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Research Article

Characterizing Nosocomial Bacterial Pathogens and Antibiotic Resistance at a Major Referral Hospital in Western Kenya

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Bacterial pathogens responsible for hospital acquired infections in Jaramogi Oginga Odinga Teaching and Referral Hospital are difficult to treat due to their tendency to resist most antibiotics used against them. This study aimed at characterizing bacterial pathogens associated with hospital acquired infections from clinical specimens and determining their antibiotic resistance. A descriptive cross-sectional study design involving bacteriological analysis of clinical samples was used to purposively select 111 patients who exhibited signs and symptoms of hospital acquired infections from whom, samples were obtained and analyzed. Tables, pie charts and bar graphs were used to summarize data. Prevalence was determined using percentages while the relationship between patient characteristics and the risk of contracting nosocomial bacteria was determined using Chi-square. *P* values ≤ 0.05 were considered statistically significant. Of the 111 samples obtained 59 (53.2%) were from females. About one-third (31%) were younger than one year while 4% were aged between 6 and 12 years. A total of 51(45.9%) of the samples yielded bacterial growth. There was an association between age and the risk of infection (*p*=0.012). Sex was not associated with the risk of infection (*p*=0.338). Most pathogens (n=27,52.9%) were isolated from surgical patients. Gram negative bacteria (n=37, 33.6%) were more prevalent with *Klebsiella spp.,* being frequently isolated. The isolated pathotypes were not significantly associated with patient category (*p*=0.774). Ampicillin, imipenem and ceftazidime showed (100%, n=17), (97.1%, n=33) and (92.1%, n=35) resistance respectively. Amikacin, Gentamycin and Meropenem showed (7.3%, n=3), (22.2%, N=8) and (24.1%, N=7) resistance respectively. A high prevalence antimicrobial resistance was detected. *Klebsiella spp*., was the most common pathotype causing nosocomial infection. We recommend continued surveillance to detect increasing resistance on Amikacin, Gentamycin and Meropenem due to empiric use as well as antimicrobial stewardship to fight threats that may emerge.

Keywords: Antimicrobial, resistance, Nosocomial, surgical, JOOTRH

INTRODUCTION

Nosocomial infection (also known as hospital acquired infection-HAI) can be defined as infections acquired during the period of hospitalization and whose clinical manifestations appear after 48hrs or more post admission or after discharge [1]. Several microbial agents do cause infection in hospitals but, the ones caused by bacteria often occur with higher frequency [2]. Nosocomial bacterial infection still remains a major challenge in both developed and developing countries

often causing increased morbidity, mortality and prolonged hospital stay [1]. Due to resistance, limited therapeutic options will be available which can possibly result in death due to treatment failure [1, 3, 4]. Even though various studies have dwelt on antibiotic characteristics of bacterial pathogens from hospitalized patients, the resistance pattern and spectra of the isolates is still not well documented [5].

The prevalence of hospital associated infections caused by bacteria have been reported in different studies across the globe. For instance, in a study involving some European countries and another one done in Serbia found a prevalence of 6.5% and 7.1%, respectively [1, 6]. In Africa, [7] found a prevalence infections acquired in the hospital setting to be 6.9%. Studies done in East Africa, have shown high prevalence of hospital acquired infections [8] and according to [9] in a study done in a Kenyan hospital, majority of the cases are due to infection with Gram-negative bacteria most of which were resistant to antimicrobial drugs.

Risk factors to healthcare associated infections

Several risk factors have contributed to nosocomial infections including lumping sick people together under one roof especially most of whom are severely ill and immunocompromised [10], Age, presence of a indwelling medical device, site affected among others [11-13]**.**

Antimicrobial resistance

Antimicrobial resistance (AMR) continues to be one of the most serious global public health threats in the current century [14]. Notably, a number of pathogens including *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella spp.,* have shown resistance to most antibiotics used to treat them which raises concerns on future of treatment for infections caused by these pathogens [14, 15]. For instance, in AMR study done in Iranian hospitals revealed that 100% of *Klebsiella pneumoniae*, and *Acinetobacter baumannii* isolates were resistant to imipenem and that 100% were resistant to oxacillin. Respiratory tract and uropathogens isolated from catheters, *Acinetobacter baumannii* from blood stream and CAUTI showed 100% and 96.4% resistant to imipenem respectively [16]

Several factors often contribute to risk of acquiring infections within the hospital, including improper waste management, immunodeficiency, surgical procedures, presence of indwelling catheters, and underlying conditions [17, 18]. Over-use of antimicrobial agents which is occasioned with the ease of accessibility of over-the-counter, presence of counterfeit and substandard antibiotics and poor infection control measures in developing countries often antagonize the fight against AMR [19]. It is unfortunate that widely studied antibiotics such as methicillin together with other antibiotics administered together with it, for first line treatment such as carbapenems have also shown resistance to common pathogens causing HAIs [20].

This study therefore isolated and determined the antimicrobial resistance of common pathogens causing infections at JOOTRH whose findings is believed will help inform antibiotic choice for empiric treatments which go along in the fight against AMR.

METHODOLOGY

The study was conducted at the HIV comprehensive care clinic of Kisii Teaching and Referral Hospital, a level 6 hospital in Kisii County. The hospital provides promotive, preventive, curative, and rehabilitative healthcare to a population of up to 3 million people. The facility also operates as medical teaching, training, and research school. The hospital's HIV program provides comprehensive prevention, care, and treatment services, including PLHIV identification, ART initiation, and ART treatment monitoring to approximately 4,500 PLHIV under a team of medical service providers from different specialties. This was a descriptive retrospective and prospective study that primarily relied on existing hospital records of PLHIV. The study reviewed 367 PLHIV files sampled from 4,452 PLHIV records to collect quantitative data through chart abstraction method. Qualitative data was collected through key informant interviews with purposively sampled health care providers (7). The study targeted PLHIV who had been on ART for at least six months and who must have attended at least two (2) clinic appointments in the year 2019. The key informants included an HTS provider, psychological counselor, health records and information officers, laboratory technologist, pharmacist, and clinician who had managed ART services in the various service delivery points for longer than 6 months and consented to participate in the study. Simple random sampling was applied to select the 1st patient file; then systematic sampling was applied where every 4th file was selected until 367 files were obtained for chart abstraction.

Regarding structural factors affecting compliance with ART service delivery guidelines, the following were

assessed: human resource, equipment/supplies, service delivery space, health information system and health policies. Regarding ART clinical service delivery processes affecting compliance with ART service delivery guidelines, the study reviewed: ART initiation, CD4 baseline testing, sCrAg Testing, nutritional assessment, adherence monitoring, viral load testing, TB screening/testing and IPT uptake. The study reviewed viral load suppression data of all PLHIV to determine viral suppression achievement against the recommendations in the guidelines. The results were documented either as service provided or not, and the frequency was captured where appropriate. The selected files were assessed against pre-determined ART service quality indicators provided in the Kenya AIDS Strategic Framework (2014/15-18/19) and the MoH guidelines.

The Quantitative data was collected on MS excel chart abstraction tool. Qualitative data was captured through face-to-face interviews with health care workers using a key informant interview guide to document health worker perspectives regarding structural factors in ART service delivery. The Quantitative data was analyzed using Statistical Package for Social Sciences (SPSS) Version 16.0, then descriptive statistics applied to summarize the data by calculating means, frequencies, and percentages. The qualitative data was analyzed by organizing the responses into themes and presented in descriptive format and direct quotations from the interviewees.

MATERIALS AND METHODS

Study area

The study was conducted at JOOTRH, which is one of the oldest and largest referral hospitals in Kenya. The hospital was fully expanded in the late 1960s to a district hospital serving a great number of people from western Kenya. The hospital is situated in Kisumu city between along Kisumu-Kakamega highway and has a bed capacity of 467 with bed occupancy of about 94.8%. The highest bed occupancy being in surgical wards and gynecology, which are 148.7% and 146% respectively (available from-http://www.newnyanzapgh.com). The hospital has six operating rooms.

Study design

A descriptive cross sectional study design involving bacteriological analysis of samples was used to carry out this research between August 2021 to December 2021. Purposive sampling was used to select patients with healthcare associated infection.

Study population

All patients admitted to surgical, medical, obstetrics and gynecology, pediatrics, NBU wards for at least 48hrs or more or those who were discharged but returned after 48hrs and were readmitted.

Sampling procedure

A total of 111 cases of hospital acquired infections were identified. Permission to obtain the relevant sample was then sought from the patient after elaborate explanation about the exercise to be undertaken. Specimens were then obtained and transported to microbiology laboratory for processing.

Specimen collection and processing

Clinical samples were collected in sterile containers following aseptic procedure and transported to microbiology laboratory for processing. Upon arrival in the laboratory clinical samples were immediately cultured on appropriate pre-prepared media (OXOID) including Mac CONKEY Agar, Blood Agar, chocolate blood agar and CLED Agar. The culture plates were then incubated at 37°C. Chocolate agar plates were incubated in anaerobic jar. Specimens for blood culture were entered in to BD™ BACTEC™ FX40 and BD™ BACTEC™ FX automated system and incubated for five days. Positive cultures were gram stained as described by [21]. Sub-cultures were then performed on MacConkey agar, blood agar and chocolate blood agar. MacConkey agar plates were incubated at 37°C in aerobic environment while BA and CBA plates were incubated in a candle jar. Colonial morphologies were studied, and Gram staining performed [21]. Following growth on agar plates, in vitro phenotypic characterization of the colonies like morphology (colonial), pigmentation and cellular morphology was done; further characterization was done using immunological, and biochemical tests such as catalase, urease, coagulase, citrate utilization, indole, fermentation of various sugars, bile solubility, motility and TSI tests as described by [22]. Serological techniques including Staphaurex Plus* and Prolex™ Latex Agglutination were used to identify *Staphylococcus aureus* and *Streptococci* species respectively.

Antimicrobial Testing

Testing for AMR was performed on Hinton Agar (OXOID) using disc diffusion technique as described by [22]. Antimicrobial discs (Oxoid) including CN

(10mcg), CXT(5mcg), CRO (30mcg), SXT (25mcg), P1 (iu), FOX (30mcg), AZM (15mcg), CAZ (10mcg), CFM (5mcg), ETP (10mcg), OX (1mcg), AMP (10mcg), AK (30mcg), TGL (15mcg), MEM (10mcg), CIP (5mcg), were then placed on the surface of the streaked plate using disk dispenser (OXOID). The plates were then incubated immediately at 35 ± 2°C and examine after 16-18 hours. Zones of inhibition were measured and then compared with the interpretation guide as per the standards chart for the determination of antibiotic sensitivity and resistance following CLSI guidelines [23].

Data analysis and presentation

Data obtained was entered into a computer as excel worksheet and imported into SPSS (version 20) for analysis. Tables and bar graphs were used to summarize data. Prevalence rates were determined using percentages while the relationship between patient characteristics and the risk of contracting nosocomial pathogen was determined using chi-square test. *P* values ≤ 0.05 were considered statistically significant.

Ethical considerations

Clearance to conduct this study was obtained from board of post graduate studies Jaramogi Oginga Odinga University of Science and Technology Board of Post-Graduate Studies, Jaramogi Oginga Odinga Teaching and Referral Hospital Ethics and Review Committee and a research permit was given by NACOSTI. Informed consent was sought from study participants prior to sample collection.

RESULTS

Sociodemographic Characteristics

In terms of gender more than half of the study participants 59(53.2%) were female while. A third, (31%) were aged less than one year while 4% were aged between six and twelve years. In total, 51(45.9%) of samples yielded bacterial growth out of which, 37(33.3%) were gram negative while 14(12.6%) were gram positive. Those aged 6-12 years were (n=4, 3.6%). 54.1% (n=60) of samples had no growth obtained. This study also found a significance associated between age of the patient and the risk of isolating nosocomial pathogen (p=0.012) but no association between Gender and the risk of contracting nosocomial pathogens (p =0.338). Table 1.

Table 1: Distribution of bacterial isolates

Age	n(%)	NG n(%)	Kleb n(%)	S.A n(%)	E.C n(%)	Ps n(%)	Pr n(%)	E.F n(%)	A.B N(%)	P-Value
<1	35(31.5)	29(82.9)	1(2.9)	3(8.6)	1(2.9)	1(2.9)	0 (0)	0 (0)	0 (0)	**0.012**
1-5	7(6.3)	5(71.4)	0 (0)	1(14.3)	1(14.3)	0 (0)	0 (0)	0 (0)	0 (0)	
6-12	4(3.6)	2(50)	0 (0)	1(25)	1(25)	0 (0)	0 (0)	0 (0)	0 (0)	
13-19	7(6.3)	2(28.6)	1(14.3)	3(42.9)	1(14.3)	0 (0)	0 (0)	0 (0)	0 (0)	
20-39	23(20.7)	9(39.1)	7(30.4)	2(8.7)	1(14.3)	0 (0)	2(8.7)	2(8.7)	0 (0)	
40-59	22(19.8)	9(40.9)	2(9.1)	2(9.1)	6(27.3)	1(4.5)	1(4.5)	0 (0)	1(4.5)	
60+	13(11.7)	4(30.8)	5(38.5)	0 (0)	2(15.4)	2(15.4)	0 (0)	0 (0)	0 (0)	
Gender										
Female	59(53.2)	33(55)	10(16.9)	8(13.6)	6(10.2)	0(0)	1(1.7)	1(1.7)	0 (0)	0.338
Male	52(46.8)	27(51.9)	6(11.5)	4(7.7)	7(13.5)	4(7.7)	2(3.8)	1(1.9)	1(1.9)	
Total	**111(100)**	**60(54.1)**	**16(14.4)**	**12(10.8)**	**13(11.7)**	**4(3.6)**	**3(2.7)**	**2(1.8)**	**1(0.9)**	

KEY: N.G= No growth; Kleb= *Klebsiella spp.;* S.A= *Staphylococcus aureus*; E.C= *E.coli*; Ps= *Pseudomonas aeruginosa*; Pr= *Proteus spp.;* E.F= *Enterococcus faecalis*; A.B= *Acinetobacter baumannii*

Klebsiella spp., was the most frequent pathogen isolated 16(31.4%) followed by *Escherichia coli* 13(25.5%) and *Staphylococcus aureus* at 12(23.5%). In terms of ward category, surgical wards recorded higher number of infections as compared to other wards 27(52.9%). Amenity and gynecology wards had 8(15.7%) and 6(11.8%) of infections respectively. The newborn unit had the least pathogens isolated, with only two percent of the samples, 1(2%) turning positive for culture. However, the ward that the patient is admitted to did not pose any risk of contracting HAIs (p =0.774). Table 2.

Table 2: Prevalence of bacterial pathogens

Pathogen	n(%)	New n(%)	Sur n(%)	Ame n(%)	Gyn n(%)	ICU n(%)	HDU n(%)	Chil n(%)	Cas n(%)	P
Klebsiella	16(31.4)	0(0)	6(37.5)	3(18.8)	3(18.8)	2(12.5)	1(6.3)	0(0)	1(6.3)	**0.774**
S. aureus	12(23.5)	0(0)	9(75)	1(8.3)	1(8.3)	0(0)	0(0)	1(8.3)	0(0)	
E. coli	13(25.5)	1(7.7)	5(38.5)	2(23.1)	1(7.7)	1(7.7)	0(0)	2(15.4)	0(0)	
P. aeruginosa	4(7.8)	0(0)	3(75)	1(25)	0(0)	0(0)	0(0)	0(0)	0(0)	
Proteus	3(5.9)	0(0)	3(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	
E. faecalis	2(3.9)	0(0)	1(50)	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	
A. baumanni	1(2.0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	
Total	**51(100)**	**1(2)**	**27(52.9)**	**8(15.7)**	**6(11.8)**	**4(7.8)**	**1(2)**	**3(5.9)**	**1(2)**	

Key: New = Newborn; Sur = Surgical; Ame = Amenity; Gyn = Gynecology; ICU = Intensive Care Unit; HDU = High Dependency Unit; Chi = Children; Cas = Casualty

Antimicrobial resistance of bacterial isolates

According to this study, all *Klebsiella spp.,* (n=16) showed resistance to SXT, IMP, AZM, AMP, CAZ, AMC; more than 50% showed resistance to CIP and CTX. AK and OF resistance were 13% and 20% respectively. Isolates of *Proteus spp.,* were all resistant n=3, to SXT, IMP and *CAZ*. Isolates of *Escherichia coli* were all resistant, (n=13) to SXT, IMP and PIP resistance was 92% while that of MEM, AK and GEN were 10%, 7.7% and 22% respectively. Isolates of *Pseudomonas aeruginosa* were all (n= 4) resistant to SXT, MEM, IMP, AMP, AZM, CEF PIP and 75% resistant to CAZ. While all isolates of *Staphylococcus aureus,* (n=12) were resistant to AMP, CEF, PIP, IMP, 83% of the isolates were resistant to Pi and 44% and 50% to OX, AZM and ERY respectively while 11% and 20% resistance were against GEN and CTX respectively. *Enterococcus faecalis* isolates were 100% (n=2) resistant to OX, CIP, SXT, CTX, MEM, AZM, FEP and AMC. There was no resistance shown against AK and GEN while there was 50% resistance against ERY. 100% (n=1) isolate of *A. baumannii* was resistant to CIP, CTX, IMP, CEF, CAZ, TAZ, AMC, PI and OF. Overally, antimicrobial resistance were considerably lower against AK 7.3% (n=3), GEN 22.2% (n=8) and MEM 24.1% (n=7).

Table 3. Trends in antibiotic resistance

Drug	No	Kleb (%)	Pr (%)	E.C (%)	Ps (%)	S.A (%)	E.F (%)	A.B (%)	TR (%)
OX	6	NT	NT	NT	0	60	100	NT	4(66.7)
CIP	42	53.80	0	69.20	NT	25	100	100	20(47.6)
SXT	17	100	100	100	100	25	100	NT	11(64.7)
CXT	39	61.50	0	76.90		20	100	100	24(61.5)
MEM	29	33.30	0	10	100	NT	100	0	7(24.1)
IMP	34	100	100	92.30	100	100	NT	100	33(97.1)
AK	41	13.30	0	7.70	100	0	0	0	3(7.3)
Pi	6	NT	NT	NT	NT	83.30	NT	NT	5(83.3)
GEN	36	45.50	0	22.20	0	11.10	0	0	8(22.2)
AZM	13	100	NT	NT	100	44.40	100	NT	8(61.5)
AMP	17	100	NT	NT	100	100	NT	NT	17(100)
CEF	29	60	0	75	100	100	NT	100	19(65.5)
ERY	3	NT	NT	NT	NT	50	50	NT	2(66.7)
CAZ	38	100	100	92.30	75	75	NT	100	35(92.1)
FEP	15	50	0	25	0	NT	100	NT	5(33.3)
TAZ	10	75	NT	75	0	NT	NT	100	7(70)
AMC	14	100	NT	75	0	33.00	100	100	10(71.4)
PIP	31	77.80	66.70	92.30	100	100	NT	100	27(87.1)
OF	30	20	0	58.30	0	0	NT	100	10(33.3)

KEY: OX= Oxacillin; CIP= Ciprofloxacin; SXT= Sulphamethoxazole-Trimethoprim; CXT= Ceftriaxone; MEM= Meropenem; IMP= Imipenem; AK= Amikacin; Pi= Penicillin G; AZM=Azithromycin; AMP= Ampicillin; CEF= Cefotaxime; FEP= Cefepime; ERY= Erythromycin; CAZ= Ceftazidime; FEP= Cefepime; TAZ= Tazobactam; AMC= Amoxyclav; PIP= Piperacillin; OF= Ofloxacin; No= Number of organisms; Kleb= *Klebsiella spp.;* S.A= *Staphylococcus aureus*; E.C= *E.coli*; Ps= *Pseudomonas aeruginosa*; Pr= *Proteus spp.;* E.F= *Enterococcus faecalis*; A.B= *Acinetobacter baumannii*; NT= Not tested; TR= Resistance

DISCUSSION

This study revealed an association between age and the risk of contracting infection during hospitalization while there was no association between gender and the risk of contracting HAIs. This finding is consistent with [11], found out that children less than one year had an increased chance of contracting nosocomial bacterial infections. Similarly, there is an increased risk elderly patients acquiring HAIs [24-26]. Individuals falling within both extremes of ages generally have a weakened body defenses which perhaps could explain the reason for susceptibility that has been noted in this study [27, 28].

As compared to patients admitted in other wards, surgical patients had a considerably higher infection rate. This finding is consistent with [29]. However, [30] found lower rates which could have been due to the scope of their study which concentrated only on caesarean cases. Similarly, in Rwanda [24] also documented lower rates which could have been due to the fact that their findings were based on estimates. Majority of cases of HAIs according to this study were due to gram negative bacteria. This agree with findings of [24, 31-35].

Generally, there has been a growing concern about increase in antibiotic resistance in hospital acquired bacteria with variability also noted in the prevalence rates which could be due to differing population characteristics, geographical distribution, bacterial detection methods as well as sample size used. However, findings of this study, reveals an overall antibiotic resistance of between 7.3-100% which is consistent with [36]. Multiple antibiotic resistance was similarly noted in this study. For instance, all *Klebsiella spp*., isolated showed high resistance to sulfamethoxazole-trimethoprim, azithromycin, and ampicillin. Findings of [37], are consistent with our study. Resistance against imipenem was also found to be high and in consistent with [25, 35]. Findings by [38] have showed high resistance to ceftazidime, amoxiclav, penicillin, cephalosporins, macrolides and tetracyclines which also agrees with findings of our study.

While high prevalence rates were observed in a number of antibiotics, our study documented low resistance to meropenem, amikacin and gentamycin. These findings agree with studies by [25, 39]. However, the findings disagree with [37] who showed higher resistance as compared to our study. This could be due to overprescription of amikacin and so there could have been room for be abuse and misuse.

CONCLUSION

This study shows high prevalence of antibiotic resistance in JOOTRH. Most cases of HAIs were caused by Gram negative bacteria of which *Klebsiella spp*., was the most common pathogen. Majority of cases of nosocomial infection occurred in the surgical ward. Meropenem, amikacin and gentamycin resistance appeared to be low.

RECOMMENDATION

We recommend further studies to guide policy with regards to empiric administration of amikacin, gentamycin and meropenem as well as surveillance to monitor any increasing resistance. Imipenem, ciprofloxacin, ceftazidime, ceftriaxone, ampicillin, sulphamethoxazole/trimethoprim, amoxiclav and piperacillin use should be reviewed due to high rates of resistance.

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Conflicts of Interest:

The authors declare no competing interests

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