Antibacterial susceptibility patterns and factors associated with secondary peritonitis among patients with acute abdomen: a cross-sectional study

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Abstract

Introduction: Secondary peritonitis is the second leading cause of sepsis worldwide. Drug-resistance to peritoneal cavity bacterial infection remains a public health threat especially in resource limited settings of Africa including Uganda. This study was aimed at determining the antibacterial susceptibility patterns and factors associated with secondary peritonitis among patients with acute abdomen who underwent surgery at a Regional Referral Hospital in Uganda.

Methods: This was a cross sectional study conducted at Hoima Regional Referral Hospital (HRRH) that enrolled 126 patients with acute abdomen. After laparotomy, clinical samples were aseptically collected from patients with secondary peritonitis for culture and sensitivity using standard Microbiological methods. Binary logistic regression was used to identify factors associated with secondary peritonitis among patients with acute abdomen.

Results: Majority of the patients were males (61.9%) with a mean age of 37.9(SD +/- 21.8). Secondary peritonitis was found in 57(45.2%) of the patients. Gram-negative bacteria were the most commonly isolated organisms with *Escherichia coli (35.8%) and Klebsiella spp (17.0%)* predominating. Imipenem 88.8%(8/9), Amikacin 88.8%(8/9), Ciprofloxacin 44.4%(4/9) and Gentamicin 44.4%(4/9) demonstrated sensitivity to the different isolated organism at varying degrees. Being a male (AOR = 3.658; 95% CI = 1.570–8.519, p = 0.003) and presenting 3 days after onset of symptoms (AOR = 2.957; 95%CI = 1.232–7.099, p = 0.015) were independently associated with secondary peritonitis.

Conclusion

Imipenem, Amikacin, Ciprofloxacin and Gentamicin should be considered for empirical therapy in cases of secondary peritonitis. Patients, more especially males with abdominal pain should be encouraged to present early to the hospital in order to minimise progression to secondary peritonitis.

Background

Acute abdomen refers to abdominal pain that starts abruptly typically lasting not more than 24 hours requiring urgent attention (1). Most of the causes of acute abdomen require surgical attention (2). Peritonitis is the inflammation of the peritoneum, the layer which encloses many organs in the abdomen (3). There are three types of peritonitis: primary (spontaneous), secondary and tertiary (4) Secondary peritonitis refers to irritation of the abdominal peritoneal lining caused by direct contact with a peritoneal contaminants from gastrointestinal, intra-abdominal inflammation or genitourinary system perforation (6). Secondary peritonitis is associated with complications such as surgical sites infection, bust abdomen, re-laparotomy, prolonged hospitalization, morbidity and mortality (2, 7).

For secondary peritonitis to occur, there are factors that get into play and these can be socio-demographic factors or medical factors (8, 9). Following secondary peritonitis, different bacterial organisms may be involved mainly depending on the cause of the peritonitis. Drug-resistant to bacteria that cause infections of peritoneal cavity are becoming a public health concern (10). Although antimicrobial resistance containment interventions in healthcare structures have mostly been implemented in high-income countries, there is a pressing need to intervene in the antimicrobial resistance problem in low and middle income countries (LMIC) such as Uganda (11).

Even though just 1% of all hospital admissions worldwide are due to secondary peritonitis, it is the most frequent reason for admission to the surgical wards, increasing workload, length of hospital stays, and consequences such sepsis, surgical site infections, and enterocutanous fistula (2). Secondary peritonitis has also been reported to be the second leading cause of sepsis worldwide (3, 10). In Africa the proportion of secondary peritonitis has been reported to vary depending on the region with Alelign (10) reporting a prevalence of 19.1% in Ethiopia, Seni et al (12) 23.7% in Tanzania and Amone et al.(13) 17.1% in Uganda.

Even though peritonitis has been extensively studied in developed countries (14) there is still a paucity of data in low and middle income countries (LMICs) including Uganda and yet peritonitis is among the leading causes of mortality and morbidity among surgical patients(15, 16). Despite the known benefits of culture and sensitivity in the management of infections caused by secondary peritonitis, there is paucity of data relating to bacterial isolates and sensitivity patterns, yet this information would be important in choosing empiric therapy. More so, an understanding of the factors associated with secondary peritonitis may help in reducing the number of patients that come in with secondary peritonitis. This study was aimed at determining the antibacterial susceptibility patterns and factors associated with secondary peritonitis among patients with acute abdomen that underwent surgery at Hoima Regional referral Hospital in western Uganda.

Materials and methods

Study design

This was a cross-sectional study in which patients undergoing surgery for acute abdomen were enrolled. At laparotomy, the patients in whom secondary peritonitis was diagnosed, a sample of peritoneal content was taken for culture and sensitivity. Also, patient data was taken for determining factors associated with secondary peritonitis.

Study setting

This study was conducted at Hoima Regional Referral Hospital (HRRH), a public hospital funded by the Uganda Ministry of Health in which general care is free. It is located in Hoima Municipality, about 198 km west of Kampala with coordinates Latitude:1.428051; Longitude:31.354451. The surgery department has one theatre room with an average of 800 surgeries per year. The department has 4 surgeons and there is 1 day per week reserved for elective general surgeries but emergency surgeries are done at time when they are sanctioned. There are seven anesthetic officers. On average, 70 patients are operated for acute abdomen per month.

Sample size determination and sampling.

The sample size was calculated using Kish Leslie's (17) formula, $n = \frac{Z^2 p(1-p)}{d^2}$ whereby, n= estimated minimum sample size required, P=proportion of characteristics in a sample (9%) Northwestern Tanzania at University Teaching Hospital (18), Z=1.96 (for 95% Confidence interval), d= Margin of error set at 5%. On substituting, n=126. Therefore the sample size required for this study was = 126. Convenience sampling was done in which consecutive patients that satisfied the eligibility criteria were enrolled till the required sample size of 126 was reached. The enrolment of participants lasted 4 months (July 2022 to October 2022).

Inclusion and exclusion criteria

All patients who presented with the clinical and/or the radiological signs of acute abdomen that underwent surgery at Hoima regional referral hospital were included if they consented. Patients with a recent history of trauma and those that had been operated within one month prior to presentation were excluded.

Data collection procedure

A checklist was used to collect data from a patient who consented to participate in the study. One part of the checklist collected information composed of Demographic factors, Socio-behavior; medical factors, and environmental factors related to the development of secondary peritonitis. Another part collected the information from resuscitation, operation, swab collection, culture and sensitivity results. The checklist was translated to Runyoro for the participants that could not understand English. The checklist was pre-tested for validity and reliability and necessary adjustments made before data collection commenced.

Patients with acute abdomen requiring surgery were admitted in A & E of HRRH and informed about the study, written consent sought, detailed history taken and documented in the checklist. In the supine position, physical examination for signs of secondary peritonitis, including guarding, rigidity, and tenderness on palpation of the abdomen, were confirmed. The diagnosis was supported by radiological evidence and confirmed by intraoperative findings. The physiological status of the patient was assessed and resuscitation done before transfer to the operating room. The procedure (exploratory laparotomy) was done under general anesthesia by a general surgeon assisted by a resident. A sterile swab stick was socked in the peritoneal fluid during laparotomy and immediately placed in the amies transport medium to ensure the possibility of capturing all the bacteria (19). The research assistant transported the sample to the lab for immediate analysis after it had been labeled with the patient's serial number. The swab was aseptically placed into the swab container with STUART media to maintain the viability of the aerobic and facultative anaerobic bacteria at the same time avoiding contamination (20). The swab containers were placed in the cool box and transported to Microbiology laboratory of KIU-TH for laboratory analysis. The patient was given treatment according to Uganda's clinical guidelines as the researcher continued to follow up on the result in the laboratory.

Samples collected using a sterile procedure with the peritoneal swab stick were inoculated on blood agar, chocolate agar, MacConkey agar, and Thayer Martin medium, and different biochemical tests were used. After that, they were incubated at 37^{o}c for 24–48 hours both aerobically and anaerobically. The shape, size, height, margin, and surface properties of the colony were observed. The methods used to identify the organisms included gram staining, catalase test, indole test, coagulase test, citrate utilization test, urease test, triple sugar iron agar fermentation test, and oxidase test.

Pure culture colonies of the organisms were tested for their antibacterial drug susceptibility against twelve commonly used antibiotics using Kirby-Bauer disc diffusion as modified by the clinical laboratory standard institute. Selection of 3 to 5 isolated colonies from the medium was done using a sterile wire loop and transferred into 3mls of nutrient broth in a bijou bottle. The broth was mixed by inversion for the complete dissolution of the colonies. The mixture was incubated for 4 minutes and was compared with 0.5% McFarland turbidity standard. A sterile inoculation glass rod was used to spread the surface of Muller-Hinton agar plates homogenously with the diluted colonies. Antibiotic discs were placed onto the inoculated Muller-Hinton agar and incubated at 37°C for 24–48 hours. The plates were examined and the diameter of zones of inhibition were measured in mm using a meter ruler and was compared to a standard chart of the corresponding antibiotics used for measuring zone of inhibition. The zone of inhibition was measured and recorded as susceptible (S), intermediate (I), or resistant (R) according to the standard chart.

Study variables

The independent variables included social demographic characteristics, medical characteristics and the behavioral characteristics of the patients presetting with acute abdomen. The dependent variable was the occurrence of secondary peritonitis. The secondary outcome variables were the bacterial isolates and susceptibility patterns.

Quality Control and analysis

The questionnaire was pretested and necessary changes made before starting data collection. The study assistants were trained before the study begun. Data was checked for completeness at the end of each entry. Laboratory analysis was done by qualified staff. The data was analyzed with the guidance of a biostatistician.

Data from the checklist were entered in Microsoft Excel 2010, and thereafter exported to SPSS version 22 for Windows. The proportion of secondary peritonitis among patients with acute abdomen who underwent surgery at Hoima regional referral hospital was computed as a percentage of patients with secondary peritonitis of all patients with acute abdomen. The bacterial isolates were summarized as percentages, and frequencies, and presented in a table. The susceptibility pattern was summarized by various bacterial isolates and presented using a table. The factors associated with secondary peritonitis were analyzed by both bivariate and multivariate backward logistic regression analysis. Biologically plausible variables and those with p values < 0.2 were considered for multivariate analysis to avoid leaving out significant variables. The variables in the final multivariate model were significant when p-value was ≤ 0.05. The measure of association was reported as odds ratios (ORs) with corresponding 95% CI and p-values. All statistical analyses were carried out in SPSS 22 series for Windows.

Ethical considerations and consent: All methods were carried out in accordance with relevant guidelines and regulations. Ethical approval was sought from the Research and Ethics Committee of Kampala International University Western Campus (**Ref No: KIU-2022-121**). Informed consent was obtained from all the participants and their legal guardian involved in the study.

Results

This study enrolled 126 patients with acute abdomen who had a mean age of 37.9(SD+/- 21.8) years, with majority being males (61.9%). Of the 126, only 57 were found to have secondary peritonitis accounting for 45.24% of the study participants. Of the 57 samples taken for culture and sensitivity, only 53 had growth and therefore, sensitivity patterns were assessed for the 53 participants. Gram-negative bacteria represented (66.7%) of isolated bacteria. Of the 53(93.0%) that had growth, *E. coli* accounted for the majority 19(35.8%) followed by *Klebsiella spp* 9(17.0%), *and others were Staphylococcus aureus* 7(13.2%), *Citrobacter spp* 5(9.4%), *Proteus spp* 4(7.5%), *Pseudomonas spp 4(7.5%), Enterobacter spp 3*(5.7%), *Enterococcus spp 1*(1.9%), and S*treptococcus spp* 1(1.9%).

Antibacterial susceptibility patterns among patients with secondary peritonitis who underwent surgery at HRRH.

In this study, all organisms isolated had complete resistance to Cloxacillin, methicillin, ceftriaxone, amoxiclav, cefixime, penicillin, ampicillin, and metronidazole. The Antibiotics that demonstrated effectiveness at varying degrees to the different organisms isolated were Gentamicin, Ciprofloxacin, Amikacin and Imipenem. *Enterobacter SSP* was only sensitive to Ciprofloxacin and Imipenem while S*treptococcus SSP* was only sensitive to Imipenem and Amikacin. The details of susceptibility testing are shown in Table 1 below.

Table 1
Antibacterial susceptibility patterns among patients with secondary peritonitis who underwent surgery at HRRH.

		Pseudomonous SPP N = 4(7.5%)	*Staphyococcus aureus N = 7(13.2%)*	*Citrobacter SSP, N = 5(9.4%)*	*Enterococcus Fecalis N = 1(1.9%)*	*E. Coli N = 19(35.8%)*	*Proteus N = 4(7.5%)*	*Enterobacter SPP N = 3(5.7%)*	*Klebshiella SPP N = 9(17.0%)*	*Streptococ SPP N = 1(1.9%)*
Imipenem	S	25.0%	85.7%	0.0%	100%	94.7%	100%	66.7%	88.9%	100%
	I	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	11.1%	0.0%
	R	75.0%	14.3%	100%	0.0%	5.3%	0.0%	33.3%	0.0%	0.0%
Amikacin	S	100%	14.3%	60.0%	100%	78.9%	50.0%	0.0%	55.6%	100%
	I	0.0%	0.0%	20.0%	0.0%	5.3%	0.0%	0.0%	0.0%	0.0%
	R	0.0%	85.7%	20.0%	0.0%	15.8%	50.0%	100%	44.4%	0.0%
Ciprofloxacin	S	0.0%	0.0%	20.0%	0.0%	5.3%	0.0%	33.3%	22.2%	0.0%
	I	0.0%	0.0%	0.0%	0.0%	5.2%	0.0%	0.0%	0.0%	0.0%
	R	100%	100%	80.0%	100%	89.5%	100%	66.7%	77.8%	100%
Gentamicin	S	50.0%	0.0%	0.0%	0.0%	31.6%	25.0%	0.0%	22.2%	0.0%
	I	0.0%	0.0%	0.0%	25%	0.0%	0.0%	0.0%	0.0%	0.0%
	R	50.0%	100%	100%	75%	68.4%	75.0%	100%	77.8%	100%
Cloxacillin	R	100%	100%	100%	100%	100%	100%	100%	100%	100%
Methicillin	R	100%	100%	100%	100%	100%	100%	100%	100%	100%
Ceftriaxone	R	100%	100%	100%	100%	100%	100%	100%	100%	100%
Amoxicluv	R	100%	100%	100%	100%	100%	100%	100%	100%	100%
Cefixime	R	100%	100%	100%	100%	100%	100%	100%	100%	100%
Penicillin	R	100%	100%	100%	100%	100%	100%	100%	100%	100%
Ampicillin	R	100%	100%	100%	100%	100%	100%	100%	100%	100%
Metronidazole	R	100%	100%	100%	100%	100%	100%	100%	100%	100%

S = sensitive, I = intermediate, R = resistant

Factors associated with secondary peritonitis among patients with acute abdomen who underwent surgery at HRRH.

At bivariate analysis, the variables that had a p value less than 0.2, and therefore qualified for multivariate analysis were; sex, marital status, education level, chronic illness, time to presentation, use of traditional medication, physical exercise, type of house and the number of meals taken per day. The results of bivariate analysis are shown in Supplementary file 1.

At multivariate analysis, the factors that were independently associated with occurrence of secondary peritonitis were sex and time to presentation. According to our findings, a male patient with acute abdomen was 3.658 (CI = 1.570–8.519, p = 0.003) times more likely to have secondary peritonitis compared to a female patient. A patient who took 3 days or more to come to the hospital after the onset of symptoms was also found to be 2.957(CI = 1.232–7.099, p = 0.015) times more likely to have secondary peritonitis compared to one that presented in less than 3 days. The rest of multivariate analysis is shown in Table 2.

Table 2
Multivariable analysis of variables associated with secondary peritonitis among patients with acute abdomen who underwent surgery at HRRH.

Characteristic	Bivariate analysis			Multivariate analysis		
	cOR	80% CI	P value	AOR	95% CI	P value
Sex						
Male	**3.484**	**1.600-7.587**	**0.002**	**3.658**	**1.570–8.519**	**0.003**
Female	Ref					
Marital Status						
Single	Ref					
Married	1.054	0.500-2.223	0.890	2.054	0.400-3.223	0.690
Widowed	0.136	0.016–1.172	0.069	0.236	0.026–2.172	0.569
Separated	0.362	0.035–3.735	0.394	0.462	0.015–4.735	0.294
Education level						
Never	Ref					
Primary	1.389	0.570–3.386	0.470	2.389	0.470–4.386	0.670
secondary	2.083	0.788–5.506	0.139	3.083	0.688–6.506	0.339
University	N/A			N/A		
Chronic illness						
None	Ref					
PUD	2.227	0.993–4.994	0.052	3.227	0.793–5.994	0.252
HIV	1.485	0.282–7.810	0.641	2.485	0.182–8.810	0.741
Diabetes Mellitus	N/A			N/A		
Time to presentation (days)						
< 3.0	Ref					
3.1+	**3.288**	**1.510–7.159**	**0.003**	**2.957**	**1.232–7.099**	**0.015**
Traditional medication						
No	Ref					
Yes	3.368	1.602–7.084	0.001	1.698	0.714–4.037	0.231
Physical exercise						
No	Ref					
Yes	3.221	0.601–17.272	0.172	3.130	0.484–20.242	0.231
House type						
Permanent	Ref					
Semi-Permanent	1.632	0.789–3.375	0.187	1.010	0.406–2.513	0.983
Meals per day						
< 3.0	Ref					
3.1+	0.605	0.285–1.281	0.189	0.741	0.308–1.783	0.504

cOR = Crude odds ratio, AOR = Adjusted odds ratio, CI = Confidence interval, Ref = Reference category, N/A = Not applicable since the category did not register one of the outcomes.

Discussion

In this study, the proportion of patient with secondary peritonitis was elevated (45.2%). Compared to the proportion found in this study, Seni et al.(12) in Tanzania found a higher proportion of secondary peritonitis (57.7%). In Ethiopia, two studies reported lower proportions of secondary peritonitis (19.3%(21) and 24%(22). A recent study in Tanzania also reported a lower proportion (21.5%) (23). Contrary to our findings, Nyundo et al.(24) in Rwanda reported a proportion of 41.5% related to poor knowledge of mid and a lower level health professionals on the diagnosis and early decision for management of acute

abdomen. The high proportion in our study could be explained by the late presentation related to some behaviour such as self-treatment, used of herbal treatment and financial constrains typical of low resource settings and low income- countries.

In this study, gram-negative bacteria represented (66.7%) of isolated bacteria which was comparable to the findings in a study conducted in Ethiopia by Alelign, (10) where gram negative organisms accounted (76.6%). *Escherichia coli* (35.8%) and *Klebsiella spp* (17.0%) were identified as the commonest bacteria cultured from infected peritoneal fluid. These findings are similar to the study done in Ethiopia by Alelign, (10) which reported *E. coli* (36.67%) and *Klebsiella* (20%) to be the commonest isolates. Furthermore, other studies conducted in Tanzania by Seni et al. (12); in Nigeria by Akujobi et al. (25) and in India by Kumar-m et al. (5) confirmed the predominance of *E. coli* followed by *Klebsiella spp* as the most frequent bacteria growth from peritoneal infected fluid associated with secondary peritonitis. In contrast, a study done In Mbarara by Mutiibwa & Tumusiime, (16) found the most common bacteria to be *Klebiella spp* (37.9%) followed *by E. coli* (26.4%) in small bowel perforation as the cause of secondary peritonitis. The predominance of these bacteria species in the infected peritoneal fluid of patient with secondary peritonitis might be due to their presence as normal flora in the gastrointestinal tract.

All the nine bacteria isolated from infected peritoneal fluid due to secondary peritonitis had resistance to Cloxacillin, methicillin, ceftriaxone, amoxiclav, cefixime, penicillin, ampicillin, and metronidazole. This implies that these commonly prescribed antibiotics will not be encouraged to be used as first line empirical therapy for secondary peritonitis, particularly in the study area. The practice of prescribing broad spectrum antibiotics with no clear indication and over the counter use of antibiotics might have contributed to resistance of these bacteria to these antibiotics which are normally readily available and affordable for the management of these strains of bacterial isolates. The Antibiotics that demonstrated effectiveness at varying degrees to the different organisms isolated were Imipenem, Amikacin, Ciprofloxacin, and Gentamicin which is slightly close to the result found by Alelign in Ethiopia and Mutiibwa at Mbarara regional referral hospital in Uganda (10, 16)

E. coli was highly sensitive to Imipenem (94.7%) and Amikacin (78.9%), with low sensitivity to Gentamycin (31.6%) and Ciprofloxacin (5.3%). This result is similar to a study done in Indian by Kumar-m et al. (26) and Sheikhbahaei et al. (27) in Iran where Imipenem and Amikacin (≥95.6%), Gentamycin and Ciprofloxacin (≥ 60%) had high sensitivities to E.coli. In this study, the difference could be explained by irrational prescription of Gentamycin and ciprofloxacin in our medical setting and also the fact that these drugs are cheap and readily available than Imipenem and Amikacin.

Klebsiella spp were sensitive to Imipenem (100%), Amikacin (55.6%) but slight sensitivity to Gentamycin (22.2%) and Ciprofloxacin (22.2%). Kumar-m et al. (5) in India reported similar result as Dwihantoro & Rochadi, (28) in Indonesia but with some differences in antibiotic susceptibilities which included cephalosporin. These differences could be explained by the fact that the practices of antibacterial use have been shown to vary which can result in different patterns of resistance.

Literature shows that many factors are known to be associated with secondary peritonitis such as demographic, social behavior, medical and even environmental factors; (8, 9). In this study, two factors male gender and time to presentation were the significant risk factors associated with secondary peritonitis.

Being a male patient with acute abdomen was 3.658 (CI = 1.570–8.519, p = 0.003) times more likely to have secondary peritonitis compared to being female. This finding is similar to the research done in Uganda by Ojuka & Ekwaro,(30) in Nsambya Hospital where the male to female ratio was 3:1 for peritonitis. Other studies conducted in Tanzania by Mabewa et al.(2) and Mukherjee & Sarkar,(31) in Indian found similar results of male to female ratios 1.8:1 and 8.4:1.6 respectively. This could also be explained by the poor health seeking behavior of males patients (2, 31) resulting in late presentation to the health facilities and the associated complications. Men are known to be unworried than women about their health, which means they might spend more time with a medical condition before they decide to search for appropriate management. A research done by Fillingim et al.(32) at Alabama University showed that women were more likely to worry about pain and feel more helpless about it, and are more likely than men to have depression and anxiety, all of which can lead to higher pain levels and health care seeking (32).

A patient who took 3 days or more to come to hospital after onset of symptoms was also found to be 2.957(CI = 1.232–7.099, p = 0.015) times more likely to have secondary peritonitis compared to the one who presented in less than 3 days which is in agreement with a study done in Mbarara regional referral hospital (16). Others studies by Nansubuga et al. (32) at Mulago national referral hospital, Mabewa et al. (2) in Tanzania and Ndayizeye et al. (34) in Rwanda also had similar findings suggesting that late presentation could reflect delay in seeking health care, attempted treatment through a traditional healer, lack of resources for transport to the health center and late referral by the peripheral health facilities. In Africa, late presentation (beyond 24 hours of the onset of the symptom) has been the norm especially in the rural areas.

5.5 Conclusion

Secondary Peritonitis is a common surgical emergency among patients with acute abdomen at Hoima regional referral hospital and its management needs urgent surgical attention. *Escherichia coli* and *Klebsiella spp* were the most common bacteria found in the infected peritoneal fluid of patients with secondary peritonitis after culture and sensitivity. These bacteria showed multiple resistances to the most commonly used antibiotics but were sensitive to Imipenem, Amikacin, Gentamicin and Ciprofloxacin at varying degrees. Male sex and time to presentation to the Hospital were the two main factors found to be independently associated with secondary peritonitis among patients having acute abdomen.

5.6 Study Limitations

This as a cross-sectional study was not able to follow up the outcome related to antibiotics found effective after culture and sensitivity. Depending on the culture media used, we were able to isolate aerobics and facultative anaerobes. Therefore, strict anaerobes and fungi were not captured which might explain why some samples did not yield any growth.

5.8 Recommendations

Imipenem, Amikacin, Ciprofloxacin and Gentamicin should be considered for empirical therapy in cases of secondary peritonitis. Patients, more especially males with abdominal pain should be encouraged to present early to hospital in order to minimise progression to secondary peritonitis. All health workers should participate in antibiotic stewardship to prevent the emergence, spread and persistence of antibiotic resistance and do further studies to determine resistant genes of the isolates. There should be periodic monitoring of antimicrobial resistance patterns to helps physicians to choose antimicrobial agents for empiric treatment of secondary peritonitis.

Abbreviations

HRRH
Hoima regional referral hospital
LMIC
Low- and middle- income countries.

Declarations

Ethics approval and consent to participate: All methods were carried out in accordance with relevant guidelines and regulations. Ethical approval was sought from the Research and Ethics Committee of Kampala International University Western Campus (**Ref No: KIU-2022-121**). Informed consent was obtained from all the participants and their legal guardian involved in the study.

Consent for publication: Not applicable

Availability of data and materials: Data is available upon request. Requests should be sent to **NBG** Via nyenkegodefroy@yahoo.fr

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Author's contributions: NBG was the principle investigator, conceived and designed the study, collected data, analysed data and wrote the draft of the manuscript. **JM** participated in data analysis, discussion of results and revised the manuscript, **EA, and XFO** supervised the work and revised the manuscript, **SFM, MAW, BPK, WM, JW and BKF** participated in data collection, revised the manuscript and all authors approved the final paper.

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