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INTRODUCTION Nosocomial infection (NI), also called "hospital-acquired infection (HAI) or healthcare-associated infection (HCAI)," is an infection acquired in a hospital by a patient who was admitted for a reason other than that infection<sup>1,2</sup>. It is also an infection(s) occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission<sup>3</sup>. These infections occur up to 48 hours after hospital admission, or three days after discharge, to 30 days after the operation.

Despite progress in public health and hospital care, infections continue to develop in hospitalized patients and may also affect hospital staff<sup>4</sup>. Globally, 2 million people are affected by nosocomial infections each year, and about 20% of them result in hospitalization<sup>5</sup>. According to Azeez-Akande<sup>6</sup>, the proportions of nosocomial staphylococcal infection range from 2-50% in Sub-Saharan Africa and speckled with the setting, with intensive care units (ICUs) having the highest incidence rates of 21.2-35.6%.

In Uganda, like other African regions, there is no comprehensive data to show the magnitude of nosocomial infection in the country. Individual research from across the country indicates a contentious rise of nosocomial infections with resistance among the bacteria species isolated<sup>7</sup>. For example, A study by Seni et al.<sup>8</sup> in Mulago National Referral Hospital, Uganda, found that about 10% of patients undergoing surgical procedures become septic. Similarly, Adam et al.<sup>5</sup> reported 30.8% strains of *S.*

*epidermidis* isolated from 363 environmental samples from wards surfaces of Kampala International University-Teaching Hospital (KIU-TH). Among these isolates, 11 (9.8%) were found resistant to cefoxitin. Many factors fuel this infection among hospitalized patients: decreased immunity among patients, increasing variety of medical procedures, invasive techniques are creating potential routes of infection, and transmission of drug-resistant bacteria among crowded hospital populations, in which poor infection control practices may facilitate transmission.

Nosocomial infections lead to a prolonged hospital stay, long-term disability, increased antimicrobial resistance, increased socio-economic disturbance, and increased mortality rate<sup>1,2,9</sup>. Hospitals have become particularly notorious for spreading lethal infections; in each hundred hospitalized patients, seven in developed and ten in developing countries may acquire HCAI<sup>10</sup>. Furthermore, most of the pathogens causing HAI developed resistance to the most commonly prescribed antibiotics in healthcare settings, making it difficult for health care providers to deliver effective health care services.

Due to that, World Health Organization recommended urgent action to prevent and control the spread of antibiotic-resistant microorganisms in health care settings which

involved researching to adapt and validate surveillance protocols based on the reality of developing countries, researching the potential involvement of patients and their families in HAI reporting and control, among the others<sup>2,11</sup>. Literature has shown that the HAI burden was more in poor-income countries, and there lacked comprehensive data on the magnitude of the infections in the region<sup>12-14</sup>.

Therefore, this study was aimed at determining the prevalence of nosocomial infections and antibiotic susceptibility profiles of bacteria isolated from Kasese District Hospitals in Western Uganda. MATERIALS AND METHODS Materials The materials used include Nutrient agar/NA, MacConkey agar/MAC, Chocolate agar/CHOC, Mueller-Hinton agar/MHA (Oxoid), 0.5 McFarland standard, antibiotics discs of penicillin, erythromycin, oxacillin, ampicillin, tetracycline, ciprofloxacin, clindamycin, chloramphenicol, cefuroxime, ceftriaxone, ceftazidime, gentamycin, and cotrimoxazole.

The main instruments used in this study were incubator, API® 20E V4.1 and API® staph V4:1 kit (bioMérieux, France). The software used for data analysis was IBM SPSS Statistics® 16.0. Methods Study design and sites The study was a descriptive cross-sectional laboratory-based study. The study was carried out in Kasese District Western Uganda, located on latitude and longitude (0.1699°N, 30.0781°E) with a population of 738,300. Three health facilities: a private, not-for-profit health facility and also a Teaching Hospital (Kagando Hospital), one government hospital (Bwera Hospital), and a private for-profit health facility (Bishop Masereka Medical Center) which a nongovernmental organization owns were studied. These health facilities were spread out and gave a good impression and representation of all facilities in Kasese District with a bed size of not less than 30 and with regular surgical operations (Figure 1).

Furthermore, these hospitals were chosen as the study sites because they were primary health care providers for both out-patients and in-patients in the district. Patients that were newly admitted and slated for operations were recruited for the study following consent. Consenting participants were then monitored from the day of admission to the time the operation was performed. Non-consenting surgical and Cesarean section (C-section) patients and a patient who was terminally ill within selected hospitals were excluded from the study.

Sample size determination A non-probability convenient sampling technique was used to achieve the predetermined sample size<sup>15</sup>. Samples size (both surgical and C-section patients) with a cumulative incidence of surgical site infection (SSIs) 10% among surgical patients in general and 9.4% among women who undergo C-section in Uganda at a national referral hospital<sup>16</sup>, as shown in Formula 1.  $n = \frac{Z^2 \cdot p \cdot q}{d^2}$  (1-??) ?? 2 . . .

[1]  $z$  = confidence limits at 95% confidence interval;  $p$  = rough estimate of the affected persons (10);  $(1-p)$  = percentage of persons not affected;  $d$  = absolute sampling error that can be tolerated  $\pm 0.1$ ;  $n$  = estimated sample size. Therefore, the minimum sample size required according to Formula 1 was shown in the following calculations:  $n = 1.96^2 \cdot \frac{0.01(1-0.10)}{0.1^2} = 345$ . Only three of the initial four health facilities proposed permitted the study, and of the 345 initial participants proposed, 303 participants were enrolled from the three study sites.

Bacterial analysis of the operation theater air and floor Bacterial loads of the air in the operation theatres of all the selected facilities were studied by the plate settling method<sup>17</sup>. Open plates of NA, MAC, and CHOC were placed in different areas of the theatre at the start of each operation and left open until the operation was completed. All plates were closed and then incubated at 37°C for 24 hours aerobically for NA and MAC, while CHOC was incubated in a jar containing 10% CO<sub>2</sub> for 24 hours. Colonies were counted and converted to colony-forming units per cubic meter (CFU/m<sup>3</sup>).

About 1 m<sup>2</sup> of theatre floor subdivided into squares was randomly swabbed to estimate the bacterial loads of the theatre floor. Moistened swabs were dispensed in 9 mL of normal saline and mixed well. As much as 1 mL of each mixture was inoculated on NA, MAC, and CHOC plates. The plates of NA and MAC have incubated aerobically at 37°C, while CHOC plates were incubated in a jar containing 10% CO<sub>2</sub> for 24 hours<sup>18</sup>. Colonies were counted and converted to colony-forming units per square meter (CFU/m<sup>2</sup>).  
Detection of surgical site nosocomial infections Operated patients in the ward were followed daily, and operations sites were inspected for signs of infection.

Patients' notes were also checked for information that indicates signs of SSIs. The classification of an operation site as positive for SSIs was based on the Centers for Disease Control and Prevention (CDC) definition, which states that "SSI is an infection that occurred within 30 days after operative procedure"<sup>19</sup>. The patient would have developed SSIs when at least one of the following was noticed: purulent discharge from the superficial incision; isolated organism from an aseptically obtained culture of fluid or tissue from the superficial surgical incision; and signs or symptoms of infection pain, tenderness, or heat and superficial incision deliberately opened by the surgeon unless the incision was culture negative.

For any occurrence of the above signs or symptoms on a patient, consultant attention would be drawn and a decision on the status of the sign made. These sites were swabbed using sterile cotton swabs and inoculated in 9 mL sterile peptone water broths, inoculated on NA, MAC, and CHOC, and incubated at 37°C for 24 hours.  
Characterization of bacterial isolates All the isolates sub-cultured onto NA slants were

characterized by their cultural, morphological, and biochemical reactions<sup>19</sup>. Bacterial species were also confirmed using API® 20E V4.1 and API® staph V4:1. A 0.5

McFarland standard suspension of each bacterial isolates was prepared and each identification strip inoculated by pipetting the suspension into each cupule as instructed by the manufacturer. This was then incubated at 35-37°C in the moist chamber for 24 hours. After 24 hours incubation period, reagents were added to the appropriate well, and the reaction of each cupule was read by comparing the color of each well to the reading table in the package insert.

Identity of the bacterial isolates was obtained by use of profiles for this combination of reactions from API web using API® 20E V4.1 and API® staph V4:120. Antibiotics susceptibility profiles of the isolates Antimicrobial susceptibility testing was done using the disk diffusion method using MHA according to the method described by the Clinical and Laboratory Standards Institute (CLSI) guidelines<sup>21</sup>. A susceptibility test was done using the stokes method. Both the standard controls and the test organism were inoculated on the same plate, and the zone of inhibition of the test organism was compared directly with that of the control<sup>22</sup>.

Antibiotics discs used were penicillin (10 µg), erythromycin (15 µg), oxacillin (1 µg), ampicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), chloramphenicol (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), gentamycin (10 µg), and cotrimoxazole (1.25/23.75 µg). After 24 and 48 hours of incubation at 37°C, all plates were read and results interpreted according to the standard procedure of CLSI. Determination of multiple antibiotics resistance index The multiple antibiotic resistance (MAR) index was determined for each of the selected bacterial isolates by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested<sup>23</sup>. Data analysis Data collected through a questionnaire and from sample analysis were entered in Microsoft Excel data set. The data set was imported into SPSS 16.0 for analysis and presented using tables and bar graphs.

Means, proportions, and medians were used to characterize the study participants<sup>24</sup>. Ethical considerations The ethical approval of the study was sought from Mbarara University of Science and Technology, Faculty of Medicine Research Committee (Ref: DMS6); Mbarara University of Science and Technology, Institutional Research and Ethics Committee (IREC) on Human Research (Ref: MUREC1/7); and final approval were obtained from all the three studied Hospitals.

All research protocols were performed by the ethical standards of committees on

human experimentation laid down in the Declaration of Helsinki.

/ Figure 1. Map of Kasese District, Uganda showing the distribution of health facilities

RESULTS AND DISCUSSION Demographic characteristic of studied participants As many as 303 patients from three selected health care facilities from Kasese District were enrolled in this study, 44 SSIs were confirmed. As many as 71.6% of participants were females. Surgical site infection was more prevalent in males 17.4%, patients who underwent explorative laparotomies 21.9%, and those with American Society of Anesthesiologists (ASA) score of 3 21.1%, as shown in Table I. The higher prevalence of SSIs found in males than their counterparts (female) was contrary to the findings of Nair et al.<sup>25</sup>, who reported a nearly equal prevalence of SSIs from a tertiary hospital in the Northern Cape Province, South Africa.

Prevalence of surgical site infections in selected hospitals Prevalence of nosocomial infection among hospitalized surgical and C-section patients among the hospitals studied was 20.9%, 12.2%, and 8.6% for Bwera, Kagando, and Bishop Masereka Hospitals, respectively (Table II). These results were in line with Yallem et al.<sup>26</sup> and Okello et al.<sup>27</sup>, who reported 14.9% (n=908) and 14.0% (n=129) prevalence of HAI from two teaching hospitals of the Amhara region in Ethiopia and large hospital in Northern Uganda, respectively. However, Nair et al.<sup>25</sup> reported a lower prevalence of 7.67% (n=326) of HAI from a tertiary hospital in the Northern Cape Province, South Africa. The prevalence of nosocomial infection found in this study was lower compared to the prevalence reported by Greco et al.<sup>14</sup> and Kesah et al.<sup>28</sup>, who reported an overall prevalence HAI of 45.8% (n= 664) and 28% (n=410) from Paediatric surgical patients at a tertiary health institution in Lagos, Nigeria and Larco Hospital Northwestern Uganda, respectively.

Bacterial loads of air and floor Bacterial contamination was noted in all the three health facilities but notably highest in Bwera Hospital operating theatre with 600 CFU/m<sup>2</sup> and >60 CFU/m<sup>3</sup> for floor and air, respectively (Figure 2). Table I. Demographic characteristics of the studied participants

Characteristics	n=303	Proportion n (%)	SSIs n (%)
Hospital			
Kagando	123 (40.6)	15 (12.2)	
Bwera	110 (36.3)	23 (20.9)	
Bishop Masereka	70 (23.1)	6 (8.6)	
Gender and age			
Male	86 (28.4)	15 (17.4)	
Female	217 (71.6)	29 (13.4)	
Median age	34	-	
ASA score			
1	11 (3.6)	2 (18.2)	
2	234 (77.2)	30 (12.8)	
3	57 (18.9)	12 (21.1)	
4	1 (0.3)	0 (0)	
Procedure			
C-section	178 (58.8)	24 (13.5)	
Amputation	1 (0.3)	1 (3)	
Hysterectomy	3 (0.99)	0 (0)	
Prostatectomy	13 (4.29)	1 (7.7)	
Herniorrhaphy	6 (1.98)	1 (16.7)	
Appendectomy	16 (5.28)	1 (6.2)	
Exploratory laparotomy	73 (24.09)	16 (21.9)	
Vesicovaginal fistulas	13 (4.29)	0 (0)	
Time taken in surgery (minutes)			
<31	20 (6.6)	3 (15)	
31-60	231 (76.24)	27 (11.7)	
>60	52 (17.16)	14 (26.9)	
Mean duration of procedure	48	-	
Overall SSIs			
Yes	44 (14.5)		
No	259 (85.5)		

The results also showed that the floor has the highest contaminant or bacterial loads compare to air from all the studied hospitals. The results of these findings were lower



compared to the findings of Tang and Wan<sup>29</sup>.

They reported higher mean and standard deviation of bacterial loads of 383.5 (2.1), 106.9 (2.0), 373.7 (1.6), 141.5 (2.2), 270.8 (1.8), 182.2 (1.8), 92.0 (2.3), 144.5 (2.0), and 87.19 (1.9) CFU/m<sup>3</sup> from air rooms of the post-operative recovery room, instrument room, supply washing room, delivery room, kidney transplant room, traumatic surgery room, and liver transplant room respectively in a Medical Center in Taiwan. Table II. Prevalence of SSIs in the three selected hospitals of Kasese District, Western Uganda

Hospital	Samples collected	SSIs n (%)	Negative n (%)	Total n (%)
Kagando Hospital	123	15 (12.2)	108 (87.8)	123 (100)
Bwera Hospital	110	23 (20.9)	87 (79.1)	110 (100)
Bishop Masereka Hospital	70	6 (8.6)	64 (91.4)	70 (100)

/ Figure 2.

Comparison of bacterial loads on air and floor of operating theaters in three selected hospitals

Contaminants isolated from air and floor of operating theatres

Coagulase-negative Staphylococci (CoNS), E. coli, and Micrococcus spp were the major contaminants isolated from air and floor in the operating theatre from the three studied hospitals, as shown in Table III. These organisms were reported to be a significant cause of nosocomial infections Onwubiko et al.<sup>30</sup>, especially among immunocompromised patients.

The results of contaminants bacterial isolates found in this study were in line with findings of 11 who reported a higher prevalence of CoNS from the air and protective wears in the operating theatre and surgical wards of two tertiary hospitals in Kano, Northwestern Nigeria. This result was contrary to the finding of Genet et al.<sup>31</sup>, who reported a higher prevalence of S. aureus (70.8%), which was coagulase-positive from operating rooms and surgical wards at Jimma University Specialized Hospital, Southwest Ethiopia. However, Najotra et al.<sup>18</sup> reported Bacillus spp as the most isolated bacterial species (87.6%) from operation theatres of a tertiary care hospital in North India. Table III.

Isolated bacteria from air/floor of operating theatres in selected hospitals of Kasese District

Hospital	Sample collected	Isolated bacteria	CoNS n (%)	Micrococcus spp n (%)	S. aureus n (%)	Proteus spp n (%)	Bacillus n (%)	E. coli n (%)
Bwera Hospital	10	6 (60)	4 (40)	4 (40)	5 (50)	4 (40)	5 (50)	
Kagando Hospital	10	5 (50)	4 (40)	3 (30)	3 (30)	2 (20)	4 (40)	
Bishop Masereka Hospital	10	2 (20)	3 (30)	1 (10)	2 (20)	1 (10)	2 (20)	

Isolated nosocomial surgical site pathogens Staphylococcus aureus (33%) was the most Gram-positive bacteria isolated, whereas E. coli (24%) was the most common Gram-negative nosocomial microorganism, as shown in Figure 3. The prevalence of S.

aureus found in this study was higher than the finding of Agaba et al.<sup>12</sup>, who reported 4.40% and 4.48% from blood and tracheal samples collected from patients in Ugandan intensive care units (ICUs). This result was lower than Al Laham's findings<sup>32</sup>, who reported Staphylococcus spp 45.31% prevalence from general operating theatres in selected hospitals in the Gaza Strip, Palestine. This study also showed that S. aureus was the predominant species of Gram-positive bacteria isolated from studied hospitals. This result was in line with the findings of Agaba et al.<sup>12</sup>, who also reported S. aureus as the commonest Gram-positive bacteria isolated from Ugandan ICUs.

/ Figure 3. Frequency of nosocomial pathogenic bacteria isolated from three selected hospitals in the Kasese District of West Uganda Susceptibility profiles of Gram-positive bacteria from surgical site The results of this study showed that there was exceptional resistance of S. aureus to gentamycin (50%), oxacillin (33%), and resistance to clindamycin (16%), with 100% sensitivity of Group A Streptococcus to erythromycin, ceftriaxone, and clindamycin, as presented in Table IV.

This result was in line with the finding of Agaba et al.<sup>12</sup>, who reported that S. aureus isolated from Ugandan ICUs was resistant to erythromycin, penicillin, and oxacillin but susceptible to gentamycin. These findings also prove that different Gram-positive bacteria from different environments may have different antibiotic susceptibility profiles, regardless of the species<sup>33</sup>. Table IV. Susceptibility profiles of Gram-positive nosocomial SSI from three studied hospitals of Kasese District, Western Uganda

Antibiotics (µg)	S. aureus n=15 (%)	S. viridans n=2 (%)	Group A Strep n=6 (%)
Ceftriaxone (30)	S 0 (0%)	I 0 (0%)	R 15 (100%)
Chloramphenicol (30)	S 0 (0%)	I 0 (0%)	R 15 (100%)
Ciprofloxacin (5)	S 3 (20%)	I 0 (0%)	R 5 (33.3%)
Clindamycin (30)	S 5 (33.3%)	I 2 (33.3%)	R 1 (6.7%)
Erythromycin (15)	S 1 (6.7%)	I 0 (0%)	R 14 (93.3%)
Gentamycin (10)	S 8 (53.3%)	I 0 (0%)	R 7 (46.6%)
Oxacillin (1)	S 8 (53.3%)	I 0 (0%)	R 4 (26.6%)
Penicillin (10)	S 1 (6.7%)	I 5 (33.3%)	R 12 (80%)
Cotrimoxazole (1.25/23.75)	S n/a	I n/a	R 0 (0%)
Tetracycline (30)	S 6 (40%)	I 1 (6.7%)	R 8 (53.3%)

DST: disc sensitivity testing; n/a: not tested; R: resistant; I: intermediate sensitivity; S: sensitive

Susceptibility profiles of Gram-negative bacteria from surgical site Gram-negative microorganisms showed 100% resistance to ampicillin.

There was 100% resistance to chloramphenicol by Proteus spp and Pseudomonas spp. Pseudomonas aeruginosa showed (50 -100%) sensitivity to gentamycin and



developing SSIs in Kasese District selected hospitals. In the bivariate analysis, the hospital attended in Kasese District Western Uganda and duration of procedure were significant risk factors for acquiring SSIs, with p-values of 0.033 (CI 0.137-0.921) and 0.006 (CI 1.338-5.791), respectively (Table VI). Multivariate analysis of risk factors for developing SSIs in Kasese District selected hospitals. The factors that had a p-value <0.05 in the bivariate analysis were included in the multivariate analysis.

Binary logistic regression was done where the model showed an 8% variation independent variable (Nagelkerke's R<sup>2</sup> 0.088). The model used in this analysis best fit data (p 0.819) and 85.5% of the participants were correctly classified as being infected. The results of multivariate analysis showed that those who had 30-60 minutes surgery were 3.501 times more likely to have SSIs, and it was statistically significant with p 0.002, as shown in Table VII. Table VI. Bivariate analysis between socio-demographic factors and SSIs

Variables	n (%)	Infected n (%)	Normal n (%)	Crude OR	95% CI	p-value
Hospitals						
_Kagando Hospital	123 (100)	15 (12.5)	108 (87.8)	0.675	0.249- 1.827	0.439
_Bwera Hospital	110 (100)	23 (20.9)	87 (79.1)	0.355	0.137- 0.921	0.033*
_Bishop Masereka Hospital	70 (100)	6 (8.6)	64 (64)	1.000	- -	-
Gender						
_Female	217 (100)	29 (13.4)	188 (86.6)	1.370	0.694- 2.705	0.365
_Male	86 (100)	15 (17.4)	71 (86.6)	1.000	- -	-
Duration of surgical procedure (minutes)						
_<30	20 (6.6)	3 (15.0)	17 (85.0)	2.088	0.530- 8.231	0.293
_30-60	231 (76.24)	27 (11.7)	204 (88.3)	2.784	1.338- 5.791	0.006*
_>60	52 (17.16)	14 (26.9)	38 (73.1)	1.000	- -	-

\*Statistically significant at 95% level of confidence Table VII.

Multivariate analysis between socio-demographic factors and SSIs

Variables	n (%)	Infected n (%)	Normal n (%)	Crude OR	95% CI	p-value
Hospitals						
_Kagando Hospital	123 (100)	15 (12.5)	108 (87.8)	0.843	0.299- 1.827	0.749
_Bwera Hospital	110 (100)	23 (20.9)	87 (79.1)	0.324	0.122- 0.859	0.23*
_Bishop Masereka Hospital	70 (100)	6 (8.6)	64 (64)	1.000	- -	-
Duration of surgical procedure (minutes)						
_<30	20 (6.6)	3 (15.0)	17 (85.0)	1.875	0.530- 8.231	0.38
_30-60	231 (76.24)	27 (11.7)	204 (88.3)	2.784	1.599- 7.667	0.002*
_>60	52 (17.16)	14 (26.9)	38 (73.1)	1.000	- -	-

\*Statistically significant at 95% level of confidence

CONCLUSION Based on the findings of this study, nosocomial infections in surgically hospitalized patients were found to be 20.9%, 12.2%, and 8.6% for Bwera Hospital, Kagando Hospital, and Bishop Masereka Hospitals, respectively. Staphylococcus aureus and E. coli were found the most common bacteria isolated. Most of the isolates were found to be resistant to more than one antibiotic drug of choice.

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