UNIVERSITY OF RWANDA College of Medicine and Health Sciences

ANTIMICROBIAL RESISTANCE PROFILE OF COMMON BACTERIAL PATHOGENS AND IMPACT ON PATIENT TREATMENT OUTCOMES AT KIGALI UNIVERSITY TEACHING HOSPITAL (KUTH)

Submitted in partial fulfillment of requirements for the degree of Master of Medicine in INTERNAL MEDICINE.

BY: Dr Cyprien NTIRENGANYA

SUPERVISOR: Dr Olivier MANZI

CO-SUPERVISORS: Dr Onyema OGBUAGU

Dr Claude MUVUNYI

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DEDICATION

To my beloved wife,

To my dear parents,

To my lovely son,

We gratefully dedicate this achievement.

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We keep in our heart all the names of close friends whose love and care directly contributed.

ABSTRACT

Introduction: Antimicrobial drug resistance (AMR) is not a new problem but remains a serious health issue. Antimicrobial resistance represents an opportunity to develop and test new antibiotics, however in developing countries, the limited availability and affordability of antimicrobial agents remains a significant barrier. We conducted a prospective observational study to assess the prevalence of AMR among common disease causing pathogens in KUTH and to assess the treatment outcomes of patients with MDR infections.

Methods: This prospective observational study evaluated culture and sensitivity results of positive bacterial cultures obtained from urine, blood, sputum and wound swabs in 141 hospitalized patients admitted to the internal medicine wards at KUTH. Sample collection, processing and antibiotic susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: 54.2 % of the 155 positive culture results at KUTH were cultured from urine specimens Blood specimens made up 25.4% followed by wound swabs with 15.5% and lastly sputum specimens at 5.1%. In UTIs, *E.coli* were the most prevalent bacteria cultured at 58.75 %. MDR *E.coli* were noted in UTIs: Ciprofloxacin (64.3 %), norfloxacin (38.6 %), amoxicillin/CA (88 %), cotrimoxazole (78 %) and ampicillin (90 %). In bacteremia, Klebsiella spp.were resistant to almost all tested antibiotics, including 3rd generation cephalosporins: cefotaxime (90%), ceftriaxone (80 %). *Klebsiella spp.* were significantly isolated across all types of clinical specimens but were more frequently identified in blood specimens (28.2%). After Klebsiella spp, S.aureus was the second most frequently cultured organism in blood cultures (23%).

In general, S.aureus was highly resistant to the penicillin class of antibiotics. ESBL E.coli and ESBL Klebsiella spp. were evaluated at 35.1% and 56.3 % respectively. MRSA was screen in 45.5 of cases. Imipenem, vancomycin and amikacin have shown to be susceptible to all isolates. The mortality rate in our study was evaluated at 19.15% and the septic shock was identified to be associated with it with a p value < 0.0001.

Conclusions and recommendations:

Antimicrobial resistance is a serious and alarming problem in KUTH. A surveillance system should be setup to monitor antibiotic prescription patterns and to inform guidelines and indications for antibiotic use.

ACCRONYMS

3GC: Third Generation Cephalosporins

Amoxicillin/CA: Amoxicillin/clavulanic acid

AMR: Antimicrobial resistance

BHI: Brain Heart Infusion

CLED: Cysteine Lactose Electrolyte Deficient

CLSI:Clinical and Laboratory Standards Institute

CoNS: Coagulase Negative Staphylococcus

COPD: Chronic Obstructive Pulmonary Disease

E.coli: Escherchia coli

ESBL: Extended Spectrum Beta Lactamase

HIV: Human Immunodeficiency Virus

IM: Internal Medicine

IQR: Interquartile Range

KPC: *Klebsiella pneumonia* producing carbapenemase

KUTH: Kigali University Teaching Hospital

LRTI: Lower Respiratory Tract Infections

MDR: Multidrug Resistance

MDRPM: Multidrug Resistant Pathogenic Microbes

MIU: Mobility Indole Urea

MRSA: Methicillin-Resistant Staphylococcus Aureus

MSA: Monitor Salt Agar

MSSA: Methicillin Sensitive Staphylococcus Aureus

NMD: New Delhi metalloproteinase

S.aureus: Staphylococcus aureus

SD: Standard Deviation

SIRS: systemic inflammatory response syndrome

spp.: species

TMP/SMX: Trimethoprim/sulfamethoxazole

TSI: Triple Sugar Iron

USA: United States of America

UTIs: Urinary Tract Infections

XLD medium: Xylose Lysine Deoxycholate

FIGURES AND TABLES

TABLE OF CONTENTS

I.INTRODUCTION

I.1 PROBLEM STATEMENT AND CONTEXTUAL FRAMEWORK

Antimicrobial drug resistance (AMR) is not a new problem but remains a serious health issue. Over several decades, pathogenic bacteria have developed resistance to antibiotics following their introduction and subsequent widespread use and antimicrobial resistance has evolved to become a formidable health threat worldwide. (1)

This emerging problem has to be tackled both with continued development of new antimicrobials as well as appropriate infection control and antimicrobial stewardship measures in order to curb the worldwide epidemic. (2)

The emergence of multidrug resistant pathogenic microbes (MDRPM) has necessitated the development of new antibiotics, however, in developing countries the limited availability and affordability of these antimicrobial agents remains a significant barrier to the effective care of MDR infections. In resource limited settings, treating infections caused by MDRPM may be impossible and the resultant consequences devastating to patients. (3)

There is very little information available concerning the prevalence of AMR among common pathogenic microorganisms in the Rwandan health care system. One of the few studies evaluating resistance patterns in Rwanda was undertaken by Muvunyi et al. in 2011 and revealed that AMR among uropathogenic *E-coli* was high where isolates were resistant to amoxicillin, ciprofloxacin and ceftriaxone 89.2%, 41.3% and 32.1% of the time respectively. (4) It is very important to generate local data on the prevalence and implications of MDRPM in order to guide allocation of resources and efforts aimed at treating these infections.

Currently, there is not a standardized system for monitoring AMR in place at KUTH. Therefore, we conducted a prospective observational study to assess the prevalence of AMR among common disease causing pathogens in KUTH and to assess the treatment outcomes of patients with MDR infections. Secondary aims of the study were to evaluate the appropriateness of the

microbiologic sample resistance testing in our laboratory and based on study results, to make recommendations for appropriate expansion of culture media availability, range of antibiotic sensitivity testing and adequacy of the hospital antibiotic formulary. The study was an opportunity to evaluate the clinical usefulness of results gained from culture and sensitivity by assessing its impact on antibiotic choice and patient treatment outcomes. Furthermore, the hospital will benefit from the information gained from this study as data on prevalence of AMR will provide guidance on infection surveillance, control and prevention measures.

I.2. BACKGROUND

I.2.1 Global epidemiology of AMR

AMR is a serious global public health concern and impacts every region of the world. Not only is the frequency of isolation of AMR pathogens increasing over time, the degree of resistance is also worsening. Resistant microbes have been shown to have spread across countries and regions. A striking example demonstrating this phenomenon was the identification of N. gonorrhea isolates that were resistant to penicillin G in Vietnam in 1967 and later found in the Philippines.(5)

The New Delhi metalloproteinase (NMD) enzymes found in *Klebsiella pneumonia* of an Indian patient with a very broad resistance to the beta lactam class of antibiotics is no longer unique to India; there are now worldwide reports of its presence.(6) In general, bacteria have developed resistance to the beta lactamase class of antibiotics and the rate of resistance development has increased since 1990.(7)

AMR usually follows widespread use of an antibiotic. While pathogens may be resistant to antibiotics even before exposure through naturally occurring mechanisms, the majority of AMR occurs secondary to drug exposure as bacteria develop multiple mechanisms to evade antibiotic activity.(8) Common Multidrug resistant (MDR) pathogens isolated in hospital settings include extended spectrum beta lactamase (ESBL) producing *Enterobacteriaceae, Klebsiella pneumonia* producing carbapenemase (KPC) and Methicillin Resistant *S. aureus* (MRSA).(6) The frequency of occurrence of these MDR pathogens in hospitals differs by region. In Sudan, *E.coli*

was significantly resistant to ceftazidime (35%), ceftriaxone (64%) and ciprofloxacin (58.4%) in a study done in Khartoum between May-August 2011.(9) A recent study conducted in Rwanda by Muvunyi in 2011 revealed that *E-coli* isolated in urine samples were resistant to a different range of antibiotics: 32% of isolates were resistant to ceftriaxone, 41.3% were resistant to ciprofloxacin and 29.1% were resistant to ceftazidime.(4)

Recent studies have shown that rates of resistance to antibiotics have been increasing in Africa. The increasing rates of antimicrobial resistance in sub-Saharan Africa is concerning and poses a serious challenge to antimicrobial selection, especially considering the limited range of antibiotics readily available. In South Africa, a study conducted in KwaZulu-Natal province in 2006 showed *MRSA* with high resistance rates to available and commonly used antibiotics including gentamycin (96.7%), erythromycin (82%), clindamycin (82%) and trimethoprim (85.2%).(10) A systematic review of studies from African countries—Tunisia, Ghana, South Africa, Ethiopia, Botswana, Libya, Nigeria, Algeria, Morocco and Egypt—showed that the prevalence of MRSA increased from 2000 to 2007.(11) Similarly, a study performed at Mulago Hospital in Uganda between September 2011 and April 2012 showed an increase in the prevalence of *MRSA* and ESBL producing *enterobacteriaceae* compared to what had been shown previously.(12)

1.2.2 Antimicrobial resistance, underlying comorbidities and clinical outcomes

Patients with certain medical comorbidities are at higher risk of acquisition of MDR pathogens and experience disproportionate morbidity and mortality from infections caused by them. A study done in Korea by Cheol-In Kang *et al.* showed that diabetes mellitus, advanced age (>65 years), malignancy, liver disease, renal disease and use of immunosuppressive drugs were associated with the acquisition of community acquired ESBL producing *E.coli*.(13) Saoraya *et al*. similarly showed that diabetes mellitus, malignancy, liver disease, COPD, chronic dialysis, HIV were risk factors for sepsis, regardless the causative pathogen.(14) In a study undertaken in Belgium, Didier *et al*. showed that diabetes mellitus, cirrhosis, heart failure, chronic renal insufficiency, severe chronic respiratory disease, haematological malignancies, urinary catheters and wounds were linked to infections with ESBL producing organisms.(15) In a study conducted

in Thailand, Bunchorntavakul *et al.* assessed pathogenic microbes in cirrhotic patients and revealed that gram negative agents were prevalent in cirrhotic patients. (16)

MDR infections have been shown to be associated with increased length of hospital stay and mortality. A study done in Europe in 2011 assessing the effect on mortality of MRSA revealed that 85(36%) of 239 *MRSA* infected patients died compared to 41(9%) of the 446 patients in the *MSSA* control group who died.(17) It has been also found that inappropriately treated infections lead to septic shock and to high mortality.(14) Infections of MRSA have also been shown to increase mortality in patients with malignancies in a study performed in the USA by Sminil *et al*.(18)

Because AMR is a worldwide concern and given that in Rwanda we do not have enough data on antimicrobial resistance profiles and MDR infections' impact on patient' care, we chose to conduct a prospective observational study aiming to identify the pattern and frequency of bacterial pathogens, their antimicrobial susceptibility and the clinical outcomes of patients at Kigali University Teaching Hospital in the department of internal medicine with positive cultures from 1st June to 31st December 2013.

I.3 OBJECTIVES OF THE STUDY

I.3.1 Research Question

What is the frequency of AMR among common bacterial pathogens causing infections in patients hospitalized in KUTH and what are the implications for their treatment outcomes?

I.3.2 General Objective

The general objective was to determine the frequency and antimicrobial susceptibility of bacterial pathogens associated with common infections in patients admitted to the internal medicine department at KUTH between July 1, 2013 and December 31, 2013.

I.3.3 Specific objectives

1. To identify the common bacterial pathogens isolated from urine, blood, wounds and sputum at KUTH
2. To determine the antimicrobial resistance profile of common pathogens isolated at KUTH on the internal medicine wards
3. To assess in-hospital mortality and discharge of patients with laboratory confirmed pathogen associated infections isolated from laboratory of KUTH.
4. To assess risk factors associated with mortality in patients with positive urine, blood, wound or sputum cultures admitted to internal medicine at KUTH.

II. METHODOLOGY

II.1. STUDY DESIGN

This is a prospective observational study conducted between July 1st and December 31st 2013.

II.2. STUDY SITE

This study was conducted at KUTH.

II.3. STUDY POPULATION

Our study population included all hospitalized patients on internal medicine wards at KUTH who had positive bacterial culture results while admitted between 1st July to 31st December 2013.

II.4. SELECTION CRITERIA

II. 4.1. Inclusion criteria

Patients admitted on internal medicine wards at KUTH with positive culture results from urine, blood, wound or sputum.

II.4.2. Exclusion criteria

1. Culture negative samples
2. Positive samples from departments other than IM
3. Mislabeled or poorly labeled specimens making identification of sampled patients difficult
4. Insufficient or inappropriately collected samples
5. Patients with missing information in the medical record on patient demographics and treatment history.

II.5 DATA COLLECTION

Before data collection, we gave a refresher in proper sample taking to the nurses and in processing to the laboratory. Doctors were reminded how to correctly fill out culture and sensitivity request forms before the start of the study. The following information was collected:

demographic data (age, gender, district of origin, code of the patient's file), information about the pathogen (date of sample collection, type of clinical sample, number and species of bacterial isolates from each sample and antimicrobial sensitivity patterns of the isolates). Patient's files were reviewed to ascertain the effect of sensitivity tests on antimicrobial treatment decisions and assess the outcome of the patients in terms of being discharged or having died in hospital as well as looking for information about risk factors or comorbidities that can predispose to infections.

II.6 LABORATORY PROCEDURE

Blood samples were collected into Brain Heart Infusion (BHI) and Schadler broth containing bottles with 25 mls of volume. Urine, wound and sputum cultures were collected in sterile containers. Laboratory materials including sterile containers, antibiotic discs and culture media were obtained from Becton Dickinson Company (USA) through Rwanda Biomedical Center/Medical Procurement and Distribution Division (RBC/MPDD and Biopharma Rwanda).

Blood cultures were directly incubated at 37°C and observed daily for 7 days looking for turbidity or hemolysis that would suggest growth and/or presence of pathogens. Samples found with bacterial growth were sub-cultured on appropriate media followed by isolation and identification of the pathogen according to KUTH standard laboratory procedures. Gram positive cocci were isolated on Monitor Salt Agar (MSA) and/ or blood agar while Mac Conkey agar and Xylose Lysine Deoxycholate agar (XLD) were used for isolation of gram negative bacilli. The identification of gram positive cocci was performed using catalase and coagulase tests. Identification of species of gram negative bacilli was done by colony morphology and conventional biochemical tests.

Urine samples, after wet mount examination, were cultured on Blood agar, chocolate agar, Cysteine Lactose Electrolyte Deficient (CLED) and Mac Conkey agar. The number of colonies was counted after 18-24 hours of incubation at 37° C. Urinary specimens with $>10^4$ CFU/ml of urine were considered as representing urinary tract infection.

For wound swabs and sputum specimens, the gram stain morphology of principal pathogens dictated the selection of appropriate medium for culture and incubated at 37°C for 24 hours. As

with other specimens, identification of bacterial species was done using catalase test for differentiating gram positive cocci, and subsequently, coagulase test to differentiate *S. aureus* from others. Biochemical tests were performed by using Triple Sugar Iron (TSI), Mobility Indole Urea (MIU) and citrate tests to identify and differentiate Enterobactericeae species.

Antibiotic susceptibility testing was performed by the Kirby Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and the following antibiotics were used: ampicillin 10 μg , ceftazidime 30 μg , cefotaxime 30 μg , ceftriaxone 30 μg, cefalothin 30 μg , cefuroxime 30 μg , ciprofloxacin 5 μg, trimethoprimsulfamethoxazole 1.25/23.75 μg , amikacin 30 μg, amoxicillin/clavulanic acid 20/10 μg, clindamycin 2 μg, cloxacillin 1 μg, erythromycin, gentamycin 10 μg, imipenem 10 μg, norfloxacin 10 μg, penicillin 10 unit, ofloxacin 5 μg, oxacillin 1 μg, piperacin 100 μg, vancomycin 30 μg and tetracycline 30 μg.

Gram negative bacilli that were resistant to three third generation cephalosporins—ceftriaxone, ceftazidime and cefotaxime—were classified as ESBL producers; confirmatory tests were not undertaken. Screening for MRSA was determined using oxacillin disk-diffusion tests.

II.7. DATA ANALYSIS

Data collection was done using data collection forms. Data was entered in Excel 2010 and exported to SPSS 18.0 for analysis. Microsoft Word and Excel 2010 were used to treat text and generate tables and graphs respectively. Rates of isolation of bacterial species were reported as a proportion of total samples within and across sample types. Chi-square was used for categorical data to compare 2 or more groups whether dependant or not. Univariate and multivariate analysis was conducted evaluating associations with mortality. Variables included in univariate analysis were: HIV, age >65, diabetes, cirrhosis, malignancy, indwelling catheter, septic shock defined as meeting SIRS criteria, pathogen resistant to antibiotics given or patient having not received antibiotics with activity against the pathogen, having at least one comorbidity and having at least 2 or more comorbidities. Logistic regression multivariate analysis included variables with p value < 0.05 in univariate analysis.

II.8. ETHICAL CONSIDERATION

We received approval from the ethics committee of Kigali University Teaching Hospital and from the research committee of the Faculty of Medicine, National University of Rwanda.

III. STUDY RESULTS

III.1: DEMOGRAPHIC DISTRIBUTION OF THE PATIENTS

Table 1: Patient demographics

Variables	No. (% or IQR)
Age (N=141)	
Median age	45 (30-66)
Mean age	47.65 (30-66)
Age <25 years	26 (18.4)
Age >65 years	37 (26.2)
Sex (N=141)	
Females	78 (55.3%)
Males	63 (44.7%)
District of origin (N=141)	
Nyarugenge	33 (23.4%)
Gasabo	22 (15.6%)
Kicukiro	16 (11.3%)
Others*	70 (49.7%)

*Other administrative districts were: Bugesera, Burera, Gakenke, Gatsibo, Gicumbi, Huye, Kamonyi, Karongi, Kayonza, Kirehe, Muhanga, Musanze, Ngoma, Nyabihu, Nyagatare, Nyanza, Nyaruguru, Rubavu, Ruhango, Rusizi, Rutsiro, Rwamagana

Positive cultures were found to have been collected from 141 unique individuals comprised of 78 females (55.3 %) and 63 males (44.7 %). All patients had requisite demographic and clinical information found in the medical records to meet inclusion criteria for the study. The median age was 45 with an IQR 0f 30-66 years. The youngest patient was 15 years old while the oldest was 89 years old. Elderly patients (>65 years of age) made up 26.2 % of the cohort.

Rwanda is divided into 30 administrative districts. Patients resided in 25 different administrative districts throughout the country, but most were from the districts within Kigali city including Nyarugenge, Gasabo and Kicukiro where 23.4%, 21.3% and 11.3% of patients resided respectively. The distribution of positive cultures was generally evenly divided among the other districts.

Table 2: Distribution of Comorbid Conditions and Predisposing Risk Factors for Infection

Underlying condition predisposing to infection	Frequency	Percentage
Elderly	33	23.4
HIV	42	29.8
Indwelling urinary catheter	20	14.2
Diabetes mellitus	25	17
Cirrhosis	7	5
Severe malnutrition	2	1.4
Malignancy	11	7.8
No presence comorbidity	33	23.4
At least one comorbidity	108	76.6
2 or more of the above comorbidities	46	32.6

We considered preexisting conditions or factors that can predispose an individual to develop infections as HIV infection, diabetes mellitus, chronic liver disease, malignancy, severe malnutrition, immunosuppressive drugs, being elderly or the presence of an indwelling urinary catheter. Among these predisposing comorbidities and risk factors, HIV was the most prevalent at 29.8%, followed by the elderly and diabetes mellitus at 23.4% and 17% respectively. It should be noted that a patient could have 1 or more predisposing factors to infection but none of our patients had more than 3 comorbidities or co-factors that could make him or her vulnerable to infections.

III.2: MICROBIAL ISOLATES AND THEIR ANTIBIOTIC SUSCEPTIBILITY

III.2.1 Profile of microbes

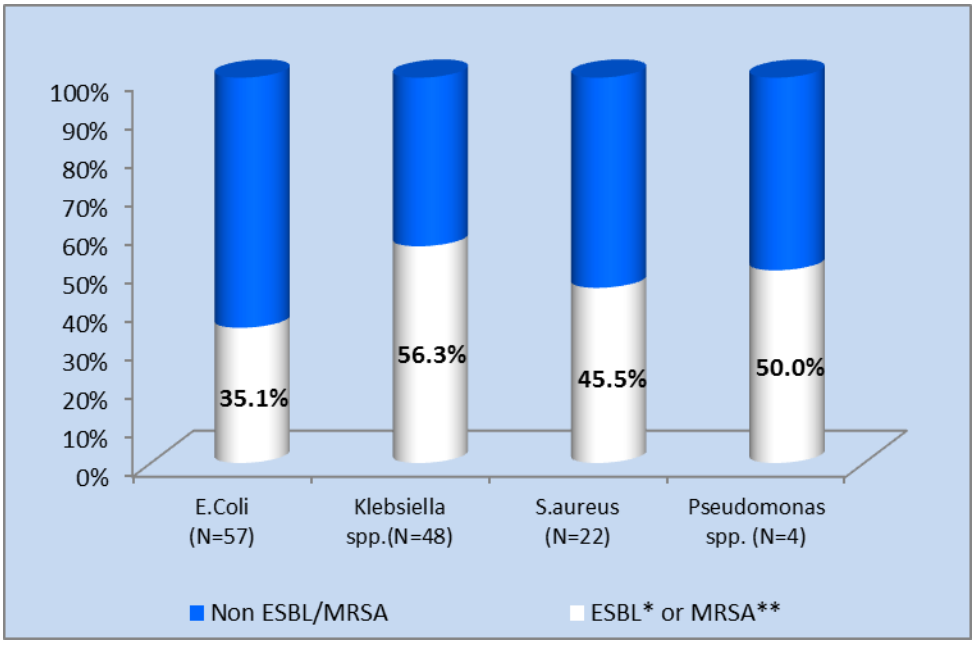
Table 3: Distribution of microbes according to their clinical specimens

Isolates	**Type of sample**				**Total**
	urine	blood	Wound	Sputum	
E.coli	49 (58.4%)	5 (12.8%)	2 (8.3%)	1 (12.5%)	**57 (36.8%)**
Klebsiella spp.	26 (31%)	11 (28.2%)	6 (25%)	5 (62.5%)	**48 (31%)**
S.aureus	4 (4.8%)	9 (23%)	7 (29.2%)	2 (25%)	**22 (14.2%)**
Proteus spp.	2 (2.4%)	1 (2.6%)	4 (16.6%)	0 (0%)	**7 (4.5%)**
Acinetobacter spp.	1 (1.2%)	3 (7.7%)	2 (8.3%)	0 (0%)	**6 (3.9%)**
CoNS	0 (0.0%)	6 (15.4%)	1 (4.2%)	0 (0%)	**7 (4.5%)**
Citrobacter spp.	1 (1.2%)	0 (0.0%)	1 (4.2%)	0 (0%)	**2 (1.3%)**
Pseudomonas spp.	1 (1.2%)	2 (5.1%)	1 (4.2%)	0 (0%)	**4 (2.6%)**
Enterobacter spp.	0 (0.0%)	1 (2.6%)	0 (0%)	0 (0%)	**1 (0.6%)**
Salmonella spp.	0 (0.0%)	1 (2.6%)	0 (0%)	0 (0%)	**1 (0.6%)**
Total	**84 (54.2%)**	**39 (25.2%)**	**24 (15.5%)**	**8 (5.1%)**	**155 (100%)**

CoNS: Coagulase negative staphylococcus

Across the four different clinical specimens (urine, blood, wound and sputum), a total of 155 microbes were isolated from 141 patients. Positive cultures most commonly came from urine specimens with a frequency of 54.2% followed by blood cultures at 25.2%. Wound and sputum specimens made up 15.5% and 5.1% of the positive cultures respectively. In our study, clinical specimens with a single identified pathogen (monomicrobe) were found in 90% of cases, while only one clinical specimen was identified as having 3 microbes. Wound cultures were generally found to have more than one isolate. *E. coli* was the most frequent pathogen isolated in urine specimens followed by *Klebsiella spp*. comprising 58.4% and 31% of urine isolates respectively. In blood specimens, *Klebsiella spp*. was the predominant pathogen among isolates at 28.2% and *S. aureus* was isolated in 23% of positive blood cultures. The most common pathogens identified

in wound cultures were *S.aureus* at 29.2% and *Klebsiella spp.* at 25%. It is notable that *Klebsiella spp.* were isolated across all types of clinical specimens.



**Extended Spectrum Beta Lactamase ** Methicillin Resistant Staphylococcus Aureus*

<u>Figure 1:</u> Distribution of MDR pathogens

ESBL *E.coli* made up 35.1% of the 57 *E.coli* isolates while ESBL *Klebsiella spp.* were the majority of isolates of that species at 56.3%. 45.5% of all *S. aureus* isolates in our study were methicillin resistant.

III.2.2.2 Overview of resistance profile of antibiotics to different microbes

Table 4 illustrates the resistance patterns of prevalent gram negative pathogens. There were high rates of resistance to ampicillin, amoxicillin/clavulanic acid (amoxicillin/CA), and trimethoprim/sulfamethoxazole (TMP/SMX). Resistance rates to aminoglycosides and imipenem were found to be very low. There was significant resistance to 2nd and 3rd generation cephalosporins.

Table 4: **Resistance profile for gram negative microbes**

Antimicrobial drug	**Isolates and their resistance rate in %**				
	E.coli (n=57)	*Klebsiella spp.(n=48)*	*Acinetobacter spp.(n=6)*	*Pseudomonas spp.(n=4)*	*Proteus spp.(n=7)*
Ampicillin	90	100	-	100	67
Amoxicillin/CA*	88	97	-	100	67
Cefalothin	25	50	-	100	100
Cefuroxime	38	67	10	100	50
Ceftazidime	33	58	20	33	33
Cefotaxime	36	64	50	100	100
Ceftriaxone	32	52	25	33	20
Ciprofloxacin	63	67	25	67	50
Norfloxacin	40	44	0	0	0
Ofloxacin	63	15	33	0	75
Amikacin	0	3	0	0	0
Gentamycin	35	48	20	25	60
Imipenem	7	0	7	0	-
Piperacillin	100	50	20	33	0
Tetracycline	100	100	75	-	-
TMP/SMX**	78	86	67	-	100

**Amoxicilline /clavulanic acid ** Trimethoprim/Sulfamethoxazole*
(-) represents having not tested for resistance

Table 5: Resistance profile for gram positive microbes

Antimicrobial drug	Isolates and their resistance rate in %	
	S.aureus (N=22)	*CoNS (N=7)*
Ampicillin	100	100
Penicillin	89	67
Oxacillin	84	100
Cloxacillin	100	100
Piperacillin	55	100
Cefalothin	35	71
Cefuroxime	23	60
Ceftriaxone	30	100
Cefotaxime	60	67
Clindamycin	100	0
Erythromycin	33	50
Ciprofloxacin	17	100
Norfloxacin	29	67
Ofloxacin	27	40
Imipenem	40	25
Vancomycin	6	0
Tetracycline	58	71
TMP/SMX	60	-

Among gram positive pathogens, *S. aureus* was the most prevalent. A high resistance against penicillin was noted. We also found high resistance rates to cephalosporins and carbapenems. Vancomycin was active against most gram positive organisms; *S.aureus* had with a low resistance rate of 6%.

III.2.2.3 Resistance profile in commonly isolated pathogens in UTIs and in bacteremia

Table 6: **Resistance profile of pathogens in urine cultures (UTIs)**

Antimicrobial drug	**Isolates and their resistance rate in %**		
	E.coli(N=49)	*Klebsiella spp.(N=26)*	*S.aureus (N=4)*
Ampicillin	91.3	100	-
Amoxicillin/CA	89.5	95.2	-
Cefalothin	25.0	50	20
Cefuroxime	36.4	66.7	-
Ceftazidime	35.7	66.7	-
Ceftriaxone	27.3	52.9	30
Cefotaxime	33.3	57.1	33.3
Ciprofloxacin	64.3	72.7	50
Norfloxacin	38.6	47.8	60
Amikacin	0.0	4.8	72.0
Gentamycin	35.7	38.9	-
Imipenem	0	0.0	32
TMP/SMX	78.3	92.9	50
Clindamycin	-	-	100
Oxacillin	-	-	100
Vancomycin	-	-	0.0
Piperacin	-	-	66.7
Penicillin	-	-	100
Tetracycline	-	-	100

In UTIs, *E.coli* was the most predominant pathogen cultured and resistance rates were quite high to all antibiotics tested, including 3rd generation cephalosporins. *E. coli* was found to be more susceptible to norfloxacin (38.6% resistance) compared with ciprofloxacin (64.3% resistance), which is commonly the first line agent used to treat UTIs. *Klebsiella sp.* cultured in urine showed a similar resistance pattern to the quinolones and was most susceptible to aminoglycosides.

E. coli and *Klebsiella spp*. UTIs were generally resistant to ampicillin and UTIs caused by *S.aureus* were widely resistant to penicillins. UTIs caused by *S.aureus* were more susceptible to cephalosporins and carbapenems with lower resistance rates of about 30%. .

Table 7: Resistance profile in blood cultures (bacteremia)

Antimicrobial drug	**Isolates and their resistance rate in %**		
	E.coli(n=5)	*Klebsiella spp.(n=11)*	*S.aureus (n=9)*
Ampicillin	80	100	100
Amoxicillin/CA	66.7	100	-
Cefalothin	33.3	75	42.9
Cefuroxime	33.3	67.7	50
Ceftazidime	25	42.9	-
Ceftriaxone	30	80	40
Cefotaxime	40	90	50
Ciprofloxacin	66.7	83.3	50
Norfloxacin	66.7	28.7	20
Gentamycin	40	66.7	-
Amikacin	0	0	0
Imipenem	0	0	33.3
TMP/SMX	75	100	100
Penicillin	-	-	88.9
Oxacillin	-	-	80
Clindamycin	-	-	100
Vancomycin	-	-	6
Piperacillin	-	-	40
Tetracycline	-	-	75

In bacteremia, *E.coli* was frequently multiresistant with resistance documented to various antibiotics tested. Only amikacin and imipenem remained generally sensitive. Cephalosporins and gentamycin can still be used compared to others. Quinolones and amoxicillin/CA had high

resistance rates and can no longer be recommended. *Klebsiella spp*. causing bacteremia showed high resistance rates to multiple classes of antibiotics. After imipenem and amikacin, which have maintained sensitivity, norfloxacin is also good option for empiric therapy when suspecting *Klebsiella spp*. or *S.aureus* to be the causative agent of bacteremia. Again, penicillin should not be not the first line choice of antibiotics in treating bacteremia caused by staphylococcal infections, as there was high resistance to penicillins found in this study.

III.3 PATIENTS ACCORDING TO INTERVENTION AND OUTCOME

Table 8: Empiric antibiotherapy given in from patients with pending culture and sensitivity testing results

Antimicrobial drug	**Patients who received empiric antibiotherapy**				
	Frequency (%)	UTI	Bacteremia	Wound	LRTI
amox/clav	5 (4.3%)	2	2	1	
ampicillin	9 (7.7%)	3	4	-	2
Lcefotaxime	5 (4.3%)	2	0	2	1
ceftriaxone	33 (28.2%)	16	12	3	2
ciprofloxacin	36 (30.7%)	25	7	3	1
cotrimoxazole	1 (0.9%)	0	1	0	0
erythromycin	5 (4.3%)	0	3	2	0
amoxicillin	1 (0.9%)	1	0	0	0
ceftriaxone/flagyl	4 (3.4%)	2	1	1	0
cipro/flagyl	6 (5.1%)	2	3	1	0
cloxacillin	7 (5.9%)	6	0	1	0
doxycycline	3 (2.6%)	0	3	0	0
ampi/genta	2 (1.7%)	1	1	0	0
Total	**117**	**60**	**37**	**14**	**5**

LRTI: Lower Respiratory Tract Infections

While results of culture and sensitivity have showed frequent resistance to ciprofloxacin in all types of clinical specimens, ciprofloxacin has been the most prescribed as empirical antibiotherapy at a frequency of 30.7%. In UTIs alone, ciprofloxacin was given in 41.7% of cases as empiric therapy. The second most commonly prescribed empiric treatment of UTI was ceftriaxone, which was prescribed 28.2% of the time. Ceftriaxone was also the most commonly used antibiotic in empiric treatment of suspected bacteremia.

Table 9: Appropriateness of empiric antibiotherapy and patient outcomes

Cultures showed sensitivity to empiric antibiotics	Frequency	Patients who died in-hospital	Discharged patients
Yes	36(30.8%)	7 (26%)	29 (32%)
No	81(69.2%)	20 (74%)	61(68%)
Total	117	27	90

A total of 117 patients out of the 141 patients found to have positive cultures were started on empiric antibiotic treatment before culture results were available and had complete demographic and clinical information documented. 69.2% of subjects were started on empiric antibiotics to which the subsequently identified disease-causing pathogen was resistant. Among the 27 patients who died, only 7 (26%) received empiric antibiotics, which were later confirmed to be active against the isolated pathogens compared to 20 patients (74%) who received empiric antibiotics for isolates ultimately found to be resistant to their prescribed antibiotic. While more patients who were empirically started on treatment that was later found to be ineffective against the identified pathogen died in hospital, this difference in mortality was not statistically significant (p-value > 0.05)

Table 10: **Intervention after results of culture/sensitivity in terms of antibiotherapy**

Targeted antibiotherapy initiated after culture and sensitivity results available	**Frequency(%)**	**Patients who died in-hospital**	**Discharged patients**	**Delay in days of starting antibiotherapy after culture results available (mean)**
Yes	96(78%)	5(50%)	91(80.5%)	
No	27(22%)	5(50%)	22(19.5%)	
Total	123	10	113	2.25 (SD= 1.20)

Of 141 patients, 123 patients received antibiotherapy after culture and sensitivity testing;18 patients died before the results of culture and sensitivity were available. Of the 123 patients, 78% ultimately received appropriate antibiotics based on culture results—either they had been on an effective antibacterial agent empirically or they were switched from an agent to which the pathogen was resistant to an effective agent based on culture and sensitivity results. The remaining 22% of patients did not receive effective antibacterial agents despite available culture results. The mean delay in starting antibiotics after results of culture were available was 2.25 days ranging from 1 to 8 days with a SD=1.178. The delay was calculated from the date of reporting the results by the laboratory until the day antibiotics—targeted or empiric—were initiated. 9 patients died after obtaining the results from culture and half of these received antibiotics to which isolates were sensitive. The difference in mortality based on receiving antibiotics that were effective against the isolated pathogen was statistically significant with a p value=0.04.

Table 11: **Patient's outcome according to their pathogens**

Isolated germs	Patient Outcome		Total
	Discharges	Deaths	
E. coli	47(41.2%)	6 (22.2%)	53 (37.6%)
ESBL/E. coli	16(14%)	4 (14.8%)	20 (14.2%)
Klebsiella spp.	32(28%)	8 (29.65%)	40 (28.4%)
ESBL/Klebsiella	18(15.8%)	6 (22.2%)	24 (17.0%)
S.aureus	18(15.8%)	4 (14.8%)	22 (15.6%)
MRSA	9(7.9%)	1 (3.7%)	10 (7.0%)
Proteus spp.	4(3.5%)	2 (7.4%)	6 (4.3%)
Acinetobacter spp.	5(4.4%)	0 (0.0%)	5 (3.5%)
CoNS	3(2.6%)	4 (14.8%)	7 (5%)
MRSE	0(0.0%)	1 (3.7%)	1 (0.7%)
Citrobacter spp.	2(1.8%)	0 (0.0%)	2 (1.4%)
Pseudomonas spp.	2(1.8%)	2 (7.4%)	2 (1.4%)
ESBL/Pseudomonas	1(0.9%)	1 (3.7%)	4 (2.8%)
Enterobacter spp.	0(0.0%)	1 (3.7%)	1 (0.7%)
Salmonella spp.	1(0.9%)	0 (0.0%)	1 (0.7%)
Total	114	27	141

Of the 27 patients that died, *Klebsiella spp.* was isolated in 29.6% of cultures and ESBL *Klebsiella spp* was cultured in 22.2%. *E.coli* was isolated in 22.2% of patients who died and 14.8% of these *E.coli* isolates were found to be ESBL producing organisms. Even though statistically not significant ($p>0.05$), it can be observed that ESBL producing E.coli and ESBL producing Klebsiella spp. were most associated with death compared to other types of microbes.

Table 12: Multivariate analysis for risk factors associated with in-hospital mortality

Variables	OR	95 CI	P-Value
Age group > 65 years(elderly)	1.029	0.223-4.741	0.971
Septic shock	39.011	10.95-138.9	0.0001
≥ 2 comorbidities	0.386	0.09-1.615	0.191
Antibiotherapy before and after not matching with culture results	3.159	0.950-10.5	0.061

Univariate analyses of comorbidities and risk factors associated with mortality were performed for the following variables: HIV; age >65; diabetes; cirrhosis; malignancy; stroke; TB; septic shock, which was defined as meeting SIRS criteria; pathogens resistant to antibiotics given or patient having not received antibiotics with activity against the pathogen; having at least one comorbidity; and having 2 or more comorbidities. Multivariate analysis was conducted including variables found to be statistically significant in univariate analyses with p-values of <0.05. Multivariate analysis showed that septic shock was associated with mortality with an odds ratio of 39 (p=0.0001). Other parameters were not significantly associated with mortality.

IV.DISCUSSION

IV.1 DEMOGRAPHIC CHARACTERISTICS

Clinical specimens yielding positive bacterial culture results obtained from 141 patients were included in our study. The median patient age of patients with positive culture results admitted to internal medicine at KUTH was 45 years (IQR 30-66) and the mean age was 47.7 years (30-66). Elderly patients (>65 years of age) were the predominant age group comprising 26.2% of patients with positive cultures. The female to male ratio was 1.24:1, 55.3% of patients with positive cultures were female. The predominance of female subjects in this study is consistent with other large observational studies in Rwanda [DHS 2010] (19) where females are overall more represented than men. Women were found to have more frequent positive urine cultures while positive sputum, wound and blood cultures were more frequently isolated in samples collected from men A study done in Republic of Korea by Cheol-In Kang *et al.* assessing the epidemiology and risk factors of community associated ESBL producing *E.coli* in specimens obtained from 2010-2011 showed comparable results as there was a higher proportion of females to males and elderly people were the most represented age group. (13)

KUTH is a national referral hospital which receives patients from different parts of the country but we found that most of our patients were coming from 3 districts of Kigali city, namely Nyarugenge (23.4%), Gasabo (15.6%) and Kicukiro (11.3%). This can be explained by the fact that our study site is located in Kigali city so it is in close proximity and more accessible to the population of the city. Of the 3 districts of Kigali city, Nyarugenge had more cases most likely because due to geographic location; KUTH is located in Nyarugene. In addition Muhima district hospital, which is the only district hospital in the Nyarugenge administrative district, does not admit patients with internal medicine problems, whereas district hospitals in other administrative regions do admit medicine patients as inpatients, and thereby, KUTH receives all of the referrals for admission from Muhima.

IV.2 FREQUENCY OF MICROBIAL ISOLATES

Urine samples yielded the most positive bacterial cultures at 54.2 %, followed by blood samples (25.2%) then wound swabs (15.5%) and, lastly, sputum samples (5.1%) These results are consistent with findings reported in previous studies. Ibrahim *et al* in Sudan found similar results where urine specimens made up 65.1% of all clinical specimens, followed by wound specimens at 22% and blood samples made up 2.2% in a study evaluating MDR E. coli in hospitals in Khartoum.(9) In this study, wound specimens were the second most frequent type of positive culture and blood samples had low frequency, which is different from the results found in our study. This can be explained by the fact that we considered only patients admitted in internal medicine wards, who are generally less concerned with wound infections, compared with patients from surgical and gynecology-obstetric wards that were included in the study done in Khartoum. Another study performed in Ethiopia by Kibret and Abera showed comparable results where urine samples comprised 45.5 % and wounds accounted for18.7%; blood samples were not evaluated in their study.(20) There is limited information available in the literature concerning positive sputum sample frequencies.

IV.3 RESISTANCE PATTERN

IV.3 .1 Urinary tract infections (UTIs)

E.coli was the most prevalent pathogen isolated in urine samples in our study at 58.4% followed by *Kebsiella spp*. at 31%. Together, *E. coli and Klebsiella spp*. made up 89.4% of all germs isolated in urine. Other studies have shown similar results. E.coli were the most predominant pathogen obtained from urine samples (50%) in hospitalized patients in the study conducted in Rwanda by Muvunyi et al. in 2011.(4) In Belgium, a study conducted by Didier *et al.* also revealed that E.coli were more frequently isolated from urine samples at 56%.(15)

In our study, *E.coli* was resistant to the commonly used antibiotics in treating urinary tract infections such as ciprofloxacin (64.3 %), and norfloxacin (38.6 %). It also showed high rates of resistance to the other commonly used oral antibiotics amoxicillin/CA (88 %), cotrimoxazole (78 %) and ampicillin (90 %). In addition to this, its potential production of extended spectrum beta

lactamase by screening method was also high as 37.7% of all *E.coli* showed this resistance pattern. These isolates showed high resistance to different generations of cephalosporins – cefalothin (25%), ceftazidime (35.7%), cefuroxime (36.4%), ceftriaxone (27.3%) and cefotaxime (33.3%). The high prevalence of resistance to commonly used antibiotics can be explained by the fact that these antibiotics—ciprofloxacin, amoxicillin/CA and cotrimoxazole—are widely prescribed to patients by health providers without the guidance of culture and sensitivity results. Quinolone usage in Rwanda has increased over the past 10 years following a study done by Nkurikiyimfura *et al*. on antimicrobial susceptibility from 1991-2000 where, among several recommendations, quinolones were proposed as empiric therapy for UTIs.(21)

The frequency of presumed ESBL producing *E.coli* reported in our study is lower than that described by other studies that used ESBL confirmatory tests. Muvunyi *et al.* in Rwanda showed that ESBL phenotypes made up 38% of *E. coli* isolates in hospitalized patients, while another study performed in the Republic of Korea by Cheol-In Kang showed that ESBL producing *E.coli* was identified at a frequency of 50.9%.(4)(13) However, in Norway, Arne *et al*. found a low frequency of ESBL producing *E.coli* at 24%.(22) It is possible that we may have underestimated the ESBL prevalence in our study by considering ESBL producing pathogen as resistant to 3 cephalosporins of 3rd generation; we did not used laboratory confirmatory tests, which can be done when one of C3G is resistant to E.coli or klebsiella spp.

The increased resistance of *E.coli* to many antibiotics has been observed in a study done in Sudan by Ibrahim *et al.* where resistance rates in adults was as follows: ciprofloxacin (62.5 %), ofloxacin (58.3 %), amoxicillin/CA (51.8 %) and cotrimoxazole (88.7 %). The resistance rate to cephalosporins was higher than that observed in our study. The authors reported a low resistance to aminoglycosides—amikacin (1.8%), gentamycin (34.5%)—as was also observed in our study where the resistance rates of *E coli* to amikacin and gentamycin were 0.0% and 35.7% respectively.(9) This can be explained by the fact that aminoglycosides are not routinely used in Rwanda. Comparable results of multidrug resistant *E.coli* have been found in Rwanda and other parts of the world (4), (13),(20),(23).

Our study has shown that MDR *E.coli*, is prevalent and a public health concern in Rwanda. There is evidence of increasing resistance rates over time. For example, in a retrospective study conducted in Rwanda by Nkurikirimfura *et al*. from 1991-2000, low resistance rates were noted to quinolones where ciprofloxacin resistance was only 1.15%. (21) Later on, data from a retrospective study conducted in Rwanda by Bayingana *et al.* found that resistance of *E.coli* urinary isolates had increased to 23.62%. (24) Our MDR *E. coli* frequencies are higher than those observed in the two above named studies and suggestive of an increase of resistance over time. Because our study only evaluated hospital specimens, this may account for the higher rates of drug resistance noted.

IV.3 .2 Bacteremia

In the blood, *Klebsiella* spp. was the most frequently isolated pathogen (28.2%) and S. *aureus* was second with a frequency of 23%. The observed resistance to 3rd generation cephalosporins by *Klebsiella spp*. is alarming; 90% of isolates were resistant to cefotaxime and 80% to ceftriaxone.

56.3% of all isolates of *Klebsiella spp*. were presumed to be ESBL producers and the high observed frequency may be explained by the prescription of 3rd cephalosporin antibiotics as empiric antibiotics in hospital settings before culture and sensitivity results are obtained. Relatively low resistance was observed to norfloxacin, which is probably due to low selective resistance pressure because this antibiotic is not included on the essential public medicine list and, therefore, less prescribed by health providers. In our study, *S. aureus* also showed high resistance to antibiotics commonly used in treating staphylococcal infections such as penicillins.

Another study performed in Rwanda by Nkurikiyimfura *et al*., from 1991 to 2000 also showed that *S.aureus* was resistant to penicillin at 77.5%, oxacillin (28.5%) amoxicillin (44.68%) and amikacin (1.5%).(21) It is notable that, in Rwanda, S.aureus was resistant to penicillins even 10 to 20 years ago and remains susceptible to amikacin; this pattern of resistance is likely due to resistance selective pressure associated with use of available antimicromial agents in the country.

Comparable results have been found by other authors in Africa (25) (10) but are different from results from developed countries where the resistance rate of *S. aureus* and *Kebsiella spp.* are lower than that observed in our study. (26) This can be explained by the strong surveillance systems, availability of more classes of antibiotics and tailored antibiotic treatment based on culture results generally found in high-income settings. (26) In our study, *S.aureus* was the predominant isolate in bacteremia compared to *Coagulase negative Staphylococcus (CoNS)* at 25% and 16.7% respectively. A similar study done in the USA in 2004 by Hilmar *et al.* came up with opposite results where *Coagulase negative Staphylococcus* was more predominant than *S. aureus* at 31% and 20% respectively.(26) This can be explained by the increased use of intravascular devices including central venous catheters in developed countries. In Egypt, a study performed by Shaaban *et al*. in 2009 documented comparable results to our findings where among gram positive microbes, MRSA was most frequent at 18.9% followed by *CoNS* at 16%.(27)

Imipenem, amikacin and vancomycin were found to be effective against almost all germs. This can be explained by their powerful potency of killing bacteria but also by a low selective pressure because they are quite expensive and are not frequently used in our country as they do not figure on the essential medicine list in the public health sector in Rwanda. It is important that we preserve the effectiveness of these agents in light of the fact that in settings where increasing resistance has been documented, it has also been associated with worse patient outcomes. Vancomycin resistance has been linked with a high mortality rate in patients with cirrhosis in a study performed in USA by Sminil et al. published in 2012.(18)

IV.4 PATIENTS' OUTCOMES

Of the 141 patients whose clinical specimens were studied, 27 (19.2 %) died in-hospital and 114 (80.9%) were discharged home. 74 of the patients received inappropriate empirical antibiotherapy before results from culture and sensitivity were available. 17 patients died before the results of culture and sensitivity were obtained. 10 patients died after their culture and sensitivity results were available—5 of them having received appropriate antibiotics according to the culture results and 5 having received antibiotics that isolates were resistant to (p value =

0.04). Of the factors considered to have potentially contributed to mortality, only the development of septic shock was statistically significant ($p<0.001$). Further evaluation of risk factors for mortality need to be considered in future studies.

Sepsis has been found to be associated with increased mortality by other authors. A study performed by Saoraya *et al*. in Thailand in 2012 found that even with appropriate antibiotics, the mortality rate was 60% in patients with sepsis. (14) Septic shock has been associated with increased mortality even in settings that used broad spectrum antibiotics like carbapenems, as reported in a study conducted by Ching-Chi Lee and published in 2010. (28)

In our study, we were not able to determine other factors shown to carry increased risk of mortality other than sepsis. However, in Africa and elsewhere, authors have identified other factors associated with mortality in patients with bacterial infections. Previously identified factors include MDR hospital acquired bacteremia, ESBL and KPC producing gram negative microorganisms and MRSA. (27) (29)(17)

IV.5 STUDY LIMITATIONS

The frequency of AMR at KUTH, which is a national referral hospital, may not reflect that of primary care centers or other health facilities located outside of Kigali. While there was very little missing data for variables collected in this study, data integrity was dependent of the quality of medical record documentation. Another limitation of our study was that the small sample size did not allow further analysis of the impact of risk factors on patient treatment outcomes. Lastly, we may have underestimated the prevalence of ESBL *Klebsiella spp.*, ESBL *E.coli* and *MRSA* by screening method because we did not use confirmatory laboratory tests.

V.CONCLUSIONS AND RECOMMENDATIONS

V.1 CONCLUSIONS

The magnitude of antimicrobial resistance in Kigali University Teaching Hospital is worrisome. In patients who are admitted to the internal medicine wards, urinary tract infections were most

common, followed by bacteremia, wound infections and then lower respiratory tract infections. *E.coli* was the most isolated pathogen followed by *Klebsiella spp*. and *S. aureus*. Other species isolated in low proportion were*: Coagulase negative Staphylococcus (CoNS), Proteus spp., Acinetobacter spp., Pseudomonas spp., Citrobacter spp., Enterobacter spp.* and *Salmonella spp.*

E. coli isolates were the most common isolate from urinary specimens, whereas Klebsiella *spp*. were isolated across all types of clinical specimens. In blood cultures yielding bacterial pathogens, *Klebsiella spp*. and *S.aureus* were isolated at similar frequencies and made up about 25% each of total microbes isolated. Wound infections and lower respiratory tract infections were not analyzed in detail given that there were few isolates obtained. *E. coli, Klebsiella spp*. and *S.aureus* species all showed high rates of resistance to frequently used antibiotics in our country. Imipenem and amikacin for gram negative organisms and vancomycin for gram positive organisms are the only antibiotics to which organisms remain largely susceptible. However, these antibiotics are very expensive and generally out of financial reach for our population given that most of our patients are subsistence farmers who rely on public insurance and have limited access to private pharmaceutical traders. These antibiotics are not on the KUTH formulary making them unavailable in the hospital pharmacy and, thereby, not covered by public insurance.

It is important to state that available antibiotics like ampicillin, amoxicillin/clavulanic acid, ciprofloxacin and cotrimoxazole, due to resistant rates described in our study, are poor choices of empiric therapy for treating E.coli and Klebsiella spp. for bacteremias or UTIs. Oxacillin, cloxacillin, ampicillin and penicillin are also poor choices to empirically treat staphylococcal infections given our study findings. Beyond the challenge of treating MDR pathogens with a limited antibiotic formulary, aggressive infection control and antimicrobial resistance surveillance systems are sorely needed to address this issue and prevent progression. There was a relatively low resistance rate to erythromycin in Staphylococcal isolates and these are better alternative empiric antibiotherapy in staphylococcal infections.

We noted an alarmingly high rate of resistance by gram negative organisms to the 3rd generation cephalosporins. The over prescription of these drugs is most likely contributing to this

observation. There is need for caution with widespread use of these drugs so as not to fuel the ongoing epidemic of drug resistance.

In our study, we observed a mortality rate of 19.2% and septic shock was found to be associated with mortality. While many of the deaths occurred before culture results were available (63%), some patients continued to receive inappropriate antibiotics when the data was available. This was mainly due to MDR pathogens resistant to antibiotics available in the hospital pharmacy; often appropriate antibiotics were only available at expensive private pharmacies and unaffordable for patients. Overall, 74 patients received inappropriate empiric antibiotics. In view of the high proportion of patients where readily available and often prescribed empiric antibiotics were ineffective, it is important that local antibiotic susceptibilities are considered when selecting antimicrobial agents for hospital formularies and in drafting national and institution specific algorithms for treatment.

V.2 RECOMMENDATIONS:

V.2 .1 To the Ministry of Health (MOH):

This study should be replicated across the country to evaluate the prevalence of antimicrobial resistance in health facilities of Rwanda including health centers, district hospitals and referral hospitals. Private hospitals should be included as well.

A surveillance system should be setup to monitor antibiotic prescription patterns and to inform guidelines and indications for antibiotic use, especially in health facilities that do not have access to well-equipped laboratories that can isolate microbes and test susceptibilities.

Laboratories in all district hospitals should be adequately equipped from both a procurement and human resource perspective to perform bacterial cultures and antimicrobial susceptibility testing.

Vancomycin, imipenem and amikacin, which have activity against multidrug resistance microbes, should be, at a minimum, included on formularies at referral hospitals so as to provide access to effective antibiotics for commonly encountered infections.

Surveillance of the private health sector and investigation of antimicrobial prescribing practices should be conducted. Over-prescription of antibiotics—in general and 3rd generation cephalosporins specifically—in the private sector may be contributing to increasing MDR and information from the private sector, in addition to the public sector, would be helpful for confirming this hypothesis.

V.2 .2 To the Kigali University Teaching Hospital (KUTH)

Overuse of 3rd generation cephalosporins should be minimized in order to limit the high resistance rates of nosocomial pathogens to these antibiotics.

Similar studies in all clinical departments of the hospital over longer periods of time should be conducted to more comprehensively assess the patterns and epidemiology of antimicrobial resistance for both in-patient and out-patient populations.

It is essential to provide the hospital pharmacy with antibiotics that have been shown to be active against the pathogenic organisms—norfloxacin among quinolones, vancomycin among glycopeptides, amikacin among aminoglycosides and imipenem among carbapenems. These above antibiotics should strictly be used for multidrug resistant organisms when isolated and shown to be susceptible to them.

For clinicians, particular attention has to be paid to septic patients. Clinical samples should be taken as soon as possible and appropriate empiric antibiotherapy initiated and guided by the results from our study taking into account the most likely offending microbes and their resistance patterns. Further, once culture and sensitivity results are available, antibiotics should be tailored to the isolated bacteria as soon as possible.

A well-powered study on the implications of antimicrobial resistance on patients' clinical outcome has to be performed over a longer period of time; this will enable further analysis of factors that are contributing to mortality in our patients. The same study can even evaluate the AMR and its economic costs and impact on length of hospital stay.

V.2 .3 To the private health sector

As most of our population was residing within Kigali city and given that many private clinics are operating in Kigali city, it is important that clinicians working in the private sector are aware of the high resistance of pathogenic microbes to the commonly used antibiotics in their usual practice. It is our hope that knowledge of local epidemiology and microbial sensitivities will reduce unnecessary antibiotic prescriptions and thereby contribute to decreasing AMR. Further, awareness of local resistance patterns may affect better patient outcomes with fewer patients being prescribed ineffective antibiotics. Continuous medical education (CME) should be based on evidence based practices and it should be emphasized that antibiotic prescribing is best done as guided by culture results.

V.2 .4 To the young researchers

The epidemiology and trends of antimicrobial resistance in Rwanda need to be explored and monitored. This study should be replicated in different settings. Also, according to the results of this study, one can carry out different studies on pertinent issues such as:

(a) Risk factors for acquisition of MDR bacterial pathogens in hospital and / or community settings,
(b) Impact of antimicrobial resistance on the clinical outcome of patients with the following medical conditions: HIV infections, chronic liver disease, malignancies, diabetes mellitus as well as other comorbidities shown to predispose to infection
(c) Describe AMR using confirmatory lab tests to more definitively and accurately identify the frequency of ESBL producing pathogens in Rwanda.

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APPENDICES

DATA COLLECTION FORM FOR ANTIMICROBIAL RESISTANCE STUDY IN KUTH

1. Study number ☐☐☐

2. Patient initials: _____

4. Gender:

☐ (1)Male

☐ (2)Female

5. Age (number in years) ☐☐

6. Patient's ID Number (File number) ☐☐☐☐☐

7. District of residence (where the patient is living): ☐

Codes of Districts

(1) Bugesera	(7) Huye	(13) Muhanga	(19)Nyarugenge	(25)Rwamagana
(2) Burera	(8) Kamonyi	(14) Musanze	(20)Nyaruguru	(26) Gisagara
(3) Gakenke	(9) Karongi	(15) Ngoma	(21)Rubavu	(27) Nyamasheke
(4) Gasabo	(10) Kayonza	(16) Nyabihu	(22)Ruhango	(28) Nyamagabe
(5) Gatsibo	(11) Kicukiro	(17) Nyagatare	(23)Rusizi	(29) Rulindo
(6) Gicumbi	(12) Kirehe	(18) Nyanza	(24)Rutsiro	(30) Nyaruguru

8. Type of sample: ☐

(1) Urine (2) Blood (3) Pus swab (4) Sputum

9. **Susceptibility to antibiotics for isolate germ**

Germ 1	Antibiotic	Susceptibility	Germ 2	Antibiotic	Susceptibility	Germ 3	Antibiotic	Susceptibility

Codes of germs

1= E.coli	4=ESBL/Klebsiella	7=Proteus	10=MRSE	13=ESBL/Pseudomonas
2=ESBL/E.coli	5=S.aureus	8=Acinetobacter	11=Citrobacter	14=Enterobacter
3=Klebiella spp.	6=MRSA	9=CoNS	12=Pseudomonas	15=Salmonella spp

Codes of antibiotics

1=amikacin	5=ceftriaxone	9=cotrimoxazole	13=penicillin	17=cipro/flagyl
2=amox/clav	6=ceftriaxone/cipro	10=erythromycin	14= amoxicillin	18=cloxacillin
3= ampicillin	7=ceftriaxone/erythromycin	11=gentamycin	15=ceftriaxone/flagyl	19=doxycycline
4=cefotaxime	8=ciprofloxacin	12=norfloxacin	16=cefuroxime	20=ampi/genta

Codes for susceptibility

(1)Susceptible (2) Intermediate (3) Resistant

12. Has the antibiotic choice been guided by culture and sensitivity after results?

☐ (1)Yes

☐ (2)No

13. If yes, the code of antibiotic given according to the culture results ☐ *(use 0 for N/A)*

14. If no, the code of empirical antibiotics after culture results ☐ *(use 0 for N/A and 99 if no antibiotic was given)*

15. Empirical antibiotics before culture results ☐☐ *(Code of antibiotics given. Use 99 if no antibiotic was given)*

16. Number of days between culture results and the start of antibiotics accordingly ☐☐

17. Clinical outcome of the patient

☐ (1) Discharged

☐ (2) Died

☐ (3) Unknown

18.If died , has he died before antibiotherapy guided by culture and sensitivity?

☐ (1) Yes

☐ (2) Not

☐ (0) N/A (not applicable)

19. Comorbidity *(Mark all that apply)*

- ☐ (1) Elderly: patient's age > 65 years
- ☐ (2) HIV
- ☐ (3) Diabetes mellitus
- ☐ (4) Cirrhosis/chronic liver disease
- ☐ (5) Malignancy
- ☐ (6) Indwelling urinary catheter
- ☐ (7) Severe malnutrition
- ☐ (8) Nephrotic syndrome
- ☐ (9) Immunosuppressive drugs
- ☐ (99) None

20. Duration in days of antibiotherapy according to the culture results before death ☐☐

(Use 00 if Not Applicable)

21. Septic shock as possible cause of death:

- ☐ (1) Yes /septic shock
- ☐ (2) No/septic shock
- ☐ (3) Unknown

22. Total duration in days of hospitalization ☐☐