 2439 per 10000 procedures within 30 days in this age group called for a review of the neonatal surgical program in our hospital. Gastroschisis, necrotizing enterocolitis, and esophageal atresia were the leading causes of mortality in these neonates who were very sick at admission. Congenital anomaly especially among neonates was associated with a 12.7fold risk of death compared with acquired conditions. The complexity of congenital anomalies, especially when cardiac defects are involved, seems to have a negative impact on postoperative outcome.^{10 15} Livingston et al^{15} observed that congenital anomalies had a higher mortality rate compared with acquired conditions. The presence of prematurity in some of the congenital cases seems to decrease their chances of survival. Weinberg and associates²⁶ identified gestational age as one of the six preoperative risk factors of postoperative morbidity and mortality. Other factors were ASA PS >3 (III), a history of cardiovascular comorbidities, and cardiovascular, neurological and orthopedic procedures. Akbilgic *et al*¹³ noted that children born prematurely had a four fold higher risk, while neonates undergoing surgery were at over 20-fold higher risk of unexpected death within 30 days following surgical procedure than older children. The burden of neonatal surgical conditions appears to overwhelm limited facilities and expertise that are available in LMICs, which have higher morbidity and mortality compared with HICs.

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Prenatal screening and diagnosis are lacking in our hospitals. These would have enabled informed decisions to be made by parents and would have allowed such pregnancies to be supervised in a specialist hospital with facilities to care for the babies and to reduce the chances of perioperative death. In addition, child birth in unorthodox health facilities, financial constraints, ignorance and poor transportation system often lead to late presentations where some babies have septicemia during admission to the hospital. As in our institution and in other health facilities in sub-Saharan Africa, other researchers have noted lack of dedicated neonatal intensive care unit, inadequate manpower (doctors, nurses and allied professionals), unavailability of parenteral nutrition and infrastructural deficits^{6–8 16 19} as factors that may increase perioperative mortality in LMICs.

A high ASA PS has been shown to predict perioperative mortality.^{3 24 25} Operative care, when stratified by ASA PS, provides insights into ways of targeting improvements to care. Approximately, 82.4% of perioperative death in our study occurred in patients with poorer ASA PS (III-V). Our findings along with other similar publications observed that surgical patients exhibiting a poorer ASA PS were more likely to experience perioperative mortality.⁴⁵ In this study, there was a seven fold increase in perioperative mortality when the ASA PS was greater than 2 (II). The risk of death was higher in those with ASA III and above. A high ASA status suggests that such patients are critically ill and may require monitoring devices, ionotropic and ventilator support. In addition, this group of patients should be recognized *ab initio*, operated on by specialist pediatric surgeons and anesthesiologists, and managed in neonatal or pediatric intensive or critical care units to reduce mortality rate. Unfortunately, the available manpower could not allow for these benefits, and some of the patients were operated on by resident doctors in surgery and anesthesia.

Our study has shown that multiple or repeated surgical procedures influenced mortality rate. The risk of death is 9.7-fold higher in those who had two or more repeated operative procedures either as a result of complications of previous surgeries, recurrence or progression of the initial pathology. This finding is consistent with literature reports from other LMICs in sub-Saharan Africa.^{10 20 27} In addition, we observed in this study as well as other reports in LMICs that postoperative septic complications were the principal indications for reoperations and eventual demise of these children.^{7 8 18 28} This contrasts with reports from developed nations where the majority of postoperative complications are non-infective.^{10 26} Though antibiotics were given to patients with postoperative sepsis, some of the parents or caregivers of these children failed to purchase antibiotics and other medications, leading to progressive deterioration of health status of these children. The national health insurance scheme that may assist in solving some of these challenges is still at its infancy in our country, and has not yet been embraced by a substantial portion of the populace.

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Parents still pay out of pocket to obtain health services. This implies that less privileged children are more likely to die from their illness in our society especially when these children have complex anomalies. We also identified that lack of pediatric trained nurses and a dearth of monitoring facilities for early detection of deterioration of medical conditions in some of the patients whose limited physiological reserve may have contributed to the POMR.

In this study, emergency operative procedures were associated with higher POMR compared with elective procedures. This result is consistent with reports from HICs as well as LMICs.³⁴¹⁰²⁴²⁷ In contrast to our previous report¹⁶ on perioperative mortality where typhoid perforation was the principal indication for emergency surgery leading to high morbidity and mortality, we observed a changing pattern towards congenital anomalies. This may be explained by an increase in the level of hygiene and provision of portable water in our environment, which has gradually reduced the incidence of typhoid septicemia.

Among the cohort of patients who had emergency operations, we found that the duration of preoperative symptoms as an independent factor did not influence surgical outcome, though the majority of the patients presented late. This result contrasts with our earlier retrospective study.¹⁶ We attributed this to the critical condition of some of the children with high ASA status who presented within 24 hours of their illness.

The major triggering factor for perioperative mortality in this study remained the underlying patient's disease. The mortality risk conferred by surgery in the current study was eight fold higher than the previous report from our center.¹⁶ This could be explained by the fact that all pediatric surgical conditions (including cardiac and neurosurgical cases) were included in the present study.

Previous reports^{29 30} noted that anesthesia-related death was 2 to 3 times higher in middle-income countries and may be 1000-fold higher in some poor countries. In the present study, anesthesia-related mortality rate was 18.9 per 10000 procedures which was at least 19 times higher than reports from HICs. Although cause of death was based on subjective interpretation, we did not find any patient in which the cause of death was solely related to surgical or anesthetic procedure. Previous studies reported that a quarter to nearly half of perioperative death could be potentially avoided by providing access to surgical care especially in low-resource countries.¹² We also believe that these deaths could be avoided if adequately trained personnel, monitoring devices and pediatric intensive care units were available and if best surgical and anesthetic practices were strictly adhered to in our setting.

In this study, there was no difference in the mortality rates between operations performed by resident doctors and consultants. Nevertheless, the fact that most of the fatalities occurred in the hands of resident trainees suggests that there is a need for more supervision to improve the care of patients. Similarly, there is a need for better patient screening to identify those at risk of preventable deaths (neonates, high ASA status III and IV, repeated operative cases, and emergencies). These ones should be anesthetized and operated by consultants when necessary so as to reduce mortality in our hospital.

Our study is limited by the fact that it is based on a single-center study.

In conclusion, POMR remains high in our institution. It is at least 4 to 5 fold higher than reports from some middle-income countries and HICs. The presence of congenital anomalies tends to impact negatively on perioperative outcome compared with acquired diseases in our setting. Availability of antimicrobial agents and postoperative intensive care unit may influence a reduction in POMR in our hospital.

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Contributors All authors made substantial contributions to the preparation of the manuscript. The study was conceptualized by TAO and SOA. All authors contributed to data curation, formal analysis and methodology. Writing–original draft was done by TAO, 000 and AOI. Writing–review and editing was done by TAO, SOA, AO and FFA. All authors have read and approved the final manuscript.

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