WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 7, 85-100. Research Article ISSN 2277– 7105

CHARACTERIZATION OF BACTERIAL INFECTIONS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF PERITONITIS POST CAESAREAN IN GYNECOLOGY AND OBSTETRIC DEPARTMENT AT UNIVERSITY TEACHING HOSPITAL OF KIGALI, RWANDA

Musabyumuremyi Celestin*[1,2], Arpita Sharma[2,3], Nyirigira John[4], Nkubana Theoneste[4], Uwamariya Chantal[4], Iradukunda Patrick[5], Musanabaganwa Clarisse[1] and Mazarati Jean Baptiste[1]

[1]Rwanda Biomedical Center, Rwanda.
[2]School of Science, Career Point University, India.
[3]Maa Bharti P. G. College, University of Kota, India.
[4]University Teaching Hospital of Kigali, Rwanda.
[5]Rwanda Food and Drugs Authority, Rwanda.

Article Received on
28 April 2020
Revised on 18 May 2020
Accepted on 08 June 2020
DOI: 10.20959/wjpr20207-17857

***Corresponding Author**
Musabyumuremyi Celestin
Rwanda Biomedical Center, Rwanda.

ABSTRACT

Introduction: Peritonitis post- caesarean infections are the second cause of maternal mortality next to postpartum hemorrhage and most of bacteria responsible for those infections are antibiotics resistances which are public health concern. **Objective:** The aim of this study is to determine the risk factors to peritonitis post-cesarean infections; bacteria profile; pattern of their antibacterial susceptibilities and the genes that are involved in Extended Spectrum Beta Lactamase production for Gram negative bacteria. **Methodology:** This study was a prospective and descriptive study conducted from October 2017 to November 2018 at University Teaching Hospital of Kigali in gynecology and obstetric department with peritonitis post caesarean section whereby a total sample of 196 participants has been recruited using convenience sampling method. The samples culture, bacteria isolation, identification and antibacterial susceptibilities were performed according to CLSI. ESBL production for Gram negative bacteria were phenotypically identified with combination disc and genotypically by PCR. The data were entered in SPSS version 22 and statistically analyzed to determine the associated factors using Chi square test. **Results:** Out of 196

samples performed, 181samples were culture positives (92.3%). Higher proportion of bacterial isolates were Gram-negative than Gram-positive bacteria 126 (69.6%). *Klebsilla pneumonia* accounted for 30.9% and was the most isolated pathogen. Imipenem was sensitive to all Gram negative bacteria while erythromycin for *Staphylococcus aureus*. 44 Gram negative bacteria (34.9%) were confirmed phenotypically as ESBL positive. The coding genes identified are: TEM accounted for 20 (45.5%), CTX- M1 for 14 (31.8%), CTX- M2 for 4 (9%); and for OXA 6(13.6%). Concerning the isolated genes of ESBL; *K.pneumonia* had TEM (5), CTX- M1 (10), CTX- M2 (1), and OXA (1); *E.coli* TEM (8) CTX- M1 (3), CTX- M2 (2) and OXA (3); *Acinetobator baumanii* TEM (3), CTX- M1 (1), CTX- M2 (1 and OXA (1); *Pseudomonas aeroginosa* TEM (3) CTX- M1 (8), and OXA 1, *Proteus mirabilis* TEM (1). **Conclusion:** High rate of antibiotic resistance has been observed in this study therefore routine screening of ESBLs production should be enhanced and also the diagnostic capacity of laboratory professionals for the detection and surveillance of antibiotic resistance should be strengthened.

KEYWORDS: Bacterial Infection Antimicrobial Susceptibility Peritonitis Caesarean.

1. INTRODUCTION

Bacterial infections during labour and the puerperium are among the leading causes of maternal mortality worldwide, accounting for about one tenth of the global burden of maternal deaths and Caesarean section is notably the most important risk factor for infection in the immediate postpartum period, with a five- to 20-fold increased risk compared to vaginal birth.[1,2] While the number of deaths arising from these infections has decreased considerably in high-income settings, the situation has not improved in resource-limited settings. Most of the estimated 75,000 maternal deaths occurring worldwide yearly as a result of infections are recorded in low-income countries.[3] Although the reported incidence in high-income countries is relatively low (between 0.1 and 0.6 per 1000 births), it is nonetheless an important direct cause of maternal mortality.[3,4] Maternal infections around childbirth also have a considerable impact on newborn mortality, and an estimated 1 million newborn deaths are associated with such infections annually.[5,6] Globally, the most common intervention for reducing morbidity and mortality related to maternal infection is the use of antibiotics for prophylaxis and treatment. Antibiotics are widely used for obstetric conditions and procedures that are thought to carry substantial risks of infection to the mother. In many low-income countries, the use of broad-spectrum antibiotics without confirmation of the

infective bacterial agent is common. Treatment of infection according to antibiotic sensitivity in this setting is constrained by poor diagnostic facilities and the need to promptly administer antibiotics to prevent severe complications. Apart from poor outcomes associated with such practice, there is increasing concern that inappropriate use and misuse of antibiotics among women giving birth could compromise public health through the emergence of resistant bacteria strains. According to the 2014 global report on surveillance of antimicrobial resistance, resistance to common bacteria has reached alarming levels in many parts of the world.[7] At University Teaching Hospital of Kigali known as CHUK no prospective study has been conducted to evaluate the extent of post-partum infection for the patients operated inside or transferred from district hospital obstetric and gynecology department for a follow up of 30 days of follow up. This study aimed to determine the risk factors that predispose to peritonitis post-cesarean section, the bacterial profile, their antibacterial susceptibilities and the genes that are involved in Extended Spectrum Beta Lactase production for isolated Gram negative bacteria. Therefore the results from this study could inform the policymakers the predominant bacteria and their antimicrobials resistance profiles, the route whereby they are spread to cause infections in obstetric and gynecologic patients and this could be used to establish appropriate strategies to reduce those infections and to make policies on infection controls and appropriate antibiotics to be administrated to the patients. Then all those data should be used to facilitate, strengthen and monitor the effectiveness of the infection prevention control committee being implemented in the various health facilities in Rwanda.

2. MATERIALS AND METHODS

2.1. Study design and sampling process

The following study was a prospective and descriptive study conducted from October 2017 to December 2018 at University Teaching Hospital of Kigali which is the main reference hospital in Rwanda serving 29 district hospitals with 560 beds capacities.

The study population included patients admitted in gynecology and obstetric department for cesarean operation after developed SSI and also other post partum patients referred for post-cesarean section peritonitis who underwent at the laparotomy at CHUK. The exclusion criteria were women with wound infections occurring after 30 days of surgery, patients under 18 years, and patient who refused to give consent. Convenience sampling method was used to recruit a sample of 196 patients.

2.2. Patients Data Collection procedures

Patients who have been referred to CHUK for the follow-up and treatment of peritonitis infection purpose, before laparotomy, they were explained about the study and requested voluntary participation in the study by signing the consent form and a sample of pus was collected using sterile cotton and transported in Laboratory immediately.

On the other side, for patients admitted at CHUK in gynecology and obstetrics department for cesarean operation, after surgery, a normal follow up has been conducted to all patients during the hospital stay. Surgical wounds and other vital signs have been inspected daily to check if there is any sign of infection such as redness, purulent discharge from operation site up to the patient is discharge and 30 days postoperatively. The patients who have been discharge from the hospital were contacted by phone and documented. If SSI was diagnosed through a structured telephone interview, the patient was referred to the nearest healthcare facility for further treatment. Structured questionnaires were used to fill the data from the participants retrieved using different sources such as open clinic, hospital medical records, surgical notes, structured interviews. The information collected were demographic data, associated risk factors (diabetes mellitus, HIV status, and smoking).The wound infection was suspected referring to CDC wound infection classification such as superficial infection, deep infection and organ or space of infection.

2.3.Specimen collection and processing

The specimens were collected aseptically on the first day when patients presented with clinical evidence of infection before the wound was cleaned with antiseptic. Using sterile cotton wool, swabs were obtained from surgical site without contaminating with skin commensals and transported to the laboratory immediately in Stuart's transport medium well labeled. Swab specimens and blood culture were processed and tested in microbiology laboratory at University Teaching Hospital of Kigali and specimens were cultured immediately when they arrived in the laboratory. The samples culture was done using standard bacteriological method for isolation and identification. The culture media used are 5% Blood agar, Chocolate agar for anaerobic isolation and identification, monnitol salt agar (MSA) for Staphylococci isolation and identification and MacConkey agar for bacilli identification. The samples cultured on chocolate blood agar were incubated at 35-37 ^{0}C in 5% Carbon dioxide and the samples cultured on Blood agar and MacConkey agar were incubated at 35 to 37 ^{0}C for 18 to 24 hours.

Antibiotic susceptibility

After isolation and identification, antibiotics susceptibility has been conducted with Kirby Bauer disc diffusion method using standard antibiotics used for antibiogram. Following isolation and identification of the bacterial isolates, a standard disc diffusion technique for drug susceptibility test (DST) was performed as recommended by Clinical and Laboratory Standard Institute (CLSI).[8] Ampicillin (10 µg), Gentamicin (10 µg), Erythromycin (15 µg), Amoxicillin- clavunilic acid (30 µg), Penicilline G_30µg, Ciproflaxin_5µg, Nitrofurane, and Oxacilline_1µg, Tetracycline_30µg were used for Gram positive isolates. Ampicillin (10 µg), Gentamicin (10 µg), Amoxacillin-clavunilic acid 30 µg, Cefotaxime_30µg, Ciprofloxacin (5 µg), Nitrofurane, Imipenem_10µg, Oxacilline_1µg, Piperacilline_10µg , Ceftazidime_30µg were tested for Gram negative isolates.

2.4.Screening and confirmation of ESBL-production bacteria

The Gram negative bacteria that showed an inhibition zone size of ≤ 22 mm with ceftazidime (30 µg) and/or ≤ 27mm with Cefotaxime (30 µg) were considered as potential of ESBL-producers and were selected for confirmation for ESBLs production. A disc of ceftazidime (30 µg), cefotaxime (30 µg), ceftazidime + clavulanic acid (30 µg), cefotaxime (30 µg) + clavulanic acid (30 µg/10 µg) were placed at appropriate distance on a MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight (18–24 h) at 37^{o} C. An increase in the inhibition zone diameter of > 5mm for a combination disc versus ceftazidime or Cefotaxime disc alone was confirmed as ESBLs production. We classified phenotypic ESBL positivity as any isolate that was resistant to either ceftriaxone or ceftazidime, and the ESBL activity was inhibited by the β-lactamase inhibitor clavulanic acid.

Testing of β-lactamase Encoding genes with Polymerase Chain Reaction

This was done in biotechnology laboratory at Career Point University (India). We performed a series of polymerase chain reaction (PCR) amplification with the Dallenne protocole on the identified phenotypically extended spectrum β-lactamase Gram negative bacteria isolates to detect β-lactamase genes (CTX-M, TEM, SHV, and OXA). DNA was extracted from one single colony of each isolate by incubation in a final volume of 100 µL of distilled water at 95 °C for 10 min followed by centrifugation. The cycling conditions were: 95 °C for 10 min, followed by 30 cycles of denaturation at 95 °C for 40s, annealing at 55 °C for 40s, elongation at 72 °C for 1 min, and a final elongation step at 72 °C for 7 min. A Positive result for polymerase chain reaction was revealed by the detection of a PCR amplicon of an appropriate

size for each target in the presence of positive and negative controls. We defined positive genotype ESBL any isolate that was PCR positive on any of CTX-M genes (CTX-M1, 2, 8, 9 or 25).

2.5.Quality control

Standard operating procedure (SOPs) for sample collection, transport, culture, isolation, identification and susceptibility testing for isolated organisms were used to ensure quality of the procedures. Quality control was performed using test strains of *E.coli* ATCC 25 922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853, *S.aureus* ATCC 29213 for MRSA, *Klebsiella pneumoniae* ATCC 700603 for ESBL.

2.6.Data analysis

The statistical analyses were performed using the SPSS, version 22.0. First, descriptive statistics, including count and percentage, were used to describe the subjects' demographic characteristics. The mean and standard deviation were computed for quantitative data variables while Qualitative data were compared using proportion. Bivariate analysis for association between potential risk factors and their potential association with SSI was performed using Chi square ($\chi 2$). P-value < 0.05 was considered statistically significant.

2.7. Ethical consideration

The study has been approved by different levels:

Rwanda National Ethic Committee (215/ RNEC/2017) National Health Research Committee (NHRC / 2017/PROT/17), Rwanda Medical Research (253/MRC/17) and University Teaching Hospital Ethic committee EC/ CHUK/487/17.

3. RESULTS

Out of 196 samples performed, 181samples were culture positives (92.3%). Higher proportion of bacterial isolates were Gram-negative than Gram-positive bacteria 126 (69.6%). *Klebsilla pneumonia* accounted for 30.9% and was the most isolated pathogen, followed by *Staphylococcus aureus* 25.9%, *Eschelichia coli* with 24.6%.Other isolated pathogens included *Acinetobater baumanii, Pseudomonas aeroginosa, Staphylococcus epidermidis, and Proteus mirabilis* with the frequency described (table 1). The sensitivity study showed that imipenem was sensitive to all Gram negative bacilli bacteria while for Gram positive bacteria erythromycin was more sensitive for *Staphylococcus aureus* but ampicillin become suitable for *Staphylococcus epidermidis*. Extended Spectrum Beta Lactamase gram negative bacteria

were phenotypically and genotypically identified. Among 126 the gram negative isolates; 55 (43.6%) were defined as presumptive ESBL production on the basis of the antimicrobial susceptibility on ceftazidime and cefotaxime testing results. This had been confirmed by the combined disk with clavulanic acid and the results showed that 44 (80.%) were confirmed phenotypically ESBL positive. Molecular analysis confirmed that these 44 isolates carried ESBL-encoding genes. TEM accounted for 20 (45.5%), CTX- M1 for 14 (31.8%), CTX- M2 for 4 (9%); and for OXA 6 (13.6%). Concerning the isolated genes of ESBL; *K.pneumonia* had TEM (5), CTX- M1 (10), CTX- M2 (1), and OXA (1); *E.coli* TEM (8) CTX- M1 (3), CTX- M2 (2) and OXA (3); *Acinetobator baumanii* TEM (3), CTX- M1 (1), CTX- M2 (1 and OXA (1); *Pseudomonas aeroginosa* TEM (3) CTX- M1 (8), and OXA 1, *Proteus mirabilis* TEM (1) (table 4 and 5). The age distribution of the participants showed that the median age was 26 years with interquartile range from 23 to 31 years. 81 (41.3%) were aged 21- 25 years, 43 (21.9%) between 26 - 30; 45 (23%) between 31 to 35 years while 7.6% were aged above 36. Infections developed were highly associated with deep infection to 90.5%. In addition 12 (6.7%) participants were HIV positive while only 2 (1.1%) had diabetes. Moreover the participants who were operated out of UTHK and transferred for post operatory peritonitis were 173 (88.2%) and those who developed post surgical infection operated at UTHK were 23 (11.7%).

Table 1: Frequency of bacteria isolated from post-operative wound infection.

No	Microorganism	Frequency N (%)
1	*K.pnemonia*	56 (30.9)
2	*S.aureus*	47 (26)
3	*E.coli*	45 (24.9)
4	*A.baumanii*	13 (7.2)
5	*P.aeruginosa*	10 (5.5)
6	*S.epidermidis*	8 (4.4)
7	*P.mirabilis*	2 (1.1)

The gram negative pathogens showed high level of resistance to Gentamicin 76.9% to *Acenotobacter baumanii*, 67.9% for *E.coli*; Ceftazidime 67.9% to *K.pneumonia*, 69.2 to *Acenotobacter baumanii*; Ciproflaxin 76.9% to *Acenotobacter baumanii*, 70% to *Pseudomonas aeroginosa,* Amoxyclavyl 62.5%, 66.7% for *K.pneumonia* and *E.coli.* Imipenem was the most sensitive antibiotic 100% for *K.pneumonia* and *E.coli* (table 2).

Table 2: Antimicrobial resistance pattern of Gram-negative aerobic bacteria in SSI patients.

Antibiotic	*K.pnemonia* N=56 (%)	*E.coli* N=45 (%)	*A.baumanii* N=13 (%)	*P.aeruginosa* N=10 (%)	*P.mirabilus* N=2 (%)	Total N=126 (%)
Piperacilline_10µg	15 (26.8)	7 (15.6)	3 (23.1)	6 (60)	1 (50)	32 (25.4)
Gentamicin_10µg	38 (67.9)	28 (62.2)	10 (76.9)	5 (50)	1 (50)	82 (65.1)
Ceftazidime_30µg	19 (67.9)	16 (35.6)	9 (69.2)	4 (40)	0 (0)	48 (38.1)
Cefotaxime_30µg	20 (35.7)	20 (44.4)	3 (23.1)	5 (50)	1 (50)	49 (38.9)
Ciproflaxin_5µg	27 (48.2)	24 (54.5)	10 (76.9)	7 (70)	0 (0)	68 (54)
Nitrofurane	24 (42.9)	23 (51.1)	2 (15.4)	2 (20)	1 (50)	52 (41.3)
Imipenem_10µg	0 (0)	0 (0)	1 (7.7)	1 (10)	0 (0)	2 (1.6)
Oxacilline_1µg	29 (52.7)	18 (40)	6 (46.1)	5 (50)	1 (50)	59 (46.8)
Amoxyclavy	35 (62.5)	30 (66.7)	2 (15.4)	4 (40)	1 (50)	72 (57.1)
Ampicillin	26 (46.4)	20 (44.4)	6 (46.1)	4 (40)	0 (0)	56 (44.4)

For gram positive bacteria, the high resistance have observed for Erythromycin 75% Nitrofurane 75%, Ciproflaxin 37.5%, Amoxyclavy 50%; 41.8% to *S.epidermidis*; while Amoxyclavy 34%, Nitrofurane 40.4%, Oxacilline 42.6 for *S.aureus* (Table 3).

Table 3: Antimicrobial resistance pattern of Gram-Positive aerobic bacteria in SSI patients.

Antibiotic	*S.aureus* N=47 (%)	*S.epidemidys* N=8 (%)	Total N=55 (%)
Erythromycin_30µg	8 (17)	6 (75)	14 (25.5)
Gentamicin_10µg	15 (31.9)	3 (37.5)	18 (32.7)
Tetracycline_30µg	16 (34)	2 (25)	1 (1.8)
Penicilline G_30µg	18 (38.3)	2 (25)	20 (36.4)
Ciproflaxin_5µg	12 (25.5)	3 (37.5)	15 (27.3)
Nitrofurane	19 (40.4)	6 (75)	25 (45.5)
Oxacilline_1µg	20 (42.6)	3 (37.5)	23 (41.8)
Amoxyclavy 30µg	16 (34)	4 (50)	20 (36.4)
Ampicillin	15 (31.9)	1 (12.5)	16 (29.1)

For phenotypic confirmation of potential ESBL-producing bacterial isolates, both cefotaxime and ceftazidime alone and in combination with clavulanic acid by disk diffusion method were used, where a >5mm increase in a zone diameter for antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirmed an ESBL-producing bacteria. Among 126 the gram negative isolates; 55 (43.6%) were defined as presumable ESBL-PE on the basis of the antimicrobial susceptibility testing results. The combined disk test indicated that (39.5%) isolates presented inhibition of clavulanic acid. The most common species presenting this study were *K. pneumonia* (80%), *Pseudomonas aeroginosa* (80%), *E. coli* (72.5%), and *Acinetobact*or *baumanii* (50%) while *P. mirabilis*

was 50%. Comparing the results of the combinations of cephalosporins with clavulanic acid those involving Cefotaxime was high that ceftazidime.

Table 4: Frequency of phenotypes ESBL-producing isolates by combined disc with clavulanic acid.

Antibiotic	*K.pnemonia* N=20 (%)	*E.coli* N=20 (%)	*A.baumanii* N=9 (%)	*P.aeruginosa* N=5 (%)	*P.mirabilus* N=1 (%)	Total N=55 (%)
Ceftazidime – clavulanic acid_30µg	15 (75)	13(65)	6(66.6)	4(80)	0	38(69)
Cefotaxime-clavulanic acid_30µg	17 (85)	16 (80)	3(33.3)	4 (80)	1	41(74.5)

Molecular analysis confirmed that these 44 isolates carried ESBL-encoding genes. TEM accounted for 20 (45.5%), CTX- M1 for 14 (31.8%), CTX- M2 for 4 (9%),; and for OXA 6(13.6%). Concerning the isolated genes ESBL, *K.pneumonia* had TEM (5) , CTX- M1 (10), CTX- M2 (1) , and OXA (1); for *E.coli* TEM (8) CTX- M1 (3), CTX- M2 (2) and OXA (3); *for Acinetobator baumanii* TEM (3), CTX- M1 (1), CTX- M2 (1 and OXA (1); *Pseudomonas aeroginosa* TEM (3) CTX- M1 (8), and OXA 1, *Proteus mirabilis* TEM (1)

Table 5: Characterization of ESBL genes for identified positives

		Class A						Class D
Isolates	**TEM**	**SHV**	**CTX M1**	**CTX M2**	**CTX M9**	**CTX M8**	**CTX 25**	**OXA**
K.pneumonia	5	0	10	1	0	0	0	1
E.coli	8	0	3	2	0	0	0	3
Acinetobator baumanii	3	0	1	1	0	0	0	1
Pseudomonas aeroginosa	3	0	0	0	0	0	0	1
Proteus mirabilis	1	0	0	0	0	0	0	0
Total sequences	20	0	14	4	0	0	0	6

The multivariate logistic regression analysis for factors associated with bacterial isolation from post operative patients, showed that the cases of deep surgical infections are statistically significance different from superficial infection ($P<0.05$). Moreover the participants who were operated out of UTHK and transferred for post operatory peritonitis are also statistically different from the post surgical infection operated at UTHK. Other variables considered such as age, HIV and Diabetes which were not statistically significance with $P>0.05$ (table 6).

Table 6: Multivariate logistic regression analysis for factors associated with bacterial isolation from post operative patients.

Variables		Bacteria isolated from SSI (n = 181)	P value
Age (Years)	> 22	12 (6.1)	0.341
	21 - 25	81 (41.3)	
	26 - 30	43 (21.9)	
	31 35	45(23%	
	36 40	8(4%)	
	41 <	7 (3.6%)	
Types of SSI	Deep	174 (88.7%)	0.011*
	Superficial	22 (11.2%)	
HIV status	Negative	184 (93.3)	0.746
	Positive	12 (6.7)	
DIABETIS	No	194 (98.9)	0.848
	Yes	2 (1.1)	
Site of operation	Out of UTHK	173(88.2%)	0.012*
	In UTHK	23(11.7%)	
* P=value less than 0.05 is statistically significant			

4. DISCUSSION

The present study shows that 88.2% of our participants with post surgical site infections were transferred from district hospitals where they have been operated for caesarean section and then after they developed peritonitis post cesarean section site. The age distribution of the participants showed that the median age was 26 years with interquartile range from 23 to 31 years with the range of 21- 25 years 81 (41.3%) the most represented age The most represented groups of age is the most active procreation of the women in Rwanda according to Rwanda Demographic Health Survey 2015.[10] The multivariate logistic regression analysis for factors associated with bacterial isolation from post operative patients, showed that the cases of deep surgical infections are statistically significance different from superficial infection (P<0.05). Moreover the participants who were operated out of UTHK and transferred for post operatory peritonitis are also statistically different from the post surgical infection operated at UTHK. Other variables considered such as age, HIV and Diabetes which were not statistically significance with P>0.05 .The finding of this study is the same as in other research where the study conducted in Kenya at Aga Khan Hospital found that the age is not the risk factor for developing post-surgical site infections.[9]

We have recorded the HIV status of the participant in the present study where the HIV positives are 12 out of 196 which represent 6.1%. This prevalence is the same as the HIV prevalence in the healthy population in Kigali city with the HIV prevalence of 6.6%.This

could be explained by the better biological and immunological follow up of those patients in providing antiretroviral drugs. The findings are this study is the same as other study like that conducted by Johanna Kate Halfon in the same hospitals where they found also that HIV is not the risk factors.[28] Higher proportion of bacterial isolates were Gram-negative than Gram-positive bacteria 126 (69.6%) and the reason for this high proportion is that the cultures that came from wounds, which were most from intra-abdominal infections. Moreover this could be attributed also to diverse habitat of Gram negative bacteria including inanimate surfaces in hospitals, multidrug resistant patterns portrayed and possible contamination from intestinal tract during surgery. For the etiological causes of the post cesarean section, the presented findings are similar to the results observed for the post cesarean section peritonitis at referral hospital in Rwanda conducted by Johanna Kate H, whereby *Klebsiella pneumoniae* was the most frequently isolated bacterial with 35.1%.[28] However, this is in contrast to the findings from the Nairobi University Hospital research on the surveillance of post surgical sites infection in general population where *Staphylococcus aureus* 30%, coagulase *Staphylococcus* 16%, *Psudomonas aeroginosa* 13%, *E.coli* 13%, *Klebsiella pneumonia* 9%, *Serratia marcescens* 3% and *Enterobacter cloacae* 3%.[9] The findings of this study also contrast the study conducted by Joel Manyahi, 2012 on the bacteriological spectrum of post operative wound infections and their antibiogram in tertiary hospital in Tanzania where *Psudomonas aeroginosa* was the most isolated followed by *S.aureus*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Acinetobater baumanii* [10.] The possible reason for variation in these studies could be attributed to differences in the populations investigated; diversity of surgical procedures performed on the study participants. In the present study the majority of the isolates were obtained from patients who were already on antimicrobials treatment, and this could have led to the low recovery of antimicrobial susceptible Gram positive pathogens. These findings demonstrated relative shift in etiological agents causing surgical site infections. Since recent studies from western Africa and Asia countries have reported increasing trend of *Klebsiella* and other enteric Gram negative rods as the common organisms causing surgical site infections.[12] The findings may be explained in the factors that the etiologic agents of surgical site infections depend on the procedures performed and whether skin was incised or gastrointestinal tract was opened and when gastrointestinal tract is opened, organisms usually include aerobic Gram negative rods. In this following study the cesarean section involved skin incision to the abdomen parts, this explains the predominance isolation increase of enterobacteriaceae in the post-surgical section for cesarean section and the skin pathogens. For antibiotic resistance, both *Klebsiella*

pneumonia and *Escherichia coli* were susceptible to imipenem. The Gram negative isolates 55 (43.6%) were defined as presumptive ESBL production on the basis of the antimicrobial susceptibility on ceftazidime and cefotaxime testing results. This had been confirmed phenotypically by the combined disk with clavulanic acid and the results showed that 44 (34.9%) Gram negative isolates presented inhibition of clavulanic acid. The present results which are higher than that of Alim 23.19% and Rahman 30.90% both in BSMMU but lower than that of Biswas of BSMMU 80% and Yasmin of Mymensingh 71.30%. by Ullah et al. (2009), in Pakistan found 58.7% ESBL producers,[11,12,15,16] and at Khartoum Teaching Hospital, Sudan with the incidence of 45.1%.[17] The prevalence of ESBLs producers in India ranges from 6.6% to 91%, in Europe from 23-25% for Klebsiella spp. and 5.4% for *E.coli* and in United States from 0 to 25%, depending on the institution.[18,19] The higher prevalence compared to western countries can be explained by the fact that western countries have strict infection control policies and practices, efficient and effective antibiotic audit systems, shorter average hospital stays, better nursing barriers, and other important health care measures which substantially decrease the chances of acquisition and spread of ESBLs strains. One of the most important factors that are responsible for ESBLs production is the selective pressure caused by the use of 3rd generation cephalosporins, lack of antibiotic surveillance, antibiotics misuse, self-medication, poor hygiene, lack of antimicrobial resistance detection tools and weak infection control measures may also contribute to the high magnitude of ESBL.[20] *Klebsiella pneumonia* and *E.coli* were the most predominant bacteria to produce phenotypically the high Extended Spectrum Beta Lactamases producers bacteria. *K. pneumonia* (80%), *E.coli (*72.5%).

Klebsiella. pneumoniae and *E. coli* are the most common species. The same observation has been reported around the world, with the prevalenceof 86.6% in India.[21] ESBLs-producing *E. coli* (39 (44%)) was the second most predominant bacterial isolate, which is in line with Shaikh et al.'s results in Australia,[22] and Ibrahimet al.'s results in Sudan.[17] In addition *Klebsiella* species have been reported common contaminants in operating room air and fomites including medical equipments in hospitals.[23] The high occurrence of ESBLs in *Klebsiella spp*. is of great concern since infections caused by this bacterium are very common and resistance of the organism may be due to the presence of capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump, easyspreading nature, pathogenic and efficient at acquiring and disseminating resistance plasmid. *Klebsiella pneumoniae* has some virulence factor like hyper-viscosity, polysaccharide capsule and

production of endotoxin, carbapenemases, which make it more resistant [24]. Molecular analysis confirmed that these 44 isolates carried ESBL-encoding genes. TEM accounted for 20 (45.5%), CTX- M1 for 14 (31.8%), CTX- M2 for 4 (9%) and OXA 6(13.6%). The majority of *K.pneumonia* carried CTX- M1 (10), TEM (5),), CTX- M2 (1) , and OXA (1); while the majority of *E.coli* possessed TEM (8) CTX- M1 (3), CTX- M2 (2) and OXA (3); *for Acinetobator baumanii* TEM (3), CTX- M1 (1), CTX- M2 (1 and OXA (1); *Pseudomonas aeroginosa* TEM (3) CTX- M1 (8), and OXA 1, *Proteus mirabilis* TEM. Among the CTX-M group, the predominance of the CTX-M-1 subgroup could suggest the presence of CTX-M-15 enzyme, which is the most widely disseminated CTX-M ESBL enzyme.[25]

Similar to our study, high proportions of blaCTX-M-15-positive clinical isolates were reported in other Sub-Saharan African countries: Cameroon (96%),[26] Gabon (84.1%).[27] The interpretation of the findings of this study is limited by the fact that there was no knowledge of the patients' previous antibiotic treatments. Indeed, antibiotic treatments prior to sample collection could have favored the transient selection of resistant bacteria, and thus increased ESBL. High rate of antibiotics resistance has been seen in post-surgical site infections in maternity and gynecology patients, and this may be due to the frequent contact with health care providers during their pregnancy. If that is the case, routine antibiotic prophylaxis at the time of cesarean section will have little effect, increasing the possibility of introducing skin bacteria into the peritoneal cavity and subsequent infection. It is possible that the emergence of antimicrobial resistance in resource limited country that is increasing the number of cesarean sections performed each year has lead to the emergence of post surgical infection on the scale documented in this study.

5. CONCLUSION AND RECOMMENDATIONS

To put in place the reporting system that could increase the transparency on surgical site infection rates at the hospital level, reducing the surgical infections rates. As 88, 2 % of peritonitis are from district hospitals further research is needed to identify methods to prevent post-cesarean section infections at the district hospital. Moreover ESBLs necessitates the strengthening of clinical bacteriology research and the diagnostic capacity of laboratory professionals for the detection and surveillance of antibiotic resistance. In addition the routine screening of ESBLs production should be enhanced.

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