College of Medicine

Healthcare-Associated Urinary Tract Infection in the Surgery Department at Queen Elizabeth Central Hospital: Deciphering Risk Factors and Antimicrobial Resistance Patterns of Isolated Bacteria

By

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CERTIFICATION OF APPROVAL

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DECLARATION

I, **Kambale Bunduki Gabriel,** hereby declare that this dissertation is my original work and has not been presented for any other awards at the College of Medicine (COM), University of Malawi (UNIMA) or any other University

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DEDICATION

I dedicate this work to my daughter Arabella Goodluck Bunduki, for whom I have been a bad dad; grown in womb, born and now close to her first birthday while I am absent at home. Know that despite all these, daddy loves you so much.

LIST OF PUBLICATIONS FROM THIS THESIS

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ABSTRACT

In this dissertation, I investigated the risk factors associated with healthcare-associated infections among patients admitted in surgical wards of the Surgery department of the Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi. A particular focus was on healthcare-associated urinary tract infection (UTI).

This dissertation is a result of three studies. The first one was a systematic review and meta-analysis of uropathogenic *Escherichia coli* (UPEC) and specifically their antimicrobial resistance and virulence profiles. The second study was a point-prevalence survey on healthcare-associated infections (HAI) and antimicrobial use in the surgery department at QECH. The third study was a cross-sectional study investigating risk factors associated with UTI and catheter-associated UTI. In addition, the study determined the antimicrobial resistance patterns of isolated bacteria from urine samples from patient suspected with hospital-acquired UTI.

From the systematic review and meta-analysis, 1,888 UPEC isolates were included in the analysis. High antimicrobial resistance rates were observed among the antibiotic class of tetracycline in 69.1% (498/721), followed by sulphonamides in 59.3% (1119/1888), quinolones in 49.4% (1956/3956), and beta-lactams in 36.9% (4410/11964). Meanwhile, virulence factors with highest prevalence were immune suppressors (54.1%) followed by adhesins (45.9%).

The point prevalence of HAI was 11.4% (n=12/105) (95% CI: 6.0%-19.1%), including 4 surgical site infections, 4 urinary tract infections, 3 bloodstream infections and 1 bone/joint infection. We identified the following risk factors for HAI; length-of-stay between 8 and 14 days (OR=14.4, 95% CI: 1.65-124.7, p=0.0143), presence of indwelling urinary catheter (OR=8.3, 95% CI: 2.24-30.70, p=0.003) and the history of surgery in the

past 30 days (OR=5.11, 95% CI: 1.46-17.83, p=0.011). 29/105 patients (27.6%) were prescribed antimicrobials, most commonly the 3rd-generation cephalosporin, ceftriaxone (n=15).

The prevalence of confirmed HA-UTI was 53.1% (179/337, 95% CI: 47.8-58.4). The CAUTI was observed in 53.9% (28/52, 95% CI: 40.0-67.1). Risk factors associated with HA-UTI and CAUTI were the age of patients, patients who are not married, low educational level (none or primary school), prostatic diseases, patients presenting UTI symptoms, hospital length of stay (>7 days).

The most frequent isolated bacteria from patient with confirmed HA-UTI were *E. coli* in 46.4% (83/179), *Klebsiella spp* in 11.7% (21/179), *Citrobacter spp* in 9.5% (17/179), *S. aureus* in 5.9% (16/179), *Enterobacter spp* in 5.5% (10/179), *Acinetobacter spp* in 5% (9/179), *Pseudomonas spp* in 3.4% (6/179) and *Enterococcus spp* in 2.8% (5/179). Other emerging bacteria with potential of causing wide ranges of infections were also observed. These included *Raoultella spp* in 2.2% (4/179), *Kluyvera ascorbata* in 1.7% (3/179), *Morganella morganii* in 0.6% (1/179) and Proteus vulgaris in 0.6% (1/179).

Resistance rates observed were 2.3% for carbapenems (meropenem and imipenem) (4/171 for each), 10.5% (18/171) for amikacin, 21.6% (36/167) for fosfomycin, 36.0% (58/161) for chloramphenicol, 50.1% (84/165) for nitrofurantoin, 53.9% (69/128) for amoxicillin-clavulanate and 54.0% (95/176) for ciprofloxacin.

Healthcare-associated infections constitute a relatively high burden in the surgical ward of QECH. Reinforcing infection prevention and control measures will help in reducing their prevalence and hence reduce antimicrobial resistance. Empiric antibiotic therapy for UTI in the Surgery Department should be revised based on the antimicrobial resistance patterns of isolated bacteria.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIC	Akaike's Information Criterion
AMR	Antimicrobial resistance
API	Analytical profile index
ABUTI	Asymptomatic bacteremic urinary tract infection
CAUTI	Catheter-associated urinary tract infection
CLABSI	Central line-associated bloodstream infection
CDC	Centre for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
COM	College of Medicine
COMREC	College of Medicine Research and Ethics Committee
CFU	Colony forming units
CLED	Cystine-Lactose-Electrolyte Deficient
ECDC	European Centre for Disease Prevention and Control
ESBL	Extended-spectrum beta-lactamase
ExPEC	Extra-intestinal pathogenic *E. coli*
GCLP	Good clinical and laboratory practice
GCP	Good clinical practice
HAI	Healthcare-associated infection
IUC	Indwelling urinary catheter

IPC	Infection prevention and control
IDSA	Infectious Diseases Society of America
ICU	Intensive care unit
IQR	Interquartile range
LOS	Length of stay
LMICs	Low- and middle-income countries
MGE	Mobile genetic element
MDR	Multidrug resistant
NOS	Newcastle-Ottawa Scale
OR	Odds ratio
PAIs	Pathogenicity islands
PPS	Point prevalence survey
PCR	Polymerization chain reaction
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
QECH	Queen Elizabeth Central Hospital
RCS	Restricted cubic splines
SSI	Surgical site infection
SUTI	Symptomatic bacteremic urinary tract infection
UTI	Urinary tract infection
UPEC	Uropathogenic *E. coli*

CHAPTER ONE: INTRODUCTION

1.1 Background

Healthcare-associated infections (HAI), also known as hospital-acquired infections or nosocomial infections, are systemic or localized conditions derived from a microbe or toxin acquired within the hospital and consecutive to patient' management. HAI include all infections acquired within the hospital or other healthcare facilities (including and also occupational infections among staff of the facility) which can appear after the patient discharge. The HAI onsets are usually after 48 hours of admission and were not in incubation period at the time of admission [1–3].

Currently, the HAI are one of the major causes of high morbidity and mortality in patients in high income settings, leading directly or indirectly to a huge increase in the costs of hospital care [4,5]. In developed countries, the prevalence of HAI is 5% to 10% in hospitalized patients in regular wards, while it can reach 50% in intensive care units [6]. In developing countries, the problem is not well described and only few studies have been done in sub Saharan Africa. A meta-analysis of 220 studies from developing countries has shown only 14 studies from Africa and the incidence of HAI was 7.4% [7]. In African countries like Malawi, this problem may be enhanced by the insufficient infection prevention and control measures. There may be inadequate and poor hygiene, resource and structural constraints, deficient surveillance data and lack of awareness regarding nosocomial infections in this country.

Urinary tract infections (UTI) represent the most frequent HAI in developed countries. This situation is similar in developing countries. In addition to that, invasive medical

procedures such as urethral catheterisation play a major role in acquiring other type of HAI like sepsis [8,9]. Nosocomial UTIs account for up to 40% of all HAI in developing countries. The associated morbidity and mortality are a major drain on hospital resources [4,5].

The organisms responsible for HA-UTI usually originate from patients' endogenous intestinal flora, but occasionally from a moist site in the hospital environment [10]. While *S. aureus* and *E. coli* and other *Enterobacteriaceae* are the most frequent bacteria for surgical nosocomial infections, it is the same microbial gallery which is responsible for UTI but with the preponderance of *E. coli* in developing countries [7,11,12].

Nosocomial pathogens causing UTIs tend to have a higher antibiotic resistance than community-acquired UTIs [10]. Due to their nosocomial transmission, these bacteria may have acquired high virulence and are multidrug resistant [13,14]. This may have an impact on the clinical features of bacterial infections as well as their treatment outcome. To date in Malawi, there is a paucity of data analysing nosocomial infection in surgical wards targeting UTI.

Several studies have shown that patients who are admitted into surgical wards of hospitals are usually at a high risk of nosocomial UTI. Letica-Kriegel et al., in a large cross-sectional study of six hospitals showed that indwelling urinary catheter play a major role in acquiring nosocomial UTI. In addition to that, they showed that female sex, paraplegia and cerebrovascular diseases are risk factors for UTI [15]. Storme et al., demonstrated that factors such as urinary incontinence, anterior vaginal wall prolapse, increased post void residual urine volume, and intermittent or permanent urinary catheterization

predispose to nosocomial UTIs [16]. Several features of pregnancy also act as predisposing factors for UTI, as well immune-compromised states (HIV, malnutrition, corticosteroid therapy, chemotherapy, etc.) [16].

1.2 Problem statement

Healthcare-associated infections (HAI) constitute a worldwide public health concern affecting hospitalised patients, individual hospitals and the health systems [4,17,18]. They increase the healthcare costs by prolonging the hospital stay and demanding the use of expensive broad-spectrum antibiotics and they are associated with high morbidity and mortality [19–22].

Increasing antimicrobial resistance is associated with inappropriate antimicrobial consumption, suggesting that restricting inappropriate antimicrobial prescription may curb the development of antimicrobial resistance [20,23]. The use of broad-spectrum antibiotics in the treatment of HAI, before the results of culture, may result in the widespread occurrence of multidrug-resistant (MDR) pathogens in hospital settings and dissemination of re/emerging infections to healthcare providers and the community. This makes the treatment of HAI more difficult and costly [19,20,24]. In Europe, about one-third of pathogens isolated in HAI are resistant to antimicrobials [25–29]. Meanwhile, higher rates of antimicrobial resistance (10 to 100%) are reported among isolates from HAI in Africa [19,30]

Data from developing countries, like Malawi, on HAI are limited and pose challenges on assessing the impact of control interventions and surveillance strategies [4,5,20,24]. The overall prevalence of HAI in Africa reach 15.5% in patients admitted in general wards

and may reach 50% in patients admitted in intensive care units (ICUs) [4,5,24,31]. High rates of HAI in Africa are due to the inexistence and/or non-adherence to infection prevention and control (IPC) policies and guidelines, exacerbated by the lack of awareness of the problem, lack of personnel, lack of antimicrobial policies resulting in the emergence of MDR pathogens, poor laboratory support, limited funding, and suboptimal adherence to safe practices by health workers and typically limited compulsion to report HAI [19,24,30,32,33].

Current evidence-based interventions can prevent about 50% of HAI [4], and surveillance of HAI is a major component of strategies to reduce HAI within hospitals [32]. Effective IPC programs require active surveillance that would generate data on prevalence and risk factors linked to HAI. Moreover, these data are useful in measuring the impact of IPC programs and are helpful while prioritizing further areas for interventions in the prevention and control of HAI [19,32,34].

The economic and public health burden of both community and hospital acquired UTIs are substantial and markedly affect the life quality of infected patients [35]. Uropathogenic *E. coli* (UPEC) are among the most common extra-intestinal pathogenic *E. coli* (ExPEC) encountered [36] and are amongst the most frequent bacteria causing urinary tract infections (UTIs) [14]. UPEC carry many virulence factors involved in the pathophysiology of UTIs such as invasion, colonization and mediation of host defences subversion [37].

Antimicrobial resistance (AMR) has an effect on both bacterial virulence and health [38,39]. The mobile genetic elements carried in UPEC may promote and contribute to the

spread and emergence of antimicrobial resistance [40–43]. Increasing AMR reported in *E. coli* worldwide harbour genes and plasmids which confer resistance to almost all antibiotics, with possibility of being transferred to other species or even other bacteria [41,43].

1.3 Aim

The aim of the study was to determine risk factors associated with HA-UTI and describe antibiotic resistance and virulence profiles of uropathogens in surgical wards in Malawi.

1.4 Specific objectives

a. To conduct a systematic review and meta-analysis on the antibiotic resistance and virulence factors of the uropathogenic *Escherichia coli* (Study I);

b. To conduct a point-prevalence survey of hospital-acquired infections in surgical wards of Queen Elizabeth Central Hospital (QECH) (Study II);

c. To determine the prevalence of catheter-associated urinary tract infection's risk factors among patients admitted in surgical wards at QECH (Study III);

d. To determine the antimicrobial susceptibility pattern of bacteria responsible for nosocomial urinary tract infections (Study III).

These objectives will be referred later in this thesis as study I to III.

1.5 Justification and relevance of the study

There is limited data regarding hospital acquired infections in Malawi. Since the burden of HAI is increasing in sub Saharan African countries like Malawi, data on HAI will be helpful in establishing infection control measures, and knowing current antimicrobial susceptibility pattern of bacteria responsible for nosocomial UTI. In addition, this study will contribute on the rational use of antibiotics and therefore prevent the emergence of multidrug resistant bacteria. The knowledge of the antimicrobial susceptibility pattern of causative bacteria will help in treating this condition efficiently; and will lead to a shortness of hospital stay, reducing the treatment cost, as well as the mortality rate. This study will also serve as a pilot study on HAI in Malawi and will be helpful for healthcare decision makers to implement measures for infection prevention and control in surgical wards. This study will decipher risk factors associated with UTI and these will help in implementing effective evidence-based related to indwelling urinary catheter.

CHAPTER TWO: LITERATURE REVIEW

2.1 Healthcare-associated infections

Healthcare-associated infections (HAI), also known as hospital-acquired infections are nosocomially acquired infections that are usually not evident or may be incubating when the patient is admitted. These infections are frequently acquired after being admitted to the hospital and manifest 48 hours later [44]. Hospitals have been concerned about HAI for decades. Several hospitals have implemented infection tracking and surveillance systems as well as robust preventative methods to lower the occurrence of HAI [45]. HAI have been related to multidrug-resistant (MDR) infections, and their impact is seen not only at the individual patient level, but also at the community level. In order to prevent and minimize HAI MDR infections, it is critical to identify patients with risk factors.

HAI results in prolonged hospital stays, increased antibiotic resistance in microbes, long-term incapacity, significant additional expenses for health systems, high expenditures for patients and their families, and avoidable deaths. Despite the fact that HAI is the most common adverse event in health care, the worldwide burden remains unclear due to the difficulties in acquiring reliable data: most countries lack HAI surveillance systems, and those with working surveillance systems struggle with the complexity and lack of universality of diagnostic criteria [7].

2.1.1 Types of healthcare-associated infections

To treat patients and help them recover, modern healthcare uses a variety of invasive devices and procedures. HAI have been linked to medical devices such as catheters and ventilators. These HAI include central line-associated bloodstream infections, catheter-

associated urinary tract infections, and ventilator-associated pneumonia. Infections may also occur at surgery sites, known as surgical site infections [44].

A central line-associated bloodstream infection (CLABSI) is a serious infection that occurs when germs (usually bacteria or viruses) enter the bloodstream through the central line. When inserting the line, healthcare providers must follow a specific process to ensure that the line remains sterile and that a CLABSI does not occur. In addition to properly inserting the central line, healthcare providers must follow strict infection control procedures every time the line is checked or the dressing is changed.

A urinary tract infection (UTI) is an infection that affects any region of the urinary system including the urethra, bladder, ureters, or kidney. The most prevalent type of HAI is UTIs. A urinary catheter, which is a tube put into the bladder through the urethra to drain urine, is associated with nearly 75% of UTIs acquired in the hospital. Urinary catheters are used by 15-25% of hospitalized patients during their stay. Prolonged usage of the urinary catheter is the most major risk factor for developing a catheter-associated UTI (CAUTI). As a result, catheters should only be used when absolutely necessary and should be removed as soon as possible. Community and hospital acquired UTIs significantly affect the life quality of infected patients [35].

A surgical site infection (SSI) is an infection that develops in the area of the body where the surgery was performed after it has been completed. Surgical site infections can be superficial infections that just affect the skin. Other surgery site infections can be more dangerous, affecting tissues underneath the skin, organs, or implanted materials. The Centre for Disease Control and Prevention (CDC) provides recommendations and tools

to the healthcare community to assist prevent SSIs, as well as information to help the general public understand these infections and take steps to protect their own health when possible.

A lung infection that develops in a person who is/was on a ventilator is known as ventilator-associated pneumonia. A ventilator is a machine that provides oxygen to a patient via a tube put in the patient's mouth or nose, or through a hole in the front of the neck. An infection may occur if microorganisms enter through the tube and get into the patient's respiratory system.

2.1.2 Risk factors

Anyone receiving medical care is at risk of contracting a HAI; however, some persons are more vulnerable than others, such as the following:

a. Very young people: premature babies and very sick children.
b. Very old people: the frail and the elderly.
c. People with certain medical conditions: such as diabetes.
d. People with weakened immune systems: from disease, or because they are getting treatments that weaken their immune system. Cancer treatments (like chemotherapy or radiation) or steroids are treatments that can weaken the immune system.

Other risk factors include:

e. Length of stay in a healthcare facility: a long hospital stay.
f. Surgery: long and complicated surgery.

g. Hand washing techniques: inadequate hand washing by hospital staff, visitors, and patients.

h. Antibiotics: overuse of antibiotics can lead to resistant bacteria, which means that antibiotics become less effective and do not work as well.

i. Equipment: medical equipment that enters the body can introduce bacteria and infection into the body. For example, urinary catheters, intravenous drips and infusions, respiratory equipment, and drain tubes.

j. Wounds: wounds, incisions (surgical cuts), burns, and skin ulcers are all prone to infection.

k. High-risk patient care areas: some patient care areas are more likely to have infections, such as hospital intensive care units.

2.2 Epidemiology

Healthcare-associated infections (HAI) constitute a worldwide public health concern affecting hospitalised patients, hospitals and the health systems [4,17,18]. They increase healthcare costs by prolonging the hospital stay and requiring the use of expensive broad-spectrum antibiotics and they are associated with high morbidity and mortality [19–22]. In a context where the microbiology service is limited, HAI are diagnosed clinically and treated empirically. This makes the treatment of HAI less effective and more costly [19,20,24]. In Europe, about 1% to 35% of pathogens isolated in HAI are resistant to antimicrobials [25–29]. Meanwhile, higher rates of AMR (10% to 100%) are reported among isolates from HAI in Africa [19,30].

Data on HAI from low and middle income countries (LMICs), like Malawi, are limited, posing challenges on assessing the impact of control interventions and surveillance

strategies [4,5,20,24]. Where studied, prevalence of HAI in Africa has been reported in up to 15.5% of patients admitted to general wards and 50% of patients admitted to intensive care units (ICUs) [4,5,24,31]. High rates of HAI in Africa are due to the paucity of infection prevention and control (IPC) policies and guidelines, exacerbated by the lack of personnel, lack of antimicrobial policies resulting in the emergence of MDR pathogens, poor laboratory support, limited funding, and suboptimal adherence to safe practices by health workers and typically limited compulsion to report HAI [19,24,30,32,33]. In addition to these, the built environment of hospitals (the structure of the hospitals, including the fixed components within the facility with which health care workers, patients, and families touch or interact as a part of the health care process) play a role in risk of acquiring HAI [46].

It has been estimated that evidence-based interventions can prevent about 50% of HAI [4], and clinical and microbiological surveillance of HAI is a major component of assessing strategies to reduce HAI within hospitals [32]. Moreover, these data are useful in prioritizing further areas for interventions in the prevention and control of HAI [19,32,34].

2.3 Pathogenic organisms causing UTI

Urinary tract infections (UTI) are among the most common hospital-acquired infections (HAI) [47]. Responsible pathogens causing UTI usually originate from patients' endogenous intestinal flora, or during invasive procedures, and occasionally from a moist site in the hospital environment [10]. The most commonly isolated microorganisms in all age groups are gram-negative enteric bacteria such as *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterococcus* or *Enterobacter* species [48]. Globally the most common cause of

UTI is *Escherichia coli* [14], a ubiquitous gram-negative pathogen and member of the family *Enterobacteriaceae*. Uropathogenic *E. coli* (UPEC) are among the most common extra-intestinal pathogenic *E. coli* (ExPEC) encountered [36].

2.4 Virulence factors of uropathogenic E. coli

E. coli is a commensal inhabitant of human and animal gastrointestinal tract and maintains the stability and homeostasis of luminal microbial flora by the symbiotic interplay with its hosts [49]. While confined in the intestinal lumen, this bacterium remains harmless in healthy individuals but some strains may cause diarrhoea in some circumstances. Meanwhile, several *E. coli* lineages have acquired specific virulence characteristics, giving them the capacity to thrive in specific niches and cause disease generally grouped in three clinical syndromes: enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis [50]. These virulence characteristics are often encoded on genetic elements that can be mobilized to establish new combinations of virulence factors in different strains, or on genetic elements that have once been mobile but now become fixed in the chromosome [50]. UPEC has large and small pathogenicity islands (PAIs), which are integrated mobile elements that encode for the key virulence factors. These allow UPEC to infect an immunocompetent host, as they encode for factors enabling it to colonize the periurethral area and ascend the urethra to the bladder [50].

Key virulence factors involved in the pathophysiology of UTIs function in invasion, colonization and mediation of host defences subversion [37]. PAIs furthermore often carry cryptic or functional genes that encode mobility factors, such as integrases, transposases and insertion sequence elements [50], which are traces from their mobile

history and may promote and contribute to the spread and emergence of antimicrobial resistance [40–43].

2.5 Resistance of uropathogens to antimicrobials

Antimicrobial resistance (AMR) has increasingly been reported in bacteria causing urinary tract infections (UTI) during the last few decades and has become a major public health concern [51].

E. coli typically acquires AMR genes through mobile genetic elements (MGE), such as plasmids, insertion sequences, transposons, and gene cassettes/integrons [52]. A large number of resistance-encoding mobile elements, in particular plasmids, are shared between different members of the *Enterobacteriaceae* and thus further promote the spread of resistance genes [53]. MGE can also encode for virulence factors, and there may be interplay between virulence and antimicrobial resistance [52].

It has been reported that *E. coli* is expected to cause loss of lives of more than three million people each year by 2050 following the increase in multi-drug resistance. A particular focus is placed to track carbapenem-resistant strains which are spreading world-wide and only leave few last-line treatment options like colistin or tigecycline, which are known for severe side-effects and not applicable for all types of bacterial infections due to reduced tissue permeation, respectively; and resistance mechanisms against both of these are increasingly observed [54].

2.6 Relationship between antimicrobial resistance and virulence factors in UPEC

This section, briefly reviews the possible relation between AMR and virulence factors in UPEC on selected examples, focusing on resistance to quinolones and beta-lactams. It discusses how harbouring virulence factors may increase or decrease the possibility of UPEC to develop resistance to antibiotics, although only aggregate data are available and trends in AMR and virulence factor carriage are not directly related in this analysis.

Previous studies on UPEC reported that quinolone-resistant isolates carried virulence factor genes related to their ability to invade the urinary tract [55]. The relevant virulence factors, like haemolysin, aerobactin, cytotoxic necrotizing factor-1 (*cnf*-1) and *sat* are chromosomally encoded in the PAIs, which can be deleted from the chromosome spontaneously and easily [56,57]. Quinolones can act by increasing the deletion and transposition of DNA regions during the development of quinolone-resistance facilitated by an exposure to quinolones [58]. While PAIs share some characteristics with bacteriophages, it has been proven that pro-phages hidden within chromosomal DNA are excised by the activation of SOS [59], a DNA repair mechanism. Quinolones likely contribute to the partial or total excision of PAIs in a SOS-dependent way because the antimicrobial agents activate the SOS system [60]. Hence, this may induce the loss of virulence factors of quinolone-resistant *E. coli* that are less able to cause invasive UTIs as this phenomenon may result in phenotypic changes in bacteria. Nevertheless, the fact that quinolone-resistance impairs the ability of UPEC to invade local tissue of the kidney and prostate does not disrupt a strain's capacity to cause bacteraemia (urosepsis) once local invasion has taken place [55].

In *E. coli*, the majority of virulence associated plasmids belong to the F incompatibility group and are often key determinants of antimicrobial resistance [61]. It is conceivable that genetic determinants of virulence may be co-mobilized under antimicrobial selective pressure if they are located on the same genetic platform as antimicrobial resistance genes (plasmids, transposons, integrons) [62]. The relationship between resistance and virulence remains uncertain and depends on the interaction between the strain's phylogenetic group and the type of resistance determinant [63]. In *Enterobactericeae*, the IncF plasmid family is very widespread and can encode aerobactin as well as other factors of putative virulence such as the *traT* virulence protein, responsible for serum resistance in *E. coli*. Extended-spectrum beta-lactamase (ESBL) producing *E. coli* are emerging and are posing challenges to the clinicians on therapeutic choices; and F-plasmids often encode for ESBL genes from the CTX-M, TEM or SHV groups, as well as genes conferring resistance to other antibiotic groups [64–67]. These few examples demonstrate how antimicrobial pressure can select for plasmids carrying virulence and resistance determinants, and hence allow virulent traits to be selected for by antimicrobial use in a bacterial population.

Some specific lineages within the *E. coli* species, such as the phylogroup B2, show high frequency of virulence factors [68–70]. Independent predictors for pathogenicity have been identified to be alpha-hemolysin, yersiniabactin receptor (*fyuA*), serum resistance-associated outer membrane protein (*traT*), and aerobactin receptor type *iutA*. In strains producing the $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$ group ESBL enzymes, respectively, *iutA* and *traT* were significantly more common among these virulence factors [71]. Similar results, where *iut* and *traT* are more prevalent, have been reported in *E. coli* CTX-M ESBL group

from UTIs [72]. The summary of UPEC virulence factors mechanisms is shown in the table below.

Table 2.1.1: UPEC virulence factors mechanisms of action

Virulence factors groups	Examples of genes	Mechanisms
Adhesins	*afa, CSH, fimH, fimP, kpsmtII, pap, sfa, traT*	UPEC adhesins can contribute to virulence in different ways: (i) directly triggering host and bacterial cell signalling pathways, (ii) facilitating the delivery of other bacterial products to host tissues, and (iii) promoting bacterial invasion [36]. Adhesins help in the adhesion of organism to epithelial cell surface, thereby it escapes from flushing action during micturition [50]. Fimbriae is responsible for adhesion, colonization, invasion of host epithelium and makes UPEC to escape from the innate immune system by internalization process within urothelial cells which is mediated by the transduction cascades [37].
Toxins	*Cnf1, hlyA, saT, vaT*	Toxins like haemolysin and Cytotoxic Necrotising Factor (CNF) act by their cytotoxicity and invasiveness. Haemolysin production could inhibit the cytokine production of host cells and promote the cytotoxicity. It causes lysis of the erythrocytes which release nutrients and other vitamins available for the bacteria. At the same time it releases inflammatory mediators and enzymes which are cytotoxic to renal proximal tubular epithelial cells, erythrocytes and leukocytes, thereby causing renal epithelial damage [36]. CNF interferes with the phagocytosis of *E. coli* by the WBCs and thus it leads to exfoliation and apoptosis of bladder epithelial cells. It further enhances the easy access of bacteria into the underlying tissue. These toxins can alter signalling pathways, provoke the inflammatory response and prevent the apoptosis thereby they cause the UPEC population to expand [51].
Siderophores	*aer, chuA, fyuA, iuD, iutA, yfcv*	Production of siderophores by *E. coli* which takes up iron from the host and helps in colonization and survival of pathogen [37,51]. They contribute to the process of nutritional passivation of metal ions, in which UPEC access these vital nutrients while simultaneously protecting themselves from their toxic potential [73]
Immune suppressors	*PAI, shiA, sisA, sisB, sivH, Eco274*	UPEC induces a non-sterilizing adaptive immune response in the bladder. Its causes long-lasting changes in the bladder urothelium, conferring resistance or increased susceptibility to subsequent infections depending on the outcomes of the first infection [74]. The invasins play a key role in suppressing the host immune response during the initial stages of infection [75].

CHAPTER THREE : MATERIAL AND METHODS

3.1 Study I

3.1.1 Study design

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [76] were used in conducting this systematic review. The protocol of this review was registered in the Research Registry (ref 5874).

3.1.2 Search strategy

The electronic bibliographic databases PubMed/MEDLINE and ScienceDirect were searched in all fields with the search terms combined as follow: Virulence factors OR virulence AND factors OR virulence factors AND associated AND anti-infective agents OR anti-infective agents OR anti-infective AND agents OR anti-infective agents OR antimicrobial AND resistance AND uropathogenic *Escherichia coli* OR uropathogenic AND *Escherichia* AND *coli* OR uropathogenic *Escherichia coli* AND UPEC.

A twenty-year time period, between 2000 and 2019, was considered for the search. This time limit was based on possible changes in the virulence, microbiology, epidemiology and antimicrobial susceptibility patterns of uropathogenic *E. coli* [77]. The number of records retrieved for each database searched was recorded. Reference lists of identified studies were checked manually to supplement the electronic search. Retrieved studies were exported into Mendeley Desktop version 1.19.4 and screened against inclusion and exclusion criteria.

3.1.3 Inclusion and exclusion criteria

Observational (cross sectional, prospective and retrospective cohort, and case-control) studies reporting the virulence and antimicrobial susceptibility patterns of uropathogenic *E. coli* isolated from human samples from patients of any age and region were included in this review. Studies published before 2000 and after 2019, and those reporting results from animal samples were excluded. Grey literature was not considered. Studies published in any other language than English and those with non-accessibility to full-texts were excluded. Only studies reporting their microbiologically confirmed UTI ($\geq 10^5$CFU/ml) using the Centre of Disease Control and Prevention's definition were included in this review [78]. This review included both inpatients and outpatients with UTIs. Hence, data from a study which used both settings were considered as two separate studies and each was counted as a single study.

3.1.4 Study selection

The identified titles and abstracts of all the studies retrieved in the electronic databases and searched manually were screened for their appropriateness and relevance to the main aim of the systematic review. Studies that were irrelevant were excluded at this stage. Full texts of potentially relevant studies were downloaded and added to a curated Mendeley library and were assessed for inclusion and exclusion criteria of this systematic review. Quality and risk bias assessment was done for included studies containing relevant data for the systematic review and meta-analysis.

I (Gabriel K. Bunduki) performed the selection process and other stages of this review. Ten percent of identified studies were screened independently for inclusion and exclusion criteria by my primary supervisor at each stage of the review. The discrepancies in either

the decision on inclusion or exclusion of studies, quality assessment or on data extraction were discussed between my supervisor and I to make the consensus for the final decision.

3.1.5 Data extraction

Data extraction was independently done by GKB and JM and was compared for matching. For variables with missing information or with disagreement between the two authors, a consensus between the authors was made for the final decision.

An Excel 2010 spreadsheet was used for data extraction and contained the following data for studies that met inclusion criteria: first author, year of publication, country/place of study, study population/sample size, patient types (inpatients or outpatients), prevalence of antimicrobial resistance of different antibiotics tested, method used for detecting virulence factors, and prevalence of virulence factors.

3.1.6 Quality assessment and risk of bias in individual studies

The Newcastle-Ottawa Scale (NOS) adapted for cross-sectional studies was used for assessing the risk of bias of included studies (Appendix 1). This scale was adapted from the NOS quality assessment scale for cohort studies. The assessment was in the area of selection (maximum of 3 points), comparability (maximum of 2 points) and outcome (maximum of 3 points). This was done by GKB and JM. Studies were classified into 4 categories: very good (9-10 points), good (7-8 points), satisfactory (5-6 points) and unsatisfactory (0-4 points).

3.1.7 Statistical analysis

We used *metaprop* and *metaprop_one* commands in Stata 16 for Windows to conduct the meta-analysis. Prevalence of antimicrobial resistance and virulence factors of UPEC were estimated using random-effects meta-analysis model. The 95% Wald confidence intervals were computed using the score statistic and the exact binomial method by incorporating the Freeman-Tukey double arcsine transformation of proportions for avoiding exclusion of studies with proportion equal to 0 or 1 from the calculation of the estimate [79]. The effect size of the prevalence was considered statistically significant when p-value was < 0.05. The proportions with 95% Wald confidence intervals were generated. I-square (I^2) statistic test was used to evaluate the proportion of statistical heterogeneity and the Cochran's Q test was used to explain the degree of heterogeneity. The funnel plot publication bias was not assessed as it is not relevant for the prevalence studies [80], however, the Egger's linear regression test was used.

3.2 Study II

3.2.1 Study setting

The study was conducted at QECH, a large urban government central hospital in Blantyre, which has a capacity of about 1,300 beds but frequently operates above its capacity. Its surgery department provides care in general surgery, orthopaedics, neurosurgery, and paediatric surgery. The 4 surgical wards at QECH, with about 190 inpatient beds were surveyed in this study. The accident and emergency wards and ear, nose and throat department were excluded as were the wards under the Mercy James Centre (MJC) for paediatric surgery and intensive care.

3.2.2 Study design

A single-day cross-sectional point prevalence survey (PPS) was conducted in different wards of the surgery department of QECH on 9th June 2020, using an adapted version of the European Centre for Disease Prevention and Control (ECDC) tool for PPS on HAI and antimicrobial use, protocol version 5.3 [2].

3.2.3 Inclusion and exclusion criteria

Patients admitted to surgical wards before or at 8 a.m. on the day of the survey and not discharged at the time of the survey were included in the study. Patients who were transferred in/out after 8 a.m. from/to another unit were excluded. In addition, all day cases patients (patients undergoing same-day treatment or surgery, patients seen at the outpatient department, patients in an emergency room and dialysis patients) were excluded.

3.2.4 Data collection

Data were collected by a clinical microbiologist, and by nurses trained in the use of the data collection tool and the case report form. Before data collection, a competency-based evaluation was undertaken. For all eligible patients with or without a HAI, a case report form was used for collecting demographic data, clinical history (length of hospital stay (LOS), surgical procedure, indwelling devices, and comorbidities), information on antimicrobial use, data on HAI if present, and results of routine microbiological tests performed if available. The McCabe score was calculated. The McCabe score categorizes the severity of underlying medical conditions into non-fatal disease (expected survival of at least five years), ultimately fatal disease (expected survival between one and five years), rapidly fatal disease (expected death within one year) and unknown [2]. In HAI PPS, the McCabe score is used as a subjective score of underlying illness severity. It is an important tool for risk stratification in infection prevention and control [81].

3.2.5 Operational definitions

The ECDC criteria and definitions for HAI [2] were used and these are based on the presence of signs and symptoms of a particular HAI on the day of the survey, with or without microbiological results. Briefly, an infection was considered to be HAI when the onset of the signs and symptoms occurred >48 hours after the current admission or became apparent within 48 hours of admission, but the patient had been discharged from an acute care hospital <48 hours before the current admission [2]. Infections were categorized into surgical site infection, bone/joint infection, urinary tract infection, sepsis or bloodstream infection, pneumonia, and other infections including skin and soft tissue infection, nervous system infections, and gastrointestinal tract infection [2,20]. For surgical site infections (SSIs), the definition included infections that occurred up to 30

days after a surgical procedure and affected either the incision or deep tissue at the surgical site, or infections related to an implant that occurred within one year. Moreover, device-associated HAI was recorded for urinary tract infections (urinary catheter in place within seven days preceding HAI onset), sepsis or bloodstream infections (vascular catheter in place within 48 hours before HAI onset) and pneumonia (intubation within 48 hours before HAI onset) [2].

3.2.6 Data analysis

Data were entered into MS Excel 2010, double-checked for coding errors, cleaned and exported into SPSS version 24 (IBM Corp., Armonk, NY, USA) and Stata 16 (StataCorp., USA) for analysis. The point prevalence of HAI was reported as the percentage of the patients with at least one clinically identified HAI divided by the total number of included patients. Prevalence rates were calculated with the exact binomial 95% confidence intervals (95% CI). Arithmetic means, median and interquartile range (IQR) were calculated to summarise continuously measured variables. Effect sizes of associations of risk factors with the outcome of HAI were reported using odds ratios (ORs). For categorical and binary variables, Fisher's exact test was used to test the null hypothesis of no association with HAI. Statistical significance was determined by p-values <0.05. Due to the small number of HAI in our data, no multivariable regression model was fitted to the data as this would likely overfit and result in overinterpretation of the results and unstable coefficient estimates.

To fit a logistic regression model for HAI against LOS using restricted cubic splines (RCS), we used R v4.0.2 [82] and the rms package v6.0.0. [83]. We used Akaike's Information Criterion (AIC) to select the number of knots, with a model with 3 knots

having the lowest AIC and having therefore been selected. To test the null hypothesis of no association between HAI and LOS we performed a likelihood ratio test, comparing the RCS model to an intercept-only model.

3.3 Study III

3.3.1 Study setting

The study setting of this study was identical to the one in the study II described above (see study II).

3.3.2 Study design

These were prospective cross-sectional study that was undertaken in different wards of surgery department of QECH from August to October 2020.

3.3.3 Inclusion and exclusion criteria

All patients who were admitted in surgical wards for more than 48 hours, with suspicion of UTI and with a positive dipstick test were included in the study. Patients meeting the following criteria were excluded:

a. Patients admitted with UTI signs/symptoms, and/or with a positive dipstick test;
b. Patients with UTI onset before 48h of admission;
c. Patient with history of recurrent UTI;
d. Patient with dipstick not suggestive of UTI.

3.3.4 Study population and sample size estimation

The study population was constituted by all patients admitted in surgical wards of the surgery department at QECH during the study period. The sample size estimation (N) was done using the Fischer's formula as stated below. The prevalence (p) of hospital-acquired UTI was assumed to be 25% at a margin error (d) of 5%, and a standard normal deviation of 1.96 with a 95% confidence interval.

$$N = \frac{z^2 \times p \times (1-p)}{d^2}$$

After calculation, the estimated minimum sample sized was of 288 patients. After added a 10% of non-respondents was added, hence the estimated minimum sample size was of 317 patients. A total of 337 patients who met the inclusion criteria were recruited.

3.3.5 Participant selection and enrolment procedures

Daily review of admitted patients was done from Monday to Friday to identify patients suspected with HA-UTI. Urine dipstick test was done for all eligible patients. All patients with negative results on dipstick testing were retested each 48 hours. Clinical features and urine samples were collected and sent to COM microbiology diagnostic laboratory for microbial culture and susceptibility testing.

3.3.6 Operational definitions

Descriptions for healthcare-associated infections have been adapted from those of the ECDC [2].

The primary outcome was hospital-acquired UTI, described as a UTI that occurs after 48 hours of hospitalization, unless the patient was transferred from another hospital and the total hospital stay exceeds 48 hours. Clinical and laboratory data helped to exclude community-acquired UTI.

The secondary outcome was the catheter-associated urinary tract infection (CAUTI). CAUTI was defined as a UTI where an indwelling urinary catheter was in place for at least 7 calendar days on the date of event, with day of device placement being day 1, and an indwelling urinary catheter was in place on the date of event or the day before. If an indwelling urinary catheter was in place for more than 7 calendar days and then removed, the date of event for the UTI must be the day of discontinuation or the next day for the UTI to be catheter-associated [2].

In this study, we distinguished asymptomatic bacteriuria UTI (ABUTI) and symptomatic bacteriuria UTI (SUTI) as defined by the Infectious Diseases Society of America (IDSA) guidelines [84]. SUTI was considered as a culture growth of $\geq 10^3$ colony forming units (CFU)/mL of uropathogenic bacteria in the presence of symptoms or signs compatible with UTI without other identifiable source in a patient with indwelling urethral, indwelling suprapubic, or intermittent catheterization. Compatible symptoms included fever, suprapubic or costovertebral angle tenderness, and otherwise unexplained systemic symptoms such as altered mental status, hypotension, or evidence of a systemic inflammatory response syndrome. However, ABUTI was considered for culture growth of $\geq 10^5$ CFU/mL of one bacterial species in a patient without signs or symptoms compatible with UTI.

3.3.7 Data capture and storage

Data was collected using KoboCollect software (version 1.27.3). Completed KoboCollect forms were pushed daily to a dedicated secured SQL database. All data on the study database were stored securely with access restricted to the study PI and the database administrators in the College of Medicine (COM) data department. Results of laboratory investigations in the microbiology diagnostic laboratory of COM were stored in a secured Excel form, anonymised and linked only to the participant unique study ID number.

3.3.8 Specimen collection and processing

A sterile clear container with cap screw was used for taking a clean-catch midstream or catheter urine sample from the recruited patients. For the patients' comfortability during the sampling process, they were informed on how the process will be done. For women, they were asked to clean the region across the urethra with a cleaning wipe, by spreading the labia of the external genitals and cleaning from front to back (towards the anus). The cleaning hand was then used to keep the spread while the container was held by the other hand to collect the sample. For men, the tip of the penis was wiped with a cleaning pad preceding to collection.

Regarding the catheter specimen sampling, urine was obtained using an aseptic technique. The closed drainage system was not interrupted during urine collection. A sterile closed urinary drainage system was maintained. The catheter port was prepared by cleaning with 70% alcohol; and allowed to air dry. The tube was clamped below the urine sample port on drainage tubing; a needle and syringe was inserted into the port at a 45° angle. The amount of urine was aspirate and then after the syringe/needle was removed. The urine

was immediately transferred into a sterile specimen container. The syringe/needle was disposed appropriately and the catheter tubing was then unclamped.

A dipstick test was done. Samples with dipstick test suggestive of UTI were sent to college of Medicine diagnostic microbiology laboratory for subsequent processes within an hour of collection. Urine samples were processed for culture according to good laboratory practice guidelines and following standard microbiological methods. Briefly, the sample was inoculated on UTI ChromAgar, MacConkey agar, Cystine-Lactose-Electrolyte Deficient (CLED) agar and blood agar using sterile and calibrated disposable inoculating loops (1 μL). Inoculated plates were incubated overnight at 35-37°C. The agar plates were observed for growth of microorganism. A colony count was done and the number of bacterial colonies was multiplied by 1000 for the estimation of bacterial load/mL of the urine sample. A sample with $\geq 10^5$ CFU/mL of pure culture or predominance of one organism was considered as positive. If the colony count was $\leq 10^2$ CFU/mL, the culture was considered as negative. Meanwhile, when the colony count was in between 10^3 and 10^4 CFU/mL, the culture was considered positive if clinical features were suggestive of UTI; otherwise it was considered as negative. Nevertheless, if cultures grew a non-uropathogen or if ≥2 organisms are isolated in the absence of a clear predominance of one organism, the culture was considered contaminated. Cultures with significant growth of more than one organism were reported as "mixed growth of n types of organisms" (polymicrobial infection) [85].

3.3.9 Bacterial identification and antimicrobial susceptibility testing

Bacteria were identified by their colonial morphology and colour produced on the chromogenic agar (UTI ChromAgar). Gram-positive bacteria were identified using

standard biochemical tests (catalase, coagulase, bile esculin, hippurate hydrolysis, bacitracin susceptibility test). Meanwhile, gram-negative bacteria (Enterobacteriaceae) were identified using the API-20E (Biomeriux, France). All the enterobacteriaceae were put in Microbank vials (Pro-Lab Diagnostics, USA) and stored at 20°C for future processing.

Antimicrobial susceptibility testing was done using the Kirby Bauer disc diffusion method and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [86]. Briefly, standard inoculum adjusted to 0.5 McFarland was swabbed on Mueller Hinton agar and was allowed to soak for 2 to 5 minutes. Antibiotic disks were then placed on the surface of media and pressed gently using an antibiotic dispenser. The Mueller Hinton agar plates were then incubated at 37◦C for 24 hours. Inhibition zones were measured and interpreted as recommended by the CLSI. The choice of antibiotics tested was guided by the Essential drugs books protocols used in Malawi and their local availability. These included ampicillin (10 µg), augmentin (20/10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), cefepime (30 µg), meropenem (10 µg), imipenem (10 µg), cefuroxime (30 µg), cefpodoxime (10 µg), amikacin (30 µg), chloramphenicol (30 µg), doxycycline (30 µg), nitrofurantoin (300 µg), fosfomycin (200 µg), and cotrimoxazole (1.25 µg /23.75 µg). Standard strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were routinely used as control.

3.3.10 Statistical analysis

We used a backward stepwise procedure by selecting all variables associated with a p <0.20 at univariate and kept in the model all those significantly associated with the outcome (p <0.05).

In order to determine discrepancies between catheterisations that resulted in a CAUTI and those that did not, we conducted a time-independent analysis. A t-test measured the differences in continuous variables and a Chi-square test evaluated the differences in categorical variables. For characterising the event-free survival, the time-independent study was followed by survival analyses considering the number of days between indwelling urinary catheter insertion and either CAUTI occurrence or without CAUTI event. As per ECDC definition of CAUTI, it is unlikely for a CAUTI to exist before day 7, because CAUTI is characterized as an infection that occurs any time after the seventh day of catheter placement. The risk for CAUTI therefore starts, by definition, at day 7 [2]. Day 7 is considered the starting time in our survival evaluations.

We computed the Kaplan-Meier estimates for assessing the instantaneous hazard rates for developing a CAUTI and the time-dependent differences in sub-populations found to be significant in the time-independent analysis. The time-dependent differences in infection-free survival rates were reported in the full population, the population stratified by gender (male versus female), and stratified by the history of undergoing surgery in the past 30 days (those who underwent surgery versus those who did not). We conducted a Cox proportional hazard analysis after checking the proportional hazard assumption and finding that it is not violated. A set of confounders was adjusted for addressing potential biases. Univariate Cox models were computed to evaluate the impact of each variable's

effect on time-to-infection. Variables assed included gender, age, marital status, education level, pregnancy, diabetes, HIV, immunosuppressive/corticoid treatment, chronic renal diseases, spinal cord injury, prostatic disease, urethral stent, urinary reflux, renal transplantation, uterine prolapse, McCabe score, urinary catheter, waiting a surgical procedure, underwent surgery in the past 30 days, symptomatic and hospital length of stay. Variables considered statistically significant in the univariate model were included in the multivariate Cox proportional hazard model.

3.4 Ethical consideration

The study was approved by the College of Medicine Research and Ethics Committee (COMREC), protocol number P.10/19/2834 (Appendix 2). Informed written consent was obtained from patients, who were free to withdraw their consent at any time. The study was done in compliance of good clinical practice (GCP) and good clinical and laboratory practice (GCLP) guidelines.

CHAPTER FOUR: RESULTS

4.1 Results of Study I

4.1.1 Study selection

The literature search using PRISMA identified a total of 2,536 studies (2,504 studies through databases searching and 32 from scrutinising the included papers). After removing duplicates, 1,053 were screened for eligibility. After the screening of titles and abstracts, 1,006 studies were excluded. Full-texts of the remaining 47 studies were read and 35 more studies were excluded. At the end, 14 studies were included in the qualitative analysis and 13 in the meta-analysis (Fig. 4.1.1).

4.1.2 Study characteristics

Study characteristics of included studies are presented in Table 4.1. The 14 studies reported in this systematic review represent 8 countries, namely Iran (6 studies), China (2), India (1), Poland (1), Jordan (1), Mexico (1), Brazil (1) and Nigeria (1). The total sample size of UPEC isolates from the fourteen studies is 1,888 (range 32-227). Nine of the 14 studies report UPEC from inpatients [14,87–94] while 5 were from outpatients [87,93,95–97]. Among the 14 studies, 2 studies reported UPEC from in- and out-patients [87,93] and were therefore considered each as single study for each category of patients. Meanwhile, 2 other studies [92,96] reported UPEC in in- and out-patients but did not specify sample size in each category of patients. After consensus of authors, one was considered as reporting in-patients [92] and another one out-patients [96]. Among the 13 studies included in the meta-analysis, one reported in- and out-patient UPEC but did not distinguish the two categories while reporting the antimicrobial resistance rate [87], and was hence considered as a single study in the meta-analysis.

Of the 14 included studies, 9 studies used the polymerization chain reaction (PCR) as method for detecting virulence factors of UPEC [14,87–89,91,93,95–97], 3 studies used phenotypic methods [90,92,94], while 2 studies used both methods [91,95].

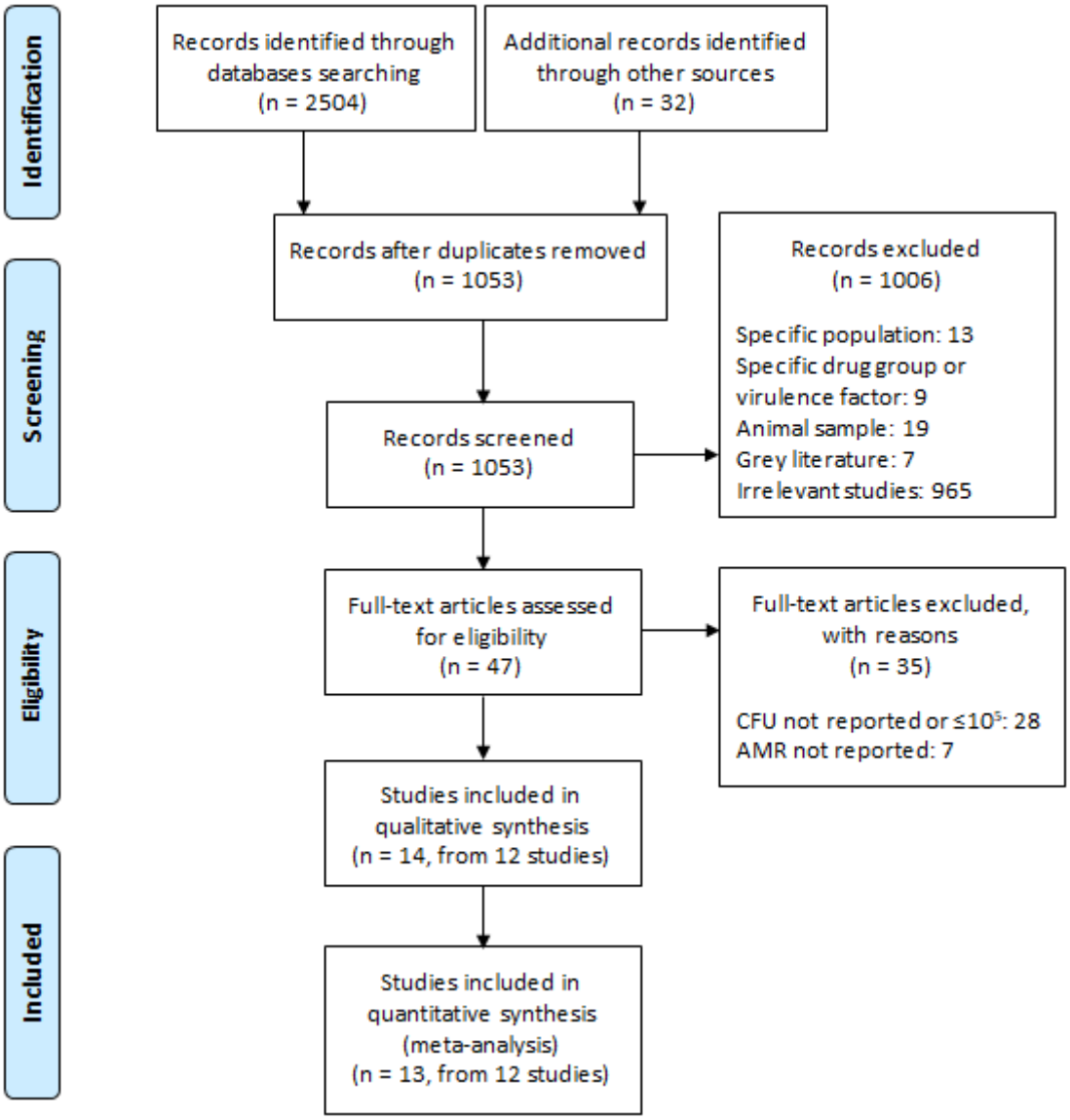


Figure 4.1.1: The PRISMA flowchart for literature search and study selection

Table 4.1.1: Characteristics of included studies after full assessment

Authors	Publication year	Country	Sample size	Type of patients	Method for VFs detection	NOS points
Ghazvini et al. (1) [87]	2019	Iran	168	Outpatients	PCR	8
Ghazvini et al. (2) [87]	2019	Iran	32	Inpatients	PCR	6
Jadhav et al. [90]	2011	India	150	Inpatients	Phenotypical	6
Kot et al. [91]	2016	Poland	173	Inpatients	Phenotypical, PCR	6
Malekzadegan et al. [89]	2018	Iran	126	Inpatients	PCR	8
Miranda-Estrada et al. [95]	2017	Mexico	107	Outpatients	Phenotypical, PCR	8
Neamati et al. [88]	2015	Iran	150	Inpatients	PCR	5
Oliveira et al. [96]	2011	Brazil	204	Outpatients	PCR	8
Olorunmola et al. [92]	2013	Nigeria	137	Inpatients	Phenotypical	5
Raeispour et al. [14]	2018	Iran	60	Inpatients	PCR	5
Shakhatreh et al. [97]	2019	Jordan	227	Outpatients	PCR	5
Tabasi et al. [94]	2015	Iran	156	Inpatients	Phenotypical	8
Wang et al. (1) [93]	2014	China	69	Inpatients	PCR	8
Wang et al. (2) [93]	2014	China	129	Outpatients	PCR	6

4.1.3 Quality assessment and bias assessment

Based on the quality assessment of studies using the NOS assessment, six studies scored 8 points [87,89,93–96], which could be regarded as good studies. While eight studies scored 5-6 points [14,87,88,90–93,97], and could be regarded as satisfactory studies. The detailed NOS assessment is found in the table 4.1.2. A bias assessment was done on the countries of origin of the included studies. The Egger's regression intercept was of -7.71, with a standard error of 2.23, 95% CI: -2.26 – 3.46, t-value of 6.0 and p=0.013. The fact that almost 50% of included studies in this meta-analysis came from a single country could have introduced a bias in the analysis.

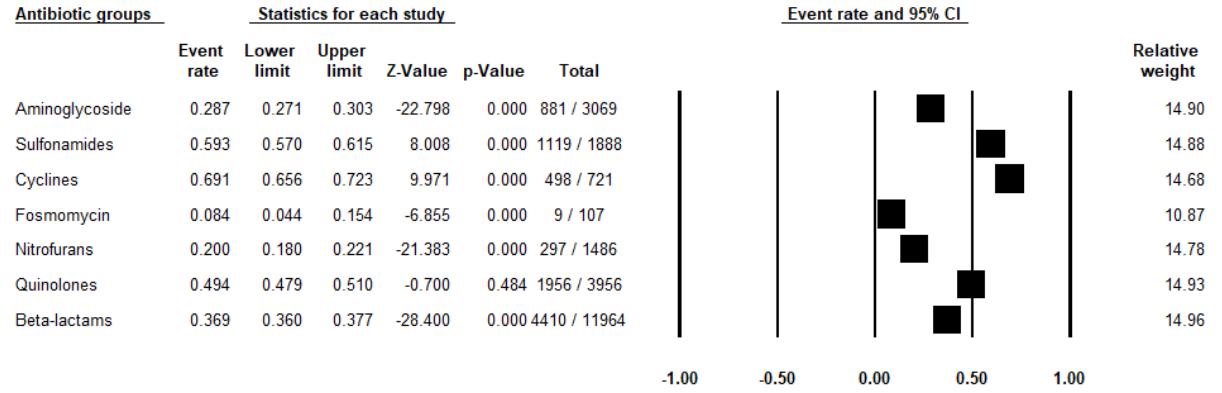
Table 4.1.2: Studies assessment using the Newcastle-Ottawa Scale adapted for assessment of cross-sectional studies

Studies	Representativeness	Sample size	Non-respondents	Ascertainment of the exposure	Comparability	Assessment of outcome	Statistical test	Total	Assessment
Ghazvini et al., 2019 (1)	1	0	0	2	2	2	1	8	Good
Ghazvini et al., 2019 (2)	1	0	0	2	0	2	1	6	Satisfactory
Jadhav et al., 2011	1	0	0	2	0	2	1	6	Satisfactory
Kot et al., 2016	1	0	0	2	0	2	1	6	Satisfactory
Malekzadegan et al., 2018	1	0	0	2	2	2	1	8	Good
Miranda-Estrada et al., 2017	1	0	0	2	2	2	1	8	Good
Neamati et al., 2015	1	0	0	2	0	2	0	5	Satisfactory
Oliveira et al., 2011	1	0	0	2	2	2	1	8	Good
Olorunmola et al., 2013	1	0	0	2	0	2	0	5	Satisfactory
Raeispour et al., 2018	1	0	0	2	0	2	0	5	Satisfactory
Shakhatreh et al., 2019	1	0	0	2	0	2	0	5	Satisfactory
Tabasi et al., 2015	1	0	0	2	2	2	1	8	Good
Wang et al., 2014 (1)	1	0	0	2	2	2	1	8	Good
Wang et al., 2014 (2)	1	0	0	0	2	2	1	6	Satisfactory

4.1.4 Antimicrobial resistance and virulence factors of UPEC

Of the 13 studies included in the meta-analysis, the pooled number of *E. coli* isolates was 1,888. Tables 2 and 3 both present the specific proportions of antimicrobial resistance and virulence factors with 95% exact confidence intervals for each antibiotic and virulence factor, and the I^2 and Q statistics which describe proportions of total variations due to inter-antibiotics/virulence factors heterogeneities. The heterogeneity tests for both antimicrobial resistance and virulence factors were significant (I^2 >75%). Highest antimicrobial resistance rates were observed among the antibiotic class of tetracyclines in 69.1% (498/721) followed by sulphonamides in 59.3% (1119/1888), quinolones in 49.4% (1956/3956), beta-lactams in 36.9% (4410/11964), aminoglycosides in 28.7% (881/3069), nitrofurans in 20.0% (297/1486) and fosfomycin in 8.4% (9/107). (Fig.4.1.2.A) Among beta-lactams, high resistance was observed in aminopenicillins in 74.3% (1157/1557), beta-lactam associated with inhibitors in 39.0% (604/1550), cephalosporins in 35.8% (2564/7155) and monobactam in 22.0% (78/354). However, carbapenems had the least rate of resistance, 0.5% (7/1348) (Fig.4.1.2.B). Among the cephalosporins, high rates of resistance were observed in the first generation cephalosporins in 38.8% (370/953) and third generation cephalosporins in 37.0% (1421/3838) (Fig.4.1.2.C). While taken individually, the highest resistance was observed in the following antibiotics: ampicillin 75.0% (835/1114, 95% CI: 0.72-0.77), amoxicillin 72.7% (322/443, 95% CI: 0.68-0.77), tetracycline 69.1% (498/721, 95% CI: 0.66-0.72), cotrimoxazole 59.3% (1119/1888, 95% CI: 0.57-0.61), nalidixic acid 59.0% (777/1317, 95% 0.56-0.62), cefpodoxime 57.8% (166/287, 95% CI: 0.52-0.63), cephalexin 56.6% (146/258, 95% CI: 0.50-0.63), and cefuroxime 55.2% (389/705, 95% CI: 0.51-0.59). Meanwhile, virtually almost all isolates were susceptible to the carbapenems with the following resistance rates: ertapenem in 0.4% (1/227, 95% CI: 0.00-0.03), imipenem 0.7% (5/567, 95% CI: 0.00-0.02), and meropenem in 0.3% (1/354, 95% CI: 0.00-0.02) (Table 4.1.3).

A



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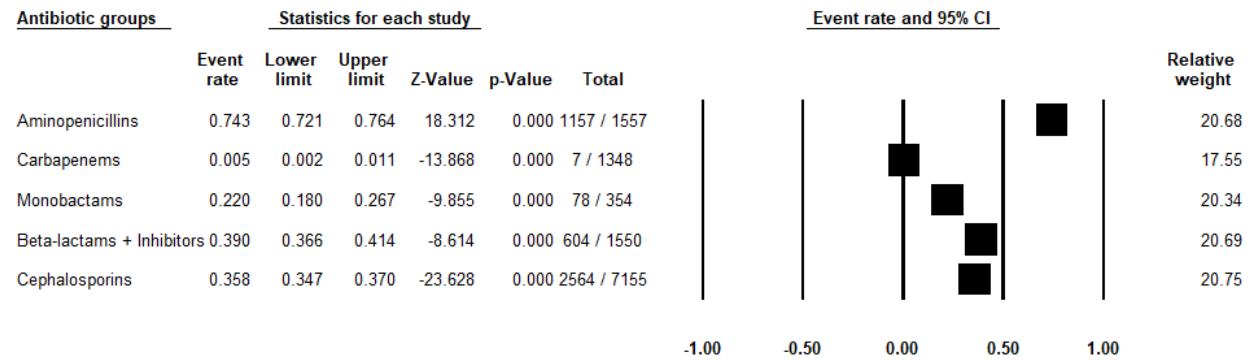

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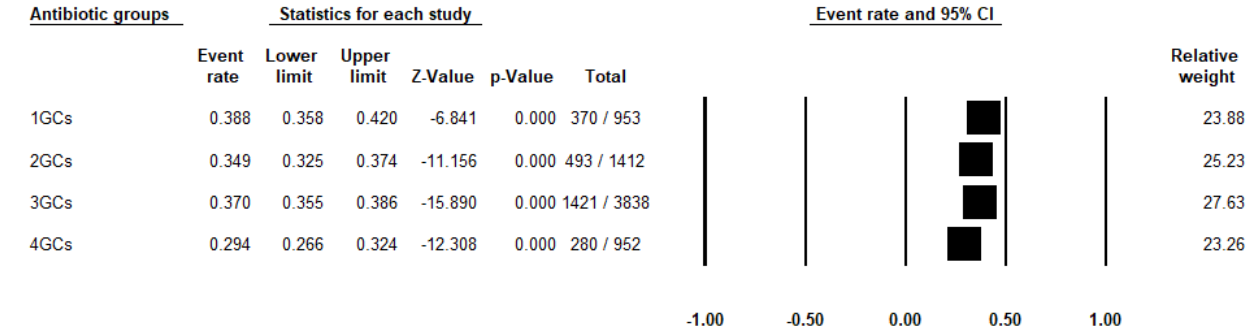

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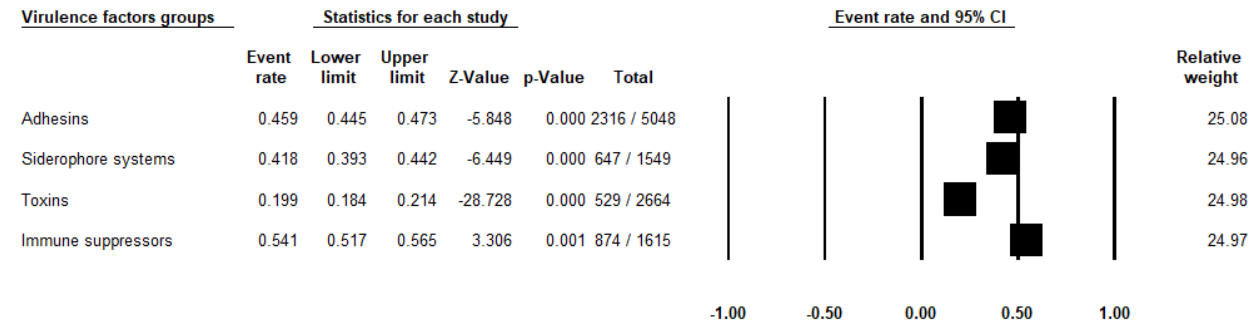


Figure 4.1.2: Forest plot of UPEC resistance to different antibiotic subgroups and virulence factors groups
(A: main antibiotic groups, B: Beta-lactams classes, C: Cephalosporins classes, D: virulence factors groups)

Table 4.1.3: Meta-analysis of antibiotic resistance for UPEC isolates from urinary tract infections

Antibiotics	No of studies	n/N	Random model		Heterogeneity		Egger's test		
			% (95% CI)	P	Q	P	I^2	t	P
Amikacin	8	214/1074	19.9 (0.18-0.22)	<0.001	344.4	<0.001	96.5	3.98	0.002
Amoxicillin	3	322/443	72.7 (0.68-0.77)	<0.001	225.6	<0.001	94.7	4.76	0.001
Amoxiclav	6	407/998	40.8 (0.38-0.44)	<0.001	406.2	<0.001	97.1	2.35	0.039
Ampicillin	8	835/1114	75.0 (0.72-0.77)	<0.001	222.9	<0.001	94.6	1.15	0.276
Ampicillin-sulbactam	3	161/354	45.5 (0.40-0.51)	0.089	178.0	<0.001	93.3	5.54	<0.001
Aztreonam	2	78/354	22.0 (0.18-0.27)	<0.001	172.8	<0.001	93.1	24.1	<0.001
Cefepime	7	280/952	29.4 (0.27-0.32)	<0.001	143.3	<0.001	91.6	3.38	0.006
Cefixime	3	120/443	27.1 (0.23-0.31)	<0.001	124.0	<0.001	90.3	5.58	0.001
Cefoperazone-sulbactam	2	36/198	18.2 (0.13-0.24)	<0.001	81.21	<0.001	85.2	24.2	<0.001
Cefotaxime	7	379/1055	35.9 (0.33-0.39)	<0.001	235.5	<0.001	94.9	3.99	0.002
Cefoxitin	4	104/707	14.7 (0.12-0.18)	<0.001	91.61	<0.001	86.9	13.6	<0.001
Cefpodoxime	2	166/287	57.8 (0.52-0.63)	0.008	182.7	<0.001	93.4	11.5	<0.001
Ceftazidime	9	509/1209	42.1 (0.39-0.45)	<0.001	212.1	<0.001	94.3	3.33	0.007
Ceftriaxone	5	247/844	29.3 (0.26-0.32)	<0.001	239.6	<0.001	95.0	5.50	<0.001
Cefuroxime	5	389/705	55.2 (0.51-0.59)	0.006	288.2	<0.001	95.8	3.16	0.009
Cephalexin	3	146/258	56.6 (0.50-0.63)	0.035	189.3	<0.001	93.7	12.8	<0.001
Cephalothin	3	82/437	18.8 (0.15-0.23)	<0.001	181.0	<0.001	93.4	3.23	0.008
Cephazolin	3	142/258	55.0 (0.49-0.61)	0.106	168.4	<0.001	92.9	13.7	<0.001
Ciprofloxacin	12	792/1781	44.5 (0.42-0.47)	<0.001	265.5	<0.001	95.5	0.54	0.602
Ertapenem	1	1/227	0.4 (0.00-0.03)	<0.001	0.799	1.000	0.00	0.49	0.634
Fosfomycin	1	9/107	8.4 (0.04-0.15)	<0.001	37.35	<0.001	67.9	21.0	<0.001
Gentamicin	13	637/1888	33.7 (0.32-0.36)	<0.001	269.6	<0.001	95.6	0.70	0.497
Imipenem	7	5/767	0.7 (0.00-0.02)	<0.001	3.719	0.988	0.00	5.02	<0.001
Meropenem	3	1/354	0.3 (0.00-0.02)	<0.001	1.416	1.000	0.00	2.40	0.035
Nalidixic acid	9	777/1317	59.0 (0.56-0.62)	<0.001	248.2	<0.001	95.2	1.70	0.118
Nitrofurantoin	10	297/1486	20.0 (0.18-0.22)	<0.001	297.1	<0.001	96.0	3.77	0.003
Norfloxacin	5	286/614	46.6 (0.43-0.51)	0.090	273.1	<0.001	95.6	3.20	0.009
Ofloxacin	2	101/244	41.4 (0.35-0.48)	0.007	153.5	<0.001	92.2	13.6	<0.001
Tetracycline	6	498/721	69.1 (0.66-0.72)	<0.001	207.3	<0.001	94.2	2.44	0.033
Tobramycin	1	30/107	28.0 (0.20-0.37)	<0.001	103.8	<0.001	88.4	35.2	<0.001
Co-trimoxazole	13	1119/1888	59.3 (0.57-0.61)	<0.001	177.1	<0.001	93.2	1.06	0.313

Regarding the virulence factors, both factors associated with *E. coli* surface cell and those secreted and exported to the site of action were observed. Taking into account the groups of virulence factors according to their action of mechanisms, a high prevalence was observed among immune suppressors in 54.1% (874/1615), followed by adhesins in 45.9% (2316/5048), siderophore systems in 41.8% (647/1549) and toxins in 19.9% (529/2664) (Fig.4.2.D). Taken individually, the most prevalent virulence factors from adhesins group were: the cell surface hydrophobicity (CSH) in 80% (120/150, 95% CI: 0.73-0.86), the fimbrial and afimbrial adhesins: *fimH/MSHA* in 75.3% (881/1170, 95% CI: 0.73-0.78), *fimP/MRHA* in 35.6% (219/616, 95% CI: 0.32-0.39), the serum resistance coded by the gene *traT* in 75.1% (266/354, 95% CI: 0.70 – 0.79), the capsular polysaccharide K antigen (kpsMTII) in 60.6% (120/198, 95% CI: 0.54-0.67) and *pap* in 30.2% (350/1158, 95% CI: 0.28-0.33). Frequencies of immune suppressors coded by the pathogenicity islands (PAIs) genes were *shiA* in 92.1% (209/227, 95% CI: 0.88-0.95), *sisA* in 72.2% (164/227, 95% CI: 0.66-0.78), *sisB* in 24.7% (56/227, 95% CI: 0.19-0.31) and *PAI* in 55.2% (265/480, 95% CI: 0.51-0.60). The secreted virulence factors exported to the site of infection were represented by toxins and siderophore molecules. The most frequent toxins observed were the haemolysin (*hlyA*) in 22.1% (334/1511, 95% CI: 0.20-0.24), the secreted autotransporter toxin (*sat*) in 26.2% (28/107, 95% CI: 0.19-0.35) and the cytotoxic necrotizing factor-1 (*cnf*-1) in 13.3% (91/682, 95% CI: 0.11-0.16). For siderophores, the aerobactin system was observed most frequently, and included outer membrane proteins genes: *iucD* in 65.7% (95% CI: 0.59-0.72), *iutA* in 61.8% (0.55-0.68), the aerobactin (*aer*) in 52.4% (130/198, 95% CI: 0.48-0.57) and the heme receptor genes (*chuA*) in 20.3% (46/227, 95% CI: 0.16-0.26) (Table 4.1.4).

Table 4.1.4: Meta-analysis of virulence factors for UPEC isolates from urinary tract infections

Antibiotics	No of studies	n/N	Random model		Heterogeneity		Egger's test		
			% (95% CI)	P	Q	P	I^2	t	P
Aer	3	229/437	52.4 (0.48-0.57)	0.315	189.2	<0.001	93.1	4.45	0.001
afa	5	98/701	14.0 (0.12-0.17)	<0.001	169.6	<0.001	92.3	4.54	0.001
chuA	1	46/227	20.3 (0.16-0.26)	<0.001	93.10	<0.001	86.0	25.9	<0.001
cnf1	5	91/682	13.3 (0.11-0.16)	<0.001	71.34	<0.001	81.8	13.2	<0.001
Colicin	1	13/137	9.5 (0.06-0.16)	<0.001	45.42	<0.001	71.4	16.9	<0.001
CSH	1	120/150	80.0 (0.73-0.86)	<0.001	242.1	<0.001	94.6	39.3	<0.001
eco274	1	99/227	43.6 (0.37-0.50)	0.055	157.9	<0.001	91.8	33.7	<0.001
fimH/MSHA	10	881/1170	75.3 (0.73-0.78)	<0.001	210.7	<0.001	93.8	0.72	0.489
fimP/MRHA	4	219/616	35.6 (0.32-0.39)	<0.001	152.0	<0.001	91.5	8.02	<0.001
fyuA	1	41/227	18.1 (0.14-0.24)	<0.001	85.68	<0.001	84.8	24.8	<0.001
hlyA	12	334/1511	22.1 (0.20-0.24)	<0.001	241.9	<0.001	94.6	2.62	0.022
iucD	2	130/198	65.7 (0.59-0.72)	<0.001	203.3	<0.001	93.6	29.9	<0.001
iutA	2	144/233	61.8 (0.55-0.68)	<0.001	198.6	<0.001	93.5	18.9	<0.001
kpsMTII	2	120/198	60.6 (0.54-0.67)	0.003	191.2	<0.001	93.2	36.4	<0.001
PAI	3	265/480	55.2 (0.51-0.60)	0.023	241.8	<0.001	94.6	3.80	0.003
pap	9	350/1158	30.2 (0.28-0.33)	<0.001	87.35	<0.001	98.9	0.54	<0.001
sat	1	28/107	26.2 (0.19-0.35)	<0.001	100.3	<0.001	87.0	25.3	<0.001
sfa	5	262/701	37.4 (0.34-0.41)	<0.001	10.08	<0.001	90.8	0.05	0.001
shiA	1	209/227	92.1 (0.88-0.95)	<0.001	292.1	<0.001	95.6	45.4	<0.001
sisA	1	164/227	72.2 (0.66-0.78)	<0.001	234.0	<0.001	94.5	40.9	<0.001
sisB	1	56/227	24.7 (0.19-0.31)	<0.001	106.9	<0.001	89.8	27.8	<0.001
sivH	1	81/227	35.7 (0.30-0.42)	<0.001	137.5	<0.001	90.6	31.5	<0.001
traT	2	266/354	75.1 (0.70-0.79)	<0.001	236.2	<0.001	94.5	40.7	<0.001
vat	1	63/227	27.8 (0.22-0.34)	<0.001	115.9	<0.001	88.8	28.9	<0.001
yfcv	1	57/227	25.1 (0.20-0.31)	<0.001	108.2	<0.001	88.0	27.9	<0.001

4.2 Results of Study II

4.2.1 Point-prevalence

A total of 113 patients were screened on the survey day (Fig. 4.2.1). The bed occupancy rate was 59.5 %. Of the 113 screened patients, 105 were hospitalised for over 48 hours and were hence included in the study. Eight of the 113 screened patients were excluded because they were admitted after 8:00 a.m. on the day of the survey (2), and/or with a hospital stays < 48 hours (5), and/or found in a ward but already discharged (1). Of the 105 included patients, 12 (11.4%) (95% CI: 6.0%-19.1%) had HAI and 29 (27.6%) (95% CI: 19.3%-37.2%) were prescribed at least one antimicrobial drug. The most frequently reported HAI were surgical site infections (n=4, 33.3%) and urinary tract infections (n=4, 33.3%), followed by bloodstream infections (n=3, 25.0%) and bone/joint infections (n=1, 8.3%) (Fig. 4.2.2). Of the 12 HAI identified, 6 (50.0%) were device-associated HAI (4 urinary tract infections and 2 bloodstream infections).

4.2.2 Patients' characteristics and risk factors for HAI

Of the 105 patients included in the survey, 58 (55.2%) were male and 47 (44.8%) were female (Table 4.2.1). The median age of the patients was 34 (IQR: 24-47) years. 77 (73.3%) patients had non-fatal diseases in the McCabe scoring. The median hospital length of stay (LOS) was 8 (IQR: 5-20) days. Of the patients surveyed, 32 (30.5%) had a peripheral venous catheter, 16 (15.2%) had an indwelling urinary catheter, 42 (40.0%) had documented comorbidities, and 27 (25.7%) had underwent surgery in the past 30 days.

The following risk factors were significantly associated with HAI: presence of indwelling urinary catheter (OR=8.3, 95% CI: 2.24-30.70, p=0.003), history of surgery in the past

30 days (OR=5.11, 95% CI: 1.46-17.83, p=0.011) and LOS for which a stay between 8 and 14 days as associated with an OR=14.4 (95% CI: 1.65-124.7, p=0.0143) (Table 4.2.1). Given the non-linear association between the probability of an HAI and LOS, we have repeated this last analysis using a logistic regression model with restricted cubic spline terms [98,99] for LOS and assessing the association between HAI and LOS using a likelihood ratio test. This confirms our finding (p=0.0035 with this model).

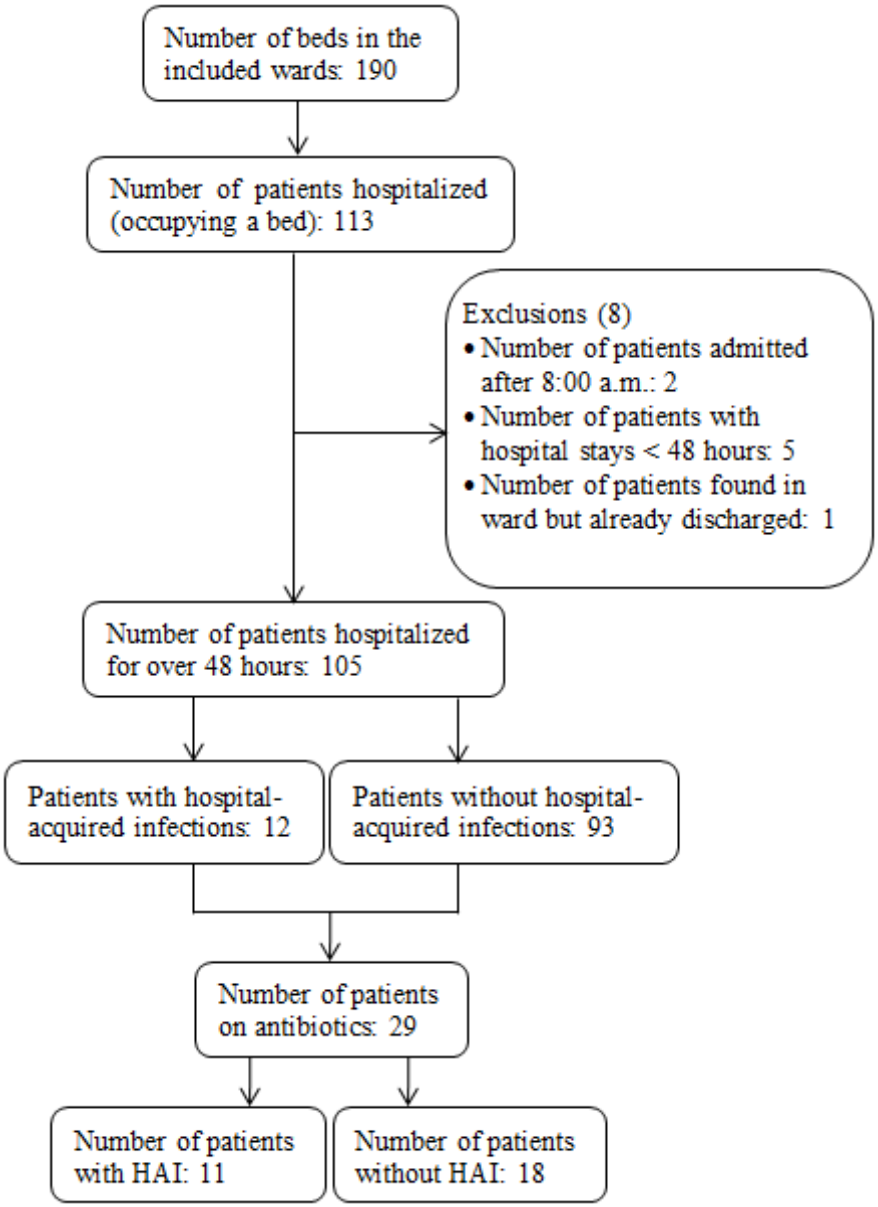


Figure 4.2.3: Flow chart of patients' recruitment

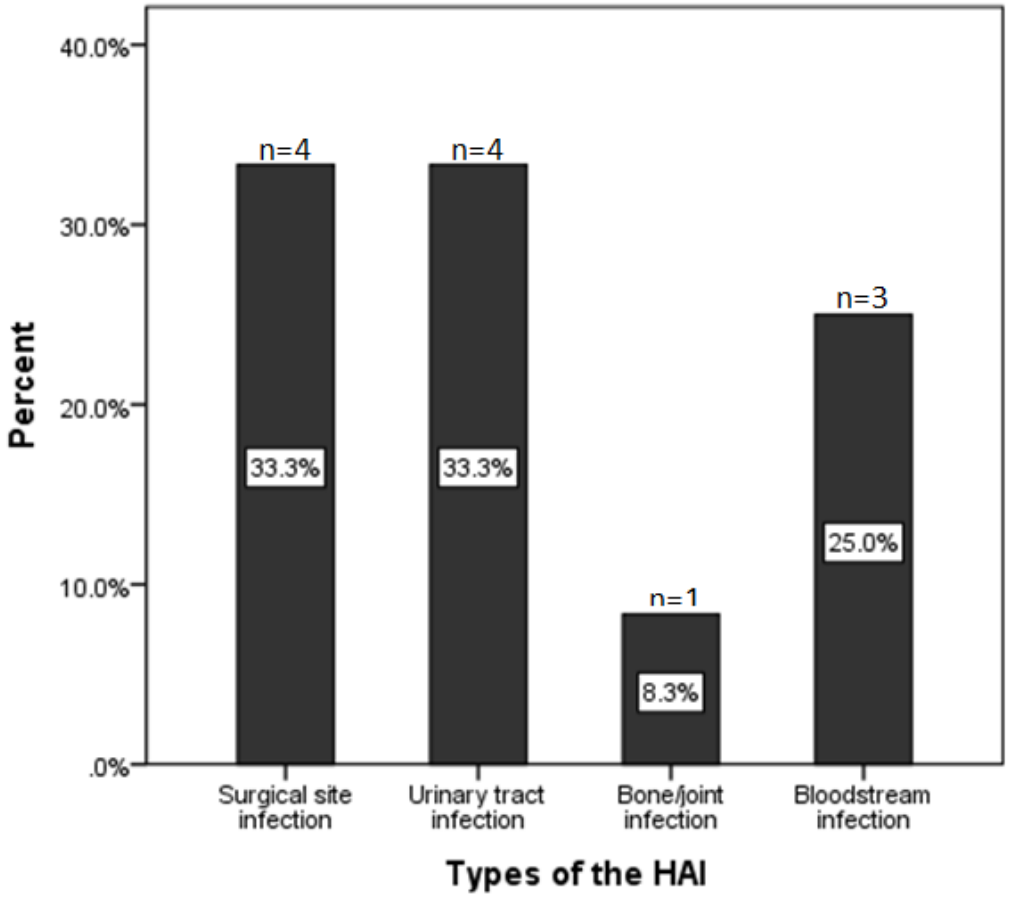


Figure 4.2.4: Distribution of different hospital-acquired infections among admitted patients in surgical wards at QECH (N=12)

Table 4.2.1: Demographic, clinical characteristics, and exposures of surveyed patients

Variables	All patients, N=105	Patients without HAI, N=93	Patients with HAI, N=12	OR (95% CI)	p-value*	Patients on AM, N=29
Patients' characteristics, n (%)						
Gender					0.764	
Female	47 (44.8)	41 (44.1)	6 (50.0)	Ref.		16 (55.2)
Male	58 (55.2)	52 (55.9)	6 (50.0)	0.79 (0.20-3.20)		13 (44.8)
Age (years), Median (IQR)	34 (24-47)				0.4901	
<20	20 (19.0)	17 (18.3)	3 (25.0)	Ref.		5 (17.2)
20-39	47 (44.8)	40 (43.0)	7 (58.3)	29.8 (6.46-201.4)		16 (55.2)
40-59	25 (23.8)	23 (24.7)	2 (16.7)	0.50 (0.038-4.88)		6 (20.7)
≥60	13 (12.4)	13 (14.0)	0 (0.0)	0.0 (0.0-3.68)		2 (6.9)
McCabe score					0.0731**	
Non-fatal diseases	77 (73.3)	68 (73.1)	9 (75.0)	Ref.		23 (79.3)
Ultimately fatal disease	16 (15.3)	16 (17.2)	0 (0.0)	0.0 (0.0-2.43)		2 (6.9)
Rapidly fatal disease	4 (3.8)	4 (4.3)	0 (0.0)	0.0 (0.0-13.10)		0 (0.0)
Unknown	8 (7.6)	5 (5.4)	3 (25.0)	4.53 (0.59-27.65)		4 (13.8)
Exposure, n (%)						
Length of stay (days), Median (IQR)	8 (5-20)				0.0143	
≤7	42 (40.0)	41 (44.1)	1 (8.3)	Ref.		11 (37.9)
8-14	27 (25.7)	20 (21.5)	7 (58.3)	14.4 (1.65-124.7)		11 (37.9)
15-21	12 (11.4)	10 (10.8)	2 (16.7)	8.20 (0.67-99.70)		4 (13.8)
≥22	24 (22.9)	22 (23.6)	2 (16.7)	3.73 (0.32-43.44)		3 (10.4)
Peripheral venous catheter					1.000	
No	73 (69.5)	65 (69.9)	8 (66.7)	Ref.		10 (34.5)
Yes	32 (30.5)	28 (30.1)	4 (33.3)	0.86 (0.21-4.24)		19 (65.5)
Indwelling urinary catheter					0.003	
No	89 (84.8)	83 (89.2)	6 (50.0)	Ref.		20 (69.0)
Yes	16 (15.2)	10 (10.8)	6 (50.0)	8.30 (2.24-30.70)		9 (31.0)
Documented comorbidities					0.537	
No	63 (60.0)	57 (61.3)	6 (50.0)	Ref.		19 (65.5)
Yes	42 (40.0)	36 (38.7)	6 (50.0)	1.58 (0.47-5.29)		10 (34.5)
Surgery in past 30 days					0.011	
No	78 (74.3)	73 (78.5)	5 (41.7)	Ref.		14 (48.3
Yes	27 (25.7)	20 (21.5)	7 (58.3)	5.11 (1.46-17.83)		15 (51.7)
Patients on AM						
Yes	29 (27.6)	18 (19.4)	11 (91.7)	-	-	-
No	76 (72.4)	75 (80.6)	1 (8.3)	-	-	-

HAI: healthcare-associated infections, IQR: interquartile range, AM: antimicrobial agent

*P-values obtained using Fisher's exact test

**After removing the unknown category in the McCabe score, the p-value is 0.5625.

4.2.3 Antimicrobial use

Of the 29 patients that received antimicrobials, 13 (44.8%) received one and 16 (55.2%) received two antimicrobial agents (Table 4.2.2). The purposes of prescribing antimicrobial agents were for prophylaxis in 3 (10.3%) cases, therapeutic in 14 (48.3%) cases and both prophylaxis and therapeutic in 12 (41.4%) cases. The third-generation cephalosporin (ceftriaxone) was used in 15 (51.7%) cases, metronidazole in 13 (44.8%) cases, amoxicillin in 7 (24.1%) cases, doxycycline in 4 (13.8%) cases, ciprofloxacin in 4 (13.8%) cases and flucloxacillin in 2 (6.9%) cases.

Table 4.2.2: Point-prevalence of antimicrobial use in Surgery Department at QECH, Malawi

Variables	All patients, N=29 n (%)	Patients with HAI n (%)**	Patients without HAI n (%)**
Number of AM prescribed			
1	13 (44.8)	4 (30.8)	9 (69.2)
2	16 (55.2)	7 (43.8)	9 (56.2)
Purpose of AM use			
Prophylactic	3 (10.3)	0 (0.0)	3 (100)
Therapeutic	14 (48.3)	4 (28.6)	10 (71.4)
Both	12 (41.4)	7 (58.3)	5 (41.7)
AM prescribed*			
Ceftriaxone	15 (51.7)	7 (46.7)	8 (53.3)
Metronidazole	13 (44.8)	4 (30.8)	9 (69.2)
Amoxicillin	7 (24.1)	3 (42.9)	4 (57.1)
Doxycycline	4 (13.8)	2 (50.0)	2 (50.0)
Ciprofloxacin	4 (13.8)	2 (50.0)	2 (50.0)
Flucloxacillin	2 (6.9)	0 (0.0)	2 (100)

*There are patients who received more than one antibiotic

**Percentages of these columns are calculated by taking corresponding lines of the first columns as the total

HAI: healthcare-associated infections, AM: antimicrobial agent

4.3 Results of Study III

4.3.1 Prevalence of UTI and CAUTI

Among 1,150 patients admitted during this study period, 1,125 were screened for UTI and urine samples of 337 patients were processed for culture. The prevalence of confirmed HA-UTI was 53.1% (179/337, 95% CI: 47.8-58.4). The SUTI was observed in 14.5 % (26/179) while 85.5% (153/179) of patients had ABUTI. Among patient whom urine culture was processed, 26.7% (90/337) had an indwelling urinary catheter in place. Of these, 57.8% (52/90) had a confirmed UTI and the CAUTI was observed in 53.9% (28/52, 95% CI: 40.0-67.1) (Fig. 4.3.1).

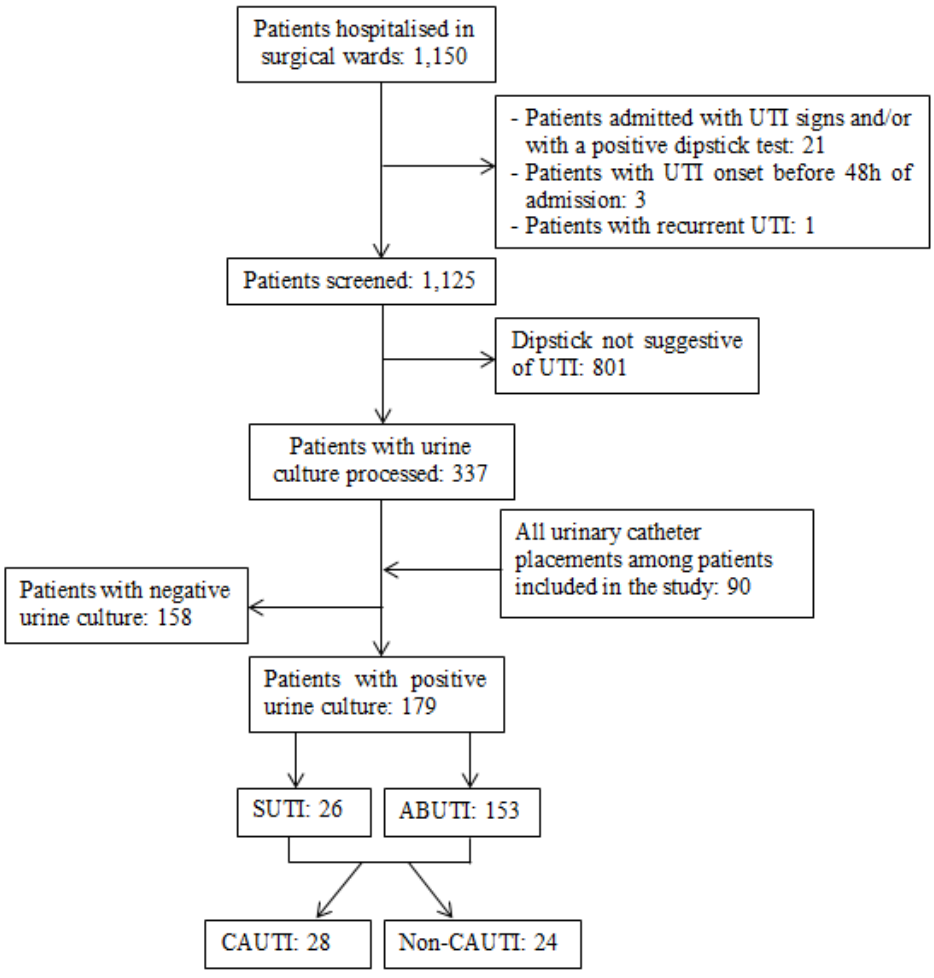


Figure 4.3.1: Flowchart of patients' recruitment

4.3.2 Demographic and clinical characteristics of patients

Of the 337 patients, 185 (54.9%, 95% CI: 49.5-60.2) were male; the mean age was 39.7±17.4, 166 (49.3%, 95% CI: 43.9-54.6) were married, and 125 (37.1%, 95% CI: 32.1-42.4) had the primary education level (Table 4.3.1).

Table 4.3.1: Socio-demographic data of patients

Variables	n	% (95% CI)
Gender		
Male	185	54.9 (49.5-60.2)
Female	152	45.1 (39.9-50.5)
Age (years), Mean ± SD	39.7±17.4	
<20	30	8.9 (6.3-12.5)
20-39	155	46.0 (40.7-51.4)
40-59	103	30.6 (25.9-35.7)
≥60	49	14.5 (11.2-18.7)
Marital status		
Single	120	35.6 (30.7-40.9)
Married	166	49.3 (43.9-54.6)
Divorced	24	7.1 (4.8-10.4)
Widow	27	8.0 (5.5-11.5)
Educational level		
None	110	32.6 (27.8-37.8)
Primary	125	37.1 (32.1-42.4
High school	75	22.3 (18.1-27.0)
College/University	27	8.0 (5.5-11.5)

Regarding risk factors and clinical features of recruited patients, 7.4% (25/337, 95% CI: 5.1-10.8) patients had diabetes, 18.7% (63/337, 95% CI: 14.9-23.2) known positive HIV patients, 11.6% (39/337, 95% CI: 8.6-15.5) patients under immunosuppressive and/or corticosteroid treatment, 32.1% (108/337, 95% CI: 27.3-37.2) patients had a non-fatal disease, 33.5% (113/337, 95% CI: 28.7-38.8) patients were waiting a surgical procedure while 43.0% (145/337, 95% CI: 37.8-48.4) patients underwent surgery in the past 30 days and the median length of stay in hospital was 5 days (IQR: 3-8). In the female population, 4 (2.3%) were pregnant. However, in the male population, 3.8% (7/185, 95% CI: 1.8-7.8) had prostatic diseases and 0.5% (1/185, 95% CI: 0.08-3.8) had a urethral stent. The

median duration of indwelling urinary catheter (IUC) to be in place was 4 days (IQR: 2-7). Majority of IUC were place in the general ward in 73.3% (66/90, 95% CI: 63.1-81.5) cases. The routine catheter changing was not performed in 78.9% (71/90, 95% CI: 69.1-86.2) cases while the drainage bag was touching the ground in 22.2% (20/90, 95% CI: 14.7-32.1) cases. Of the 337 recruited patients, 31 (9.2%, 95% CI: 6.5-12.8) were symptomatic and the common symptoms encountered included dysuria in 71.0% (22/31, 95% CI: 52.1-84.6), pelvic pain in 61.3% (19/31, 95% CI: 42.7-77.1) and pollakiuria in 51.6% (16/31, 95% CI: 33.9-69.0). The median time for symptoms onset from the admission date was 7 days (IQR: 5-9) (Table 4.3.2).

Table 4.3.2: Risk factors and clinical data of patients

Variables	n	% (95% CI)
Diabetes		
Yes	25	7.4 (5.1-10.8)
No	312	92.6 (89.2-95.0)
HIV		
Yes	63	18.7 (14.9-23.2)
No	217	64.4 (59.1-69.3)
Unknown	57	16.9 (13.3-21.3)
Immunosuppressive/corticoids treatment		
Yes	39	11.6 (8.6-15.5)
No	298	88.4 (84.5-91.4)
Chronic renal diseases		
Yes	4	1.2 (0.4-3.1)
No	333	98.8 (96.9-99.6)
Spinal cord injury		
Yes	5	1.5 (0.6-3.5)
No	332	98.5 (96.5-99.4)
McCabe score		
Non-fatal disease	108	32.1 (27.3-37.2)
Ultimately fatal disease	32	9.5 (6.8-13.1)
Rapidly fatal disease	11	3.3 (1.8-5.8)
Unknown	186	55.2 (49.8-60.4)
Length of stay in hospital, Median (IQR)	5 (3-8)	
≤7	238	70.6 (65.5-75.3)
8-17	54	16.0 (12.5-20.4
15-21	20	5.9 (3.9-9.0)
≥22	25	7.5 (5.1-10.8)
Patients waiting surgery procedure		
Yes	113	33.5 (28.7-38.8)
No	224	66.5 (61.3-71.3)
Patient underwent surgery in the past 30 days		
Yes	145	43.0 (37.8-48.4)
No	192	57.0 (51.6-62.2)
Pregnancy [a]		
Yes	4	2.3 (0.1-6.9)
No	148	97.4 (93.2-99.0)
Prostatic diseases [b]		
Yes	7	3.8 (1.8-7.8)
No	178	96.2 (92.4-98.2)
Urethral stent [b]		
Yes	1	0.5 (0.08-3.8)
No	184	99.5 (96.2-99.9)
Presence of indwelling urinary catheter		
Yes	90	26.7 (22.2-31.7)
No	247	73.3 (68.3-77.8)
Indwelling urinary catheter placement duration, Median, IQR) [c]	4 (2-7)	

<7	65	72.2 (62.0-80.1)
≥7	25	27.8 (19.4-38.0)
Place where catheterisation was performed [c]		
Ward	66	73.3 (63.1-81.5)
Emergency ward	15	16.7 (10.2-26.0)
Operating room	5	5.6 (2.3-12.8)
Outpatient clinic	3	3.3 (1.1-10.0)
ICU	1	1.1 (0.2-7.7)
Routine catheter changing [c]		
Yes	19	21.1 (13.8-30.9)
No	71	78.9 (69.1-86.2)
Drainage bag well positioned [c]		
Yes	62	68.9 (58.5-77.7)
No	28	31.1 (22.3-41.5)
Drainage bag touching the ground [c]		
Yes	20	22.2 (14.7-32.1)
No	70	77.8 (67.9-85.3)
Symptomatic patients		
Yes	31	9.2 (6.5-12.8)
No	306	90.8 (87.2-93.5)
Symptoms encountered [d, e]		
Dysuria	22	71.0 (52.1-84.6)
Pelvic pain	19	61.3 (42.7-77.1)
Pollakiuria	16	51.6 (33.9-69.0)
Back pain	12	38.7 (22.9-57.3)
Sense of incomplete bladder emptying	11	35.5 (20.4-54.2)
Fever	8	25.8 (13.1-44.6)
Time of symptoms onset (days), Median (IQR)[d]	7 (5-9)	
≤7	18	58.0 (39.7-74.4)
8-14	11	35.5 (20.4-54.2)
15-21	0	0
≥22	2	6.5 (15.3-23.5)
Sampling done from		
Midstream urine	247	73.3 (68.3-77.8)
Catheter	90	26.7 (22.2-31.7)
Culture results		
Positive	179	53.1 (47.8-58.4)
Negative	158	46.9 (41.6-52.3)

[a] Frequencies of these variables are reported only among female population

[b] Frequencies of these variables are reported only among male population

[c] Frequencies of these variables are reported only for patients with indwelling urinary catheter

[d] Frequencies of these variables are reported only among for patients presenting symptoms

[e] Patients have reported more than one symptom

4.3.3 Analysis of associated risk factors with UTI and CAUTI

Risk factors for HA-UTI and CAUTI were analysed. A p-value <0.2 was considered as significant in the univariate analysis while a p-value <0.05 was considered as significant in the multivariate analysis. In the univariate analysis, the following factors were statistically significant and associated with HA-UTI: age of patients (20 to 39 years: OR=0.53, 95% CI: 0.23-1.24, p=0.145; 40 to 59 years: OR=0.39, 95% CI: 0.16-0.93, p=0.034), single patients (OR=1.93, 95% CI; 1.19-3.12), educational level (none: OR=2.36, 95% CI: 1.00-5.56, p=0.051; primary school: OR=1.98, 95% CI: 0.85-4.60, p=0.114), prostatic diseases (OR=0.19, 95% CI: 0.23-1.62, p=0.129), symptomatic patients (OR=0.19, 95% CI: 0.72-0.51, p=0.001), hospital length of stay (LOS of 8-14 days: OR=2.91, 95% CI: 1.54-5.50, p=0.001; LOS of 15-21 days: OR=6.94, 95% CI: 1.98-24.3, p=0.002; LOS ≥22 days: OR=2.60, 95% CI: 1.08-6.26, p=0.033) (Table 4.3.3 and appendices 3 and 4). None of these factors were statistically significant and associated with HA-UTI in the multivariate logistic regression analysis. Risk factors significantly (p<0.2) associated with CAUTI in the univariate analysis included female gender (OR=0.1, 95% CI: 0.08-1.15, p=0.080), single (OR=4.52, 95% CI: 1.37-14.98, p=0.013), HIV patients (OR=2.30, 95% CI: 0.99-5.33, p=0.052), immunosuppressive and/or corticoids treatment (OR=0.19, 95% CI: 0.19-1.79, p=0.145), prostatic diseases (OR=0.19, 95% CI: 0.02-1.80, p=0.147), symptomatic patients (OR=0.06, 95% CI: 0.01-0.54, p=0.012) and age of patients (20 to 39 years: OR=0.38, 95% CI: 0.73-1.99, p=0.253; 40 to 59 years: OR=0.26, 95% CI: 0.48-1.39, p=0.115; ≥60 years: OR=0.21, 95% CI: 0.03-1.49, p=0.119) .

Table 4.3.3: Summary of univariate and multivariate analysis of associated risks to UTI and CAUTI

Variables	OR (95% CI)	p-value*	AOR (95% CI)	p-value**
A. Risk factors associated with UTI				
Age 20-39	0.53 (0.23-1.24)	0.145	1.27 (0.41-4.01)	0.66
Age 40-59	0.39 (0.16-0.93)	0.034	1.20 (0.43-3.36)	0.734
Marital status single	1.93 (1.19-3.12)	0.008	1.33 (0.53-3.31)	0.545
Education: None	2.36 (1.00-5.56	0.051	2.11 (0.57-7.90)	0.267
Education: Primary	1.98 (0.85-4.60)	0.114	2.46 (0.75-8.11)	0.140
Prostatic disease	0.19 (0.23-1.62)	0.129	0.29 (0.27-3.14)	0.309
Symptomatic patients	0.19 (0.72-0.51)	0.001	0.24 (0.24-2.29)	0.212
LOS 8-14	2.91 (1.54-5.50)	0.001	1.86 (0.70-4.95)	0.215
LOS 15-21	6.94 (1.98-24.3)	0.002	8.92 (1.03-77.37)	0.047
LOS ≥22	2.60 (1.08-6.26)	0.033	2.36 (0.75-7.47)	0.144
Indwelling urinary catheter ≥7	0.03 (0.004-0.25)	0.001	4.01 (0.07-0.15)	0.067
B. Risk factors associated with CAUTI				
Female	0.31 (0.08-1.15)	0.080	1.03 (0.75-2.04)	0.245
Age 40-59	0.26 (0.48-1.39)	0.115	1.37 (0.62-0.99)	0.893
Age ≥60	0.21 (0.03-1.49)	0.119	1.01 (0.92-1.11)	0.862
Marital status single	4.52 (1.37-14.98)	0.013	2.53 (0.11-54.7)	0.551
Known HIV +	2.30 (0.99-5.33)	0.052	1.42 (0.26-7.65)	0.687
Immunosuppressive/corticoid	0.19 (0.19-1.79)	0.145	1.01 (0.15-1.55)	0.893
Prostatic disease	0.19 (0.02-1.80)	0.147	0.03 (0.004-1.70)	0.087
Ultimately fatal disease	3.67 (0.56-24.13)	0.177	5.24 (0.18-154.0)	0.337
Symptomatic patients	0.06 (0.01-0.54)	0.012	0.17 (0.23-0.91)	0.254
LOS 8-14	22.75 (4.37-118.3)	0.0002	25.9 (2.8-75.5)	0.129
LOS 15-21	11.67 (1.79-76.01)	0.010	10.0 (0.99-24.8)	0.079

*The p-value was considered significant when <0.2

** The p-value was considered significant when <0.05

4.3.4 Time-independent and time-to-event analyses

This analysis was done in alignment with the ECDC definition of CAUTI. Hence, only patients with indwelling urinary catheter duration of at least 7 days were considered to have CAUTI. Overall, the median duration of IUC in patients with confirmed UTI was 4 days (IQR: 2-7); this was of 11 (IQR: 8-14) days among patients with CAUTI and 3 (IQR: 2-5) in patients without CAUTI (Appendix 4).

The Kaplan-Meier curve for patients with catheterisation is displayed in figure 4.3.2. CAUTI-free survival rate was 76.9% (CI: 52.8 to 89.7) at 7 days, 26.6% (4.8 to 56.1) at 14 days and 26.6% (4.8 to 56.1) at 21 days. Minimal survival rate of 26.6%% is reached on day 30 when 2 patients remain in the study sample. The instantaneous hazard analysis (Fig. 4.3.3) showed, from the left to the right, a decrease number of patients with many days of catheterisation; proven by the widen CI for the smoothed model. The highest instantaneous hazard in the B-spline smoothing model was at around 7 days.

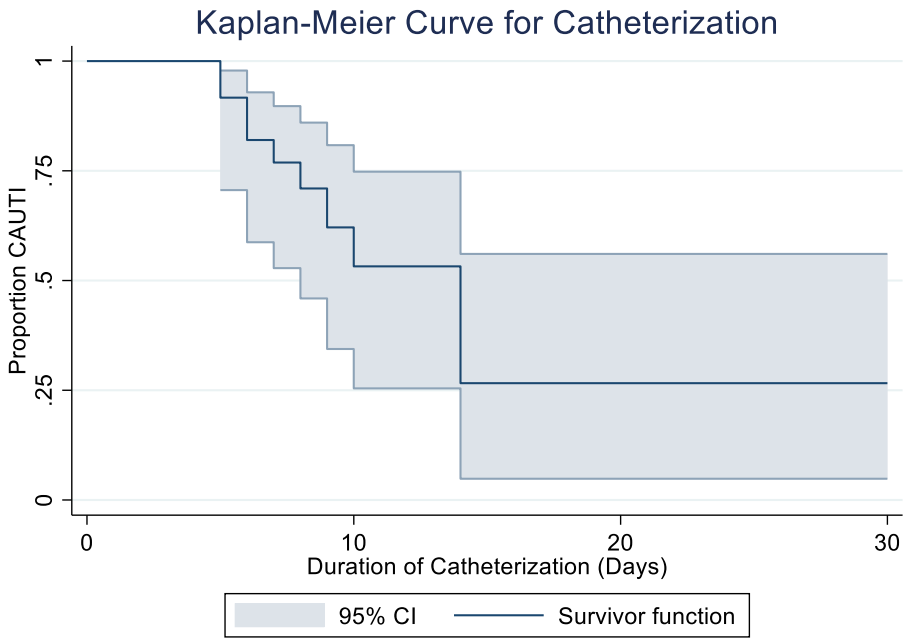


Figure 4.3.2: Kaplan-Meier survival curve for patients with catheterisation

(The 95% CI of survival function starts on day 7 at the x-axis as only patients who had been catheterised for at least 7 days were considered to develop a CAUTI as per the ECDC definition).

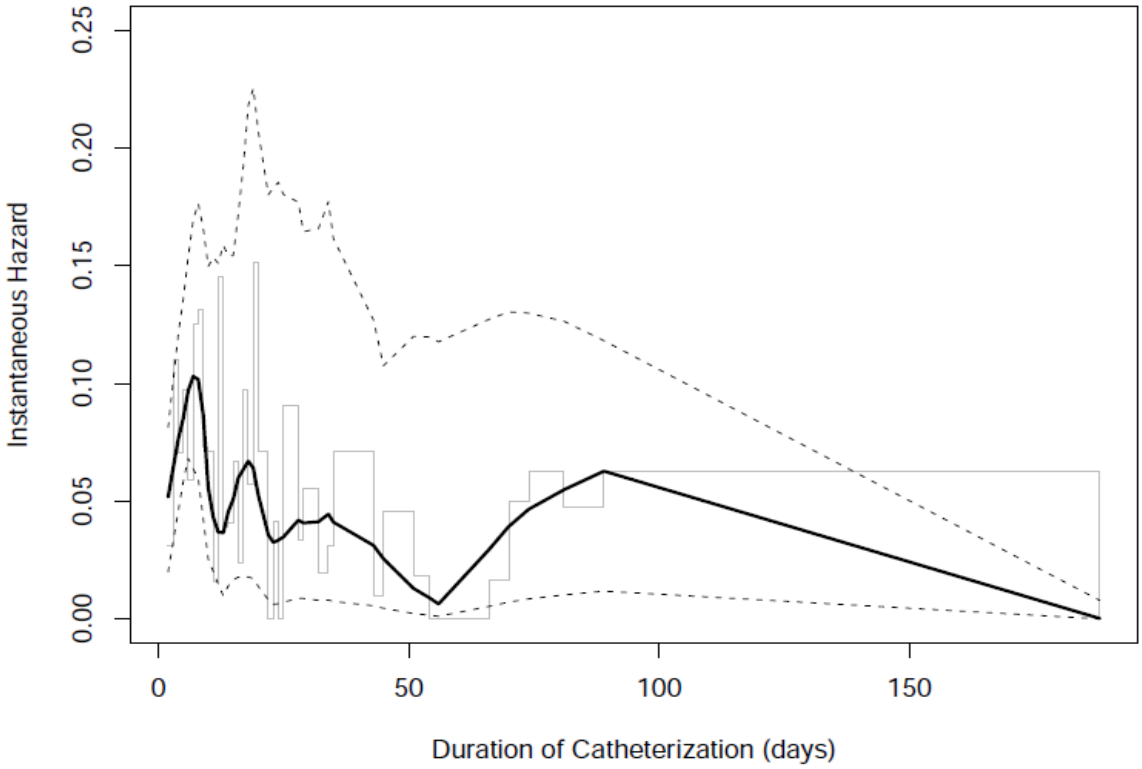
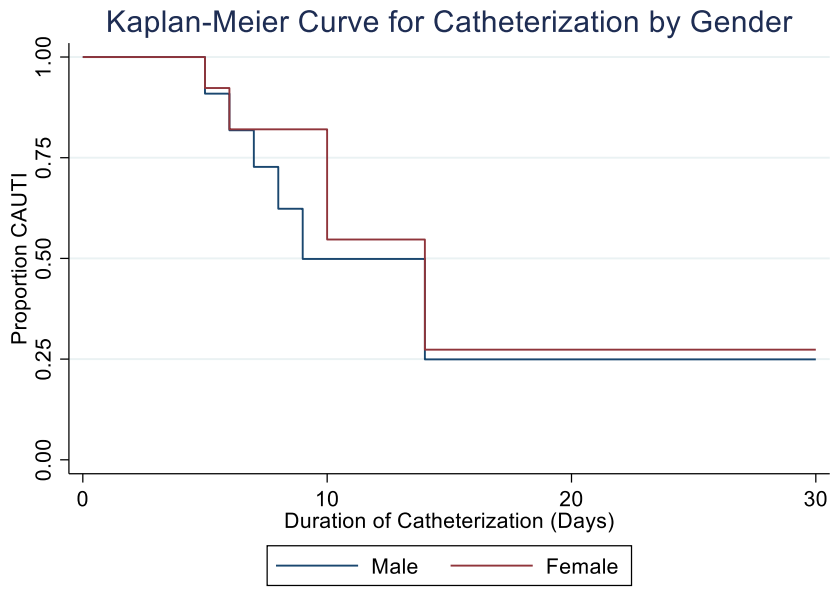


Figure 4.3.3: Instantaneous hazard curve derived using the Kaplan-Meier estimates

The disease-free survival analysis of patients, considering their gender and using the Kaplan-Meier estimate (Fig. 4.3.4.A), demonstrated that no difference in risk of developing a CAUTI (log-rank p=0.625) was identified for the groups. The Kaplan-Meier infection-free probability estimates (as per gender) at 14 days were 24.9% (95% CI: 1.5 to 63.0) for male and 27.4% (95% CI: 22.7 to 69.1) for female with the largest difference in survival occurring between days 10 and 14. In addition, there was no statistical significant difference for patients who underwent surgery in the past 30 days to develop CAUTI (log-rank p=0.996) (Figure 4.3.4.B). The Kaplan-Meier infection-free probability estimates at 14^{th} day were 59.1% (95% CI: 16.0 to 86.0) for patients who underwent surgery and 33.2% (95% CI: 5.6 to 65.7) for patients who did not, with the largest difference in survival occurring between days 14 and 30.

Cox proportional hazards models coefficients were used for identifying risk factors associated with the onset time of infection among symptomatic patients. The univariate Cox proportional hazards models found the following variables as statistically significant (with $p<0.2$): pregnancy (HR: 0.10, 95% CI: 0.01-1.65, p=0.108), waiting a surgical procedure (HR: 0.15, 95% CI: 0.03-0.78, p=0.024) and hospital length of stay ≥7 day (HR: 0.34, 95% CI: 0.13-0.92, p=0.034). None of the variables included in the multivariate Cox model were statistically significant (Appendix 5).

A



B

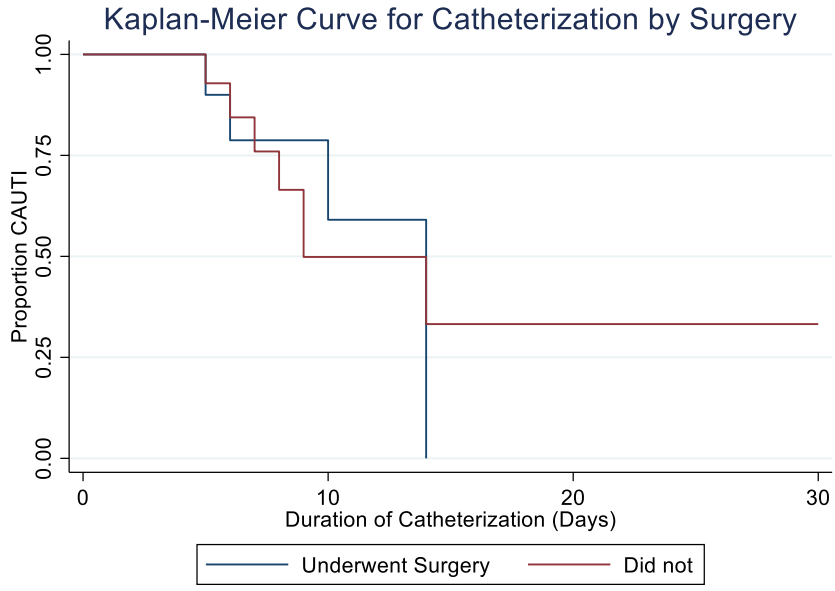


Figure 4.3.4: Infection-free survival stratified by gender and the history of surgery done in the past 30 days

(A) Kaplan-Meier survival curve comparing male versus female groups (log-rank p=0.625). (B) Kaplan-Meier survival curve comparing patients who underwent surgery in the past 30 days and those who did not (log-rank p=0.997)

4.3.5 Antimicrobial resistance patterns of isolated bacteria

The most frequent isolated bacteria from patient with confirmed HA-UTI were *E. coli* in 46.4% (83/179), *Klebsiella spp* in 11.7% (21/179), *Citrobacter spp* in 9.5% (17/179), *S. aureus* in 5.9% (16/179), *Enterobacter spp* in 5.5% (10/179), *Acinetobacter spp* in 5% (9/179), *Pseudomonas spp* in 3.4% (6/179) and *Enterococcus spp* in 2.8% (5/179). Other emerging bacteria with potential of causing wide ranges of infections were also observed. These included *Raoultella spp* in 2.2% (4/179), *Kluyvera ascorbata* in 1.7% (3/179), *Morganella morganii* in 0.6% (1/179) and *Proteus vulgaris* in 0.6% (1/179).

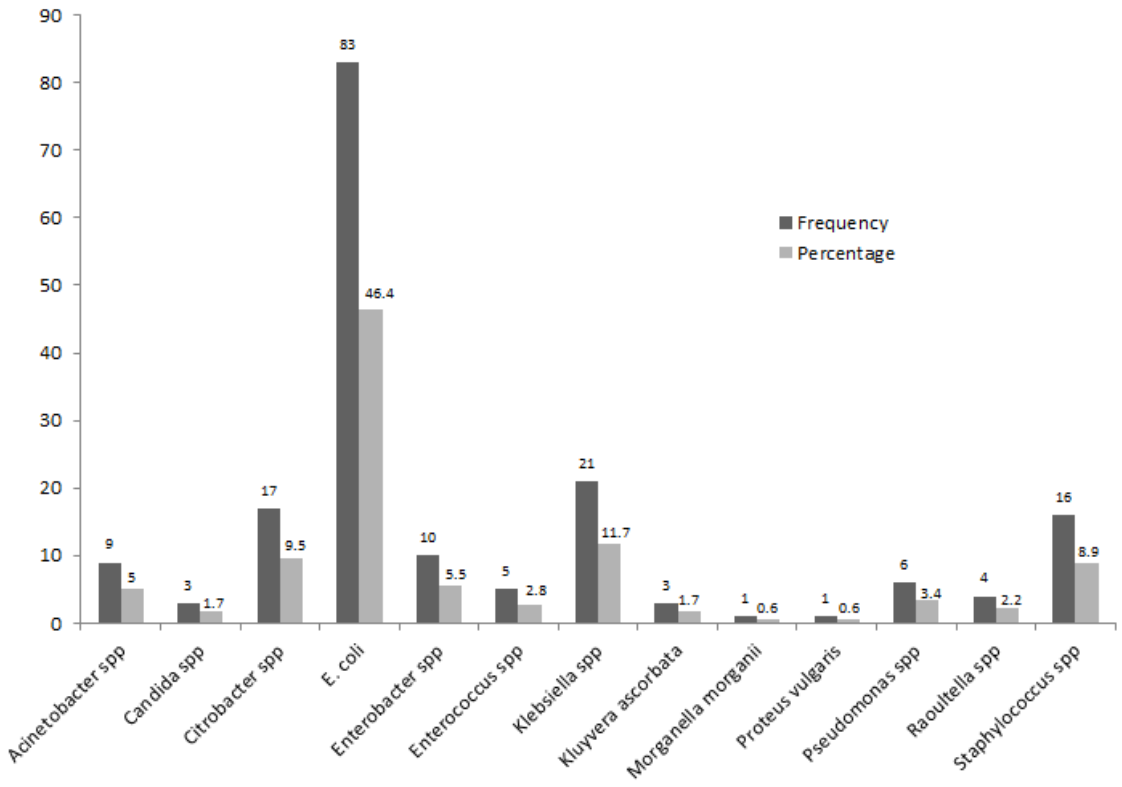


Figure 4.3.5: Isolated pathogens from urine sample processed for culture

E. coli exhibited a high resistance to ampicillin (78/83, 94.0%), cephalosporins, cotrimoxazole (78/83, 94.0%), gentamicin (45/83, 54.2%) and doxycycline (67/83, 80.7%). However, it is susceptible to imipenem and meropenem in 98.8% (82/83) for each. A low resistance was also observed for amikacin (12/83, 14.5%) and fosfomycin (12/83, 14.5%). *Klebsiella* species showed a high resistance among cephalosporins (varying from 61.9% to 76.2%), cotrimoxazole (16/21, 76.2%), nitrofurantoin (16/21, 76.2%), gentamicin (15/21, 71.4%). Amikacin (3/21, 14.3%), ciprofloxacin (7/21, 33.3%) and doxycycline (9/21, 42.9%) had a low rate of resistance. *Citrobacter* species, *Acinetobacter* species and *Enterobacter* species had high rates of resistance to cephalosporins. Emergent bacteria like *Kluyvera ascorbata*, *Raoultella* species, and *Morganella morganii* showed high resistance to most of the tested antibiotics.

In overall, low resistance rates were observed in carbapenems (meropenem and imipenem) in 2.3% (4/171) for each, amikacin in 10.5% (18/171), fosfomycin in 21.6% (36/167), chloramphenicol in 36.0% (58/161), nitrofurantoin in 50.1% (84/165), augmentin in 53.9% (69/128) and ciprofloxacin in 54.0% (95/176) (Table 4.3.4)

Table 4.3.4: Antimicrobial resistance patterns in hospital-acquired UTI

Antibiotics	*Total, n/N (%)*	*E. coli, N=83*	*Klebsiella spp, N=21*	*Citrobacter spp, N=17*	*Acinetobacter spp, N=9*	*Enterobacter spp, N=10*	*Kluyvera ascorbata, N=3*	*Morganella morganii, N=1*	*Proteus vulgaris, N=1*	*Pseudomonas spp, N=6*	*Raoultella spp, N=4*	*Staphylococcus spp, N=16*	*Enterococcus spp, N=5*
Ampicillin	91/111 (82.0)	78 (94.0)	NR	NR	NR	NR	2 (66.7)	NR	NR	NR	4 (100)	3 (18.8)	4 (80)
Augmentin	69/128 (53.9)	46 (55.4)	14 (66.7)	NR	NR	NR	3 (100)	NR	1 (100)	NR	3 (75)	2 (12.5)	NR
Cefuroxime	99/133 (70.0)	62 (74.7)	16 (76.2)	NR	NR	NR	2 (66.7)	NR	NR	6 (100)	3 (75)	10 (62.5)	NR
Cefpodoxime	110/146 (75.3)	63 (75.9)	16 (76.2)	11 (64.7)	NR	8 (80)	2 (66.7)	0 (0.0)	1 (100)	6 (100)	3 (75)	NT	NT
Cefotaxime	104/156 (66.7)	62 (74.7)	14 (66.7)	12 (70.6)	NR	8 (80)	2 (66.7)	0 (0.0)	1 (100)	NR	3 (75)	2 (12.5)	NR
Ceftazidime	114/171 (66.7)	57 (68.7)	16 (76.2)	13 (76.5)	8 (88.9)	8 (80)	2 (66.7)	0 (0.0)	0 (0.0)	4 (66.7)	3 (75)	3 (18.8)	NR
Ceftriaxone	105/156 (67.3)	61 (73.5)	15 (71.4)	12 (70.6)	NR	8 (80)	2 (66.7)	0 (0.0)	1 (100)	NR	3 (75)	3 (18.8)	NR
Cefepime	92/171 (53.8)	45 (54.2)	13 (61.9)	10 (58.8)	6 (66.7)	6 (60)	1 (33.3)	0 (0.0)	1 (100)	5 (83.3)	3 (75)	2 (12.5)	NR
Meropenem	4/171 (2.3)	1 (1.2)	1 (4.8)	0 (0.0)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NR
Imipenem	4/171 (2.3)	1 (1.2)	1 (4.8)	0 (0.0)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NR
Gentamicin	94/171 (55.0)	45 (54.2)	15 (71.4)	8 (47.1)	7 (77.8)	5 (50)	2 (66.7)	1 (100)	1 (100)	3 (50.0)	4 (100)	3 (18.8)	NR
Amikacin	18/171 (10.5)	12 (14.5)	3 (14.3)	1 (5.9)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	NR
Cotrimoxazole	142/165 (86.1)	78 (94.0)	16 (76.2)	16 (94.1)	6 (66.7)	7 (70)	3 (100)	1 (100)	1 (100)	NR	3 (75)	11 (68.8)	NR
Fosfomycin	36/167 (21.6)	12 (14.5)	11 (52.4)	1 (5.9)	NR	3 (30)	0 (0.0)	1 (100)	1 (100)	6 (100)	1 (25)	0 (0.0)	0 (0.0)
Nitrofurantoin	84/165 (50.1)	28 (33.7)	16 (76.2)	8 (47.1)	NR	10 (100)	2 (66.7)	NR	NR	6 (100)	3 (75)	10 (62.5)	1 (0.0)
Ciprofloxacin	95/176 (54.0)	45 (54.2)	7 (33.3)	11 (64.7)	6 (66.7)	6 (60)	3 (100)	1 (100)	1 (100)	5 (83.3)	1 (25)	8 (50)	1 (10)
Doxycycline	135/175 (77.1)	67 (80.7)	9 (42.9)	14 (82.4)	3 (33.3)	9 (90)	3 (100)	1 (100)	NR	6 (100)	3 (75)	15 (93.8)	5 (100)
Chloramphenicol	58/161 (36.0)	19 (22.9)	11 (52.4)	9 (52.9)	NR	4 (40)	1 (33.3)	1 (100)	1 (100)	NR	2 (50)	8 (50)	2 (20)

NR: Not reported

CHAPTER FIVE: DISCUSSION

5.1 Systematic review and meta-analysis

In this study, we reported a systematic review and meta-analysis on virulence factors and antimicrobial resistance of 1,888 UPEC isolates. High antimicrobial resistance rates were observed among the antibiotic class of tetracycline in 69.1% (498/721), followed by sulphonamides in 59.3% (1119/1888), quinolones in 49.4% (1956/3956), and beta-lactams in 36.9% (4410/11964). Meanwhile, virulence factors with highest prevalence were immune suppressors (54.1%) followed by adhesins (45.9%).

Uropathogenic *Escherichia coli* (UPEC) are the primary bacterial type associated with urinary tract infection (UTI) [51]. They include diverse *E. coli* phylogroups that express a wide range of virulence factors and resistance genes that can increase its pathogenicity and resistance to antimicrobials [38,39,52,100,101]. During the last few decades, the emergence of high rates of antimicrobial resistance and multidrug resistance (MDR) phenotype reported in UPEC has become a major concern worldwide [102,103].

The study of AMR showed variable proportions of resistance in different antimicrobial categories. High resistance rates were observed to aminopencillins, tetracyclines, cotrimoxazole, nalidixic acid and cephalosporins. Several studies have reported high resistance rates of UPEC on these antibiotics and by different mechanisms [38,95,102,104]. This study showed high resistance to beta-lactam antibiotics. The increasing rate of 3rd-generation cephalosporin resistance, suggesting extended-spectrum beta-lactamase (ESBL) producing *E. coli* is of concern worldwide. It has been reported that carbapenems are the best options for treating ESBL UPEC-producers [51,105], and our findings report similar results with susceptibility rates to carbapenems close to 100%.

However, the irrational use of 3rd generation cephalosporins and carbapenem increase the risk of spreading of ESBL and carbapenem resistant bacteria. Using carbapenems as first-line antimicrobial treatment does not make them the best option as first line over oral agents like nitrofurantoin and/or fosfomycin in treating UTIs, and reserving carbapenem use for extensively drug resistant isolates with few or no other treatment alternatives.

Regarding virulence factors of UPEC, this study showed a high prevalence of fimbriae (*fimH/MSHA:* 75%). P fimbriae and type 1 fimbriae are known to play a key role in the pathogenesis by facilitating the attachment of *E. coli* to the uroepithelium [106]. The *fimH* adhesion mediates the adherence of UPEC to the bladder epithelium as well as the invasion of bladder epithelial and mast cells into caveolae, which has been reported to protect the bacteria from host defences and antimicrobials [107]. In addition to that, the P-fimbrial adhesins, encoded by the *papG* gene, mediate the attachment to the P-blood group antigens on uroepithelial cells [107]. The expression of *E. coli* surface adhesins is increased by initiating the close contact of the bacteria with the host cell wall. Receptors for S- and P-fimbriae are located in UPEC pathotypes, on the surface of epithelial cells lining the host urinary tract [41], and the high hydrophobicity of bacterial cell promotes the adherence of UPEC to mucosal epithelial cells surfaces [108]. UPEC pathotypes carry significantly higher numbers of fimbrial gene clusters compared to faecal/commensal pathotypes [109]. Siderophores bind ferric iron and iron-siderophore complexes are recognised by cognate outer-membrane receptors. UPEC pathotypes encode the proteins required for the biosynthesis and uptake of several siderophores, such as enterobactin, aerobactin, yersiniabactin and salmochelin [109]. Haemolysin and siderophores are secreted virulence factors that enable the UPEC to colonize the urinary tract and persist despite the effectively functioning host immune defence mechanism [102]. The iron

uptake systems of UPEC are mediated by the siderophore aerobactin synthesized by a number of *iuc* genes and proteins encoded by *iut* genes mediate its transport [110,111]. This study showed prevalence of *iucD* and *iutA* genes of 66% and 62 %, respectively. The toxins produced by UPEC inflict tissue damage and are involved in the host-pathogen interplay [109]. This is mediated by the haemolysin (*hlyA*), in addition to its cytolytic effect. The *hlyA* was the most reported toxin in this review, followed by *sat* and *cnf-1*. The *cnf-1* help the UPEC to survive even in the presence of neutrophils [109]. However, the invasins like the *sisA* and *sisB* play a key role in suppressing the host immune response during the initial stages of infection [75]. Virulence factors and antimicrobial resistance patterns of UPEC is varying from a region to another. A local and/or national antimicrobial resistance and UPEC virulence factors study may be useful for staying abreast regarding the trend for the UTIs' empirical treatment [40]. Intervention strategies on virulence factors that govern the UPEC-mediated UTIs symptomatology may protect against a wide range of UTI syndromes.

5.2 Point-prevalence survey

In this single-day cross-sectional PPS, we observed an estimated point prevalence of HAI of 11.4% (n=12/105) (95% CI: 6.0%-19.1%), including 4 surgical site infections, 4 urinary tract infections, 3 bloodstream infections and 1 bone/joint infection. We identified the following risk factors for HAI; length-of-stay between 8 and 14 days (OR=14.4, 95% CI: 1.65-124.7, p=0.0143), presence of indwelling urinary catheter (OR=8.3, 95% CI: 2.24-30.70, p=0.003) and the history of surgery in the past 30 days (OR=5.11, 95% CI: 1.46-17.83, p=0.011). 29/105 patients (27.6%) were prescribed antimicrobials, most commonly the 3rd-generation cephalosporin, ceftriaxone (n=15).

This survey focused on surgical wards in an urban teaching hospital in Malawi. Similar studies reported HAI prevalence rates of 16.4% in Burkina Faso [112], 14.3% in Nigeria [19] and 11.9% in Ethiopia [113].

The most frequent HAI were surgical site infections (SSI) and urinary tract infections (UTI) (33.3% each), which is comparable to other settings [1,7,19]. Half of the reported infections were device-associated HAI, and thus preventable. Prevention of HAI in surgical patients requires integrated IPC measures before, during and after surgery [114,115]. This particularly applies while using medical devices, however, a previous study has reported a low adherence to hand hygiene practice by clinicians and medical students at QECH [116]. A recent report has proposed IPC among top priorities for patient-centred surveillance of drug-resistant infections and we echo this call [117].

The rate of antimicrobial prescribing (27.6%) found in this survey is relatively similar to that reported in Switzerland (27.6%) [21] but somewhat lower than that reported in China (46.2%) [20]. Our survey considered only the surgery wards, while these other studies were conducted within all the hospital wards and on a larger scale including several hospitals. The most frequently prescribed antimicrobial was ceftriaxone. Since January 2020, QECH has recommended the use of cefazolin in surgical prophylaxis. The exceptional use of ceftriaxone is only in case of established infection before the surgery is done or in cases where the patients were already on ceftriaxone before the surgery, suggesting much of this use was contrary to QECH guidelines, however this study did not analyse the appropriateness of the use of antibiotics nor availability of cefazolin. A recent study from the Democratic Republic of the Congo reported the use of ceftriaxone in non-compliance with surgical antimicrobial prophylaxis guidelines [118].

In many sub-Saharan African healthcare facilities, third-generation cephalosporins are the first choice for antibiotics used in the empiric treatment of acute and severe infections [119]. In Malawi, lack of alternatives have been reported as a reason for preventing the broad use of third-generation cephalosporins in most hospitals [120]. An antimicrobial stewardship program implemented in adult medical wards at QECH was effective in reducing the use of third-generation cephalosporins [121]. Such a program should be extended to other departments for promoting the rational use of antibiotics as inappropriate use of third-generation cephalosporins may facilitate the emergence of multi-drug resistant pathogens. Certainly, increased incidence of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* has been reported since the introduction of ceftriaxone into the Malawian formulary [122].

We report hospital length of stay, presence of indwelling urinary catheter and history of surgery in past 30 days as risk factors significantly associated with HAI, consistent with other reports [17,24,123–128]. Indwelling urinary catheter exposure is a well-established risk factor for UTI, associated with extra hospital length stay and healthcare-cost [18,123,126,127]. Recent surgery may be a proxy for high-risk procedures such as central/peripheral vein catheter, urinary catheter and endotracheal intubation during the surgical procedure. Placement of these invasive medical devices requires strict hygiene measures because they are key risk factors for HAI.

There were some limitations to our study; first, the sample size was small and the survey was not repeated. Second, as the survey was conducted during the COVID-19 pandemic, infection prevention and control (IPC) measures had recently been reinforced in hospital, which might have influenced the HAI prevalence. Further the admission rate during this

period was decreased so nursing staff had fewer patients to care for. Lastly, we could not assess the appropriateness of antimicrobial use due to poor documentation, although this is typically a marker of poor practice in antimicrobial prescribing.

5.3 Risk factors of UTI and antimicrobial resistance of isolated bacteria

The prevalence of confirmed HA-UTI was 53.1% (179/337, 95% CI: 47.8-58.4). The CAUTI was observed in 53.9% (28/52, 95% CI: 40.0-67.1). Risk factors associated with HA-UTI and CAUTI were the age of patients, patients who are not married, low educational level (none or primary school), prostatic diseases, patients presenting UTI symptoms, hospital length of stay (>7 days).

The most frequent isolated bacteria from patient with confirmed HA-UTI were *E. coli* in 46.4% (83/179), *Klebsiella spp* in 11.7% (21/179), *Citrobacter spp* in 9.5% (17/179), *S. aureus* in 5.9% (16/179), *Enterobacter spp* in 5.5% (10/179), *Acinetobacter spp* in 5% (9/179), *Pseudomonas spp* in 3.4% (6/179) and *Enterococcus spp* in 2.8% (5/179). Other emerging bacteria with potential of causing wide ranges of infections were also observed. These included *Raoultella spp* in 2.2% (4/179), *Kluyvera ascorbata* in 1.7% (3/179), *Morganella morganii* in 0.6% (1/179) and Proteus vulgaris in 0.6% (1/179).

Resistance rates observed were 2.3% for carbapenems (meropenem and imipenem) (4/171 for each), 10.5% (18/171) for amikacin, 21.6% (36/167) for fosfomycin, 36.0% (58/161) for chloramphenicol, 50.1% (84/165) for nitrofurantoin, 53.9% (69/128) for amoxicillin-clavulanate and 54.0% (95/176) for ciprofloxacin.

Indwelling urinary catheter associated UTI (CAUTI) is one of the most common infections acquired by patients in health care facilities. The most critical measures for preventing bacteriuria and infection are to limit indwelling catheter usage and, where catheter usage is necessary, removing the catheter as soon as it is clinically indicated. IPC programs in health care facilities must implement and monitor strategies to prevent CAUTI, such as catheter use surveillance, appropriate catheter indications, and possible complications [129]. In this study, the overall prevalence of CAUTI was 53.9%. This finding is relatively high to others findings reported in the literature [130,131]. The difference might be associated with the nature of this study which involved only patients admitted in surgical wards; whereas, the study by Anggi et al. [130] included patients in intensive care unit (ICU) and the study of Omer et al [131] included patients in medical wards and ICU . Furthermore, the difference may be explained by the study designs and geographical location. Studies conducted among young or elderly population [132] and in urologic specialised hospitals [133] may report relatively high prevalence.

The catheterization duration is a major determinant of bacteriuria and it increases the daily risk of acquiring bacteriuria by 3 to 7% [129]. Prompt removal of indwelling urinary catheter at the earliest possibility has been a cornerstone of CAUTI reduction programmes in the published literature [15]. Our study evidenced and upholds the additive hazard ratio of catheterization on acquiring CAUTI in patients admitted in surgical wards. However, there is no statistical difference between male and females.

There are some general HAI risk factors linked to patient characteristics and included age, underlying disease, comorbidities, and weakened host defences [134]. Our findings echo with this. In addition to these factors, our findings showed that the length of hospital

stay multiply the risk of acquiring UTI by 2.91; 6.94 and 2.6 respectively for a length of hospital stay more than 7 days, 14 days and 21 days. Several studies have shown that a length of hospital stay of 7 days and more increases the risk of acquiring an HAI [7,134–136]. This may be explained by the fact that being in hospital exposes the patient to microorganisms that may be found in the hospital in-built environment, especially when the compliance to IPC measures is low.

Regarding bacteria causing UTI, our findings showed that E. coli is the predominant bacteria followed by *Klebsiella spp, Citrobacter spp, Enterobacter spp, Acinetobacter spp,* and *Pseudomonas spp*. Among gram-positive bacteria, the encountered bacteria were *S. aureus* and *Enterococcus spp*. However, other emerging bacteria with potential of causing wade ranges of infections were also observed in this study. These included *Raoultella spp, Kluyvera ascorbata* in, *Morganella morganii* and *Proteus vulgaris*.

It has been reported in the literature that UTIs are caused by both gram-negative and gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in prevalence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp. For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *S. aureus*, *P. mirabilis*, *P. aeruginosa* and GBS [7,37,137].

Knowledge of local resistance patterns of bacteria causing healthcare-associated UTI is important for the selection of appropriate empirical therapy. We observed high AMR estimates among the isolated bacteria across different classes of antibiotics. However, few antibiotics exhibited low rates of resistance; these include meropenem, imipenem, amikacin, fosfomycin, chloramphenicol, nitrofurantoin, augmentin and ciprofloxacin. Similar results have been reported in the literature [37,137–141]. However, there is a paucity of surveillance data on AMR in healthcare-associated UTI in the African region. Hence, these findings will contribute on the literature of the region and will help to established local guidelines for empirical treatment of UTI.

The treatment of UTI is getting exponentially difficult because of the widespread emergence of an array of antibiotic resistance mechanisms. Members of the *Enterobacteriaceae* family, such as *E. coli* and *Klebsiella* species, are of special concern since they have both acquired plasmids encoding extended-spectrum -lactamases (ESBLs). These plasmids facilitate a rapid spread of resistance to third-generation cephalosporins and other antibiotics. Other members of the *Enterobacteriaceae* family produce class C β-lactamases (AmpC enzymes) that are active against cephamycin in addition to third-generation cephalosporins and confer resistant to β-lactamases inhibitors [37].

The emergence of antibiotic-resistant bacteria can be a result of spontaneous chromosomal mutations that confer selective benefits, allowing organisms to shift the drug's site of action (target), accelerate its elimination, or limit its availability inside the organism. This resistance can also be acquired from foreign genetic information through transposons and plasmids, which are transferred among organisms of the same or

different species. These plasmids can carry, concurrently, resistance genes to different antibiotics, creating multiple resistances [142].

The increased antibiotic resistance among uropathogenic bacteria is multifactorial. It is frequently fuelled by prolonged and, at times, inappropriate use of antibiotics [118]. As a matter of fact, in conditions that require prolonged use of chemoprophylaxis; such as urinary malformations, vesico-ureteral reflux, recurrent UTI, neurogenic bladder and some cases of urosepsis follow-up; antibiotic use should be assessed for their rational use [142]. Considering uropathogens causing healthcare-associated UTI, antimicrobial resistance may be fuelled by the use of broad spectrum antibiotics, prolonged hospital stay, critical patients, abdominal surgery, ventilator support, vascular catheters and urinary catheterization [142,143].

Emerging bacteria (*Raoultella spp*, *Kluyvera ascorbata* in, *Morganella morganii* and *Proteus vulgaris*) isolated among patient included in this study are of concern as they may cause wide ranges of infections. Few cases of infections caused by *Kluyvera ascorbata* have been reported to date. These include sepsis, soft tissue infection, urinary tract infection, biliary tract infection, and mediastinitis, with varying severity and a wide range in patient age [144–152]. *K. ascorbata* should not be neglected when isolated in the clinical setting as it may be potentially life-threatening in immunocompromised patients despite it has been regarded alternatively as saprophyte, opportunistic or pathogenic [146]. This pathogen has the ability to transfer the gene encoding for CTX-M-type ESBL to other Enterobacteriaceae [149]. *K. ascorbata* also bears the blaTEM-1, aacC2, and armA genes, as well as integronic aadA2, dfrA12, and sul1, which together confer resistance to the majority of beta-lactams, aminoglycosides, and trimethoprim-

sulfamethoxazoles [153]. Clinicians should be aware of its potential pathogenic role and provide appropriate antimicrobial therapy.

Regarding *M. morganii*, although it is a common microorganism found in nature and in human habitats, it is rarely responsible for community-acquired infections. Instead, it often causes nosocomial infections [154]. It belongs to the tribe *Proteeae* of the *Enterobacteriaceae* family and this species is considered as an unusual opportunistic pathogen that mainly causes nosocomial surgical site and urinary tract infections following surgery [155]. Some *M. morganii* clinical isolates present resistance to multiple antibiotics by carrying various homologous resistant genes (such as blaCTX-M, blaNDM-1, and qnrD1) shared within members of the tribe *Proteeae*. These genes are acquired from horizontal gene transfer via conjugative integration or mobile transposition. As for *M. morganii*, its drug resistance was introduced via extragenetic elements and/or mobile elements [155,156]. As a matter of fact, this bacterium is able to produce beta-lactamases that can break down the extended spectrum beta-lactamase antibiotics; hence posing a serious challenge for treatment and clinical infection control.
In recent years, *Raoultella spp* strains have been recognized as important emerging pathogens and should be seriously considered in cases of infection [157]. Globally, it is an emerging hospital-acquired infection and is particularly associated with invasive procedures [158]. Majority of patients with an indwelling urinary catheter will experience bacteriuria due to this organism and may progress to catheter-associated urinary tract infection (CAUTI) [84,159].

The antimicrobial susceptibility patterns of the reported *Raoultella* spp have shown resistance to penicillins and cephalosporins. Several case reports have described

Raoultella isolates producing extended-spectrum beta-lactamases belonging to the SHV, TEM CTX-M and AmpC [160–163]. Intrinsic resistance to ampicillin and ticarcillin exhibited by *Raoultella* spp is similarly to some *Klebsiella* species, and is the result of chromosomally encoded beta-lactamases that confer inherent resistant to several antibiotics [164].

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

The systematic review and meta-analysis results demonstrated an increased antibiotic resistance of UPEC isolates and suggested a need for reassessment of empirical therapies in urinary tract infections treatment caused by this pathogen. In addition, this pathotype exhibited diverse surface and secreted virulence factors.

The prevalence rates of HAI and antimicrobial use in surgery wards at QECH are relatively high. Acquiring HAI was significantly associated with length of stay, the presence of indwelling urinary catheter, and the history of surgery in the past 30 days. Hospital infection prevention and control measures should be strengthened for reducing HAI burden at QECH. Interventions supporting improved IPC should be implemented at QECH.

Indwelling urinary catheter, the hospital length of stay >7 days, history of surgery in the past 30 days and prostatic diseases constituted major risk factors of acquiring UTI and CAUTI in our study population. *E. coli* was the most prevalent bacteria causing UTI in our setting. Isolated bacteria showed high resistance rate to first line antibiotics used in empirical therapy of UTI in Malawi. This suggests a reassessment of UTI treatment guidelines in our setting.

Future work

From the antimicrobial susceptibility results of isolated bacteria in this study, I will assess the potential Enterobacteriaceae for being ESBL-producer and the quinolone and plasmid-mediated resistance. In addition, E. coli being the most frequent isolated bacteria, I will characterise its phylogenetic diversity and determine its virulence genes.

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APPENDICES

Appendix 1: Newcastle-Ottawa Scale adapted for cross-sectional studies

Selection:

1. Representativeness of the sample:
    a. Truly representative of the average in the target population. * (all subjects or random sampling)
    b. Somewhat representative of the average in the target group. * (non-random sampling)
    c. Selected group of users/convenience sample.
    d. No description of the derivation of the included subjects.
2. Sample size:
    a. Justified and satisfactory (including sample size calculation). *
    b. Not justified.
    c. No information provided
3. Non-respondents:
    a. Proportion of target sample recruited attains pre-specified target or basic summary of non-respondent characteristics in sampling frame recorded. *
    b. Unsatisfactory recruitment rate, no summary data on non-respondents.
    c. No information provided
4. Ascertainment of the exposure (risk factor):
    a. Registry/clinic registers/hospital records only. **
    b. Parental or personal recall and hospital records. *
    c. Parental/personal recall only.

Comparability: (Maximum 2 stars)

1. Comparability of subjects in different outcome groups on the basis of design or analysis. Confounding factors controlled.
    a. Data/ results adjusted for relevant predictors/risk factors/confounders e.g. age, sex, etc. **
    b. Data/results not adjusted for all relevant confounders/risk factors/information not provided.

Outcome:

1. Assessment of outcome:
    a. Independent blind assessment using objective validated laboratory methods. **
    b. Unblinded assessment using objective validated laboratory methods. **
    c. Used non-standard or non-validated laboratory methods with gold standard*
    d. No description/non-standard laboratory methods used.
2. Statistical test:
    a. Statistical test used to analyse the data clearly described, appropriate and measures of association presented including confidence intervals and probability level (p value). *
    b. Statistical test not appropriate, not described or incomplete.

Assessment:

Very Good Studies: 9-10 points
Good Studies: 7-8 points
Satisfactory Studies: 5-6 points
Unsatisfactory Studies: 0 to 4 points

Appendix 2: Ethics approval certificate

CERTIFICATE OF ETHICS APPROVAL

This is to certify that the College of Medicine Research and Ethics Committee (COMREC) has reviewed and approved a study entitled:

P.10/19/2834 - Pattern of hospital-acquired urinary tract infection in surgical wards at Queen Elizabeth Central Hospital in Malawi: a pilot study by Gabriel Bunduki

On 22-Nov-19

As you proceed with the implementation of your study, we would like you to adhere to international ethical guidelines, national guidelines and all requirements by COMREC some of which are indicated on your study

Dr. YB. Mlombe - Chairperson (COMREC)

22-Nov-19

Date

Approved by
College of Medicine
22-Nov-2019
(COMREC)
Research and Ethics Committee

Appendix 3: Patients' clinical characteristics and risk factors associated with UTI

Variables	Absence of UTI	Presence of UTI	OR (95% CI)	p-value
Gender				
Male	84	101	Ref	
Female	74	78	0.88 (0.57-1.35)	0.548
Age (years), Mean ± SD	41.4±16.6	38.1±17.9		
<20	9	21	Ref	
20-39	69	86	0.53 (0.23-1.24)	0.145
40-59	54	49	0.39 (0.16-0.93)	0.034
≥60	26	23	0.38 (0.15-0.99)	0.048
Marital status				
Single	43	77	1.93 (1.19-3.12)	0.008
Married	86	80	Ref	
Divorced	13	11	0.91 (0.39-2.15)	0.829
Widow	16	11	0.74 (0.32-1.69)	0.473
Educational level				
None	42	68	2.36 (1.00-5.56	0.051
Primary	53	72	1.98 (0.85-4.60)	0.114
High school	47	28	0.87 (0.35-2.13)	0.755
College/University	16	11	Ref	
Pregnancy*				
Yes	2	2	1.06 (0.15-7.69)	0.957
No	72	76	Ref	
Diabetes				
Yes	10	15	0.74 (0.32-1.70)	0.475
No	148	164	Ref	
HIV				
Yes	30	33	1.11 (78-1.59)	0.560
No	104	113	Ref	
Unknown	24	33		
Immunosuppressive/corticoids treatment				
Yes	19	20	1.09 (0.59-2.12)	0.807
No	139	159	Ref	
Chronic renal diseases				
Yes	2	2	1.14 (0.16-8.15)	0.900
No	156	177	Ref	
S**pinal cord injury**				
Yes	1	4	0.28 (0.31-2.52)	0.255
No	157	175	Ref	
Prostatic diseases **				
Yes	1	6	0.19 (0.23-1.62)	0.129
No	83	95		
Urethral stent**				
Yes	0	1	1 (0.89-1.59)	1.000
No	84	100	Ref	
McCabe score				

Non-fatal disease	57	51	Ref	
Ultimately fatal disease	18	14	0.87 (0.93-1.92)	0.730
Rapidly fatal disease	6	5	0.93 (0.27-3.24)	0.911
Unknown	77	109	1.58 (0.98-2.55)	0.060
Presence of indwelling urinary catheter				
Yes	38	52	0.77 (0.48-1.26)	0.301
No	120	127	Ref	
Indwelling urinary catheter placement duration, Median, IQR) [c]				
<7	1	24	Ref	
≥7	37	28	0.03 (0.004-0.25)	0.001
Patients waiting surgery procedure				
Yes	51	62	0.90 (0.57-1.42)	0.647
No	107	117	Ref	
Patient underwent surgery in the past 30 days				
Yes	65	80	0.87 (0.56-1.33)	0.511
No	93	99	Ref	
Symptomatic patients				
Yes	5	26	0.19 (0.72-0.51)	0.001
No	153	153	Ref	
Length of stay in hospital, Mean ± SD (IQR)				
≤7	131	107	Ref	
8-14	16	38	2.91 (1.54-5.50)	0.001
15-21	3	17	6.94 (1.98-24.3)	0.002
≥22	8	17	2.60 (1.08-6.26)	0.033

Appendix 4: Patients' clinical characteristics and risk factors associated with CAUTI

Variables	With CAUTI	Without CAUTI	OR (95% CI)	p-value
Gender				
Male	17	20	Ref	
Female	11	4	0.31 (0.08-1.15)	0.080
Age (years), Mean ± SD (IQR)	44.9±22.1	31.8±209		
<20	3	7	Ref	
20-39	9	8	0.38 (0.73-1.99)	0.253
40-59	10	6	0.26 (0.48-1.39)	0.115
≥60	6	3	0.21 (0.03-1.49)	0.119
Marital status				
Single	7	15	4.52 (1.37-14.98)	0.013
Married	19	9	Ref	
Divorced	1	0	1	-
Widow	1	0	1	-
Educational level				
None	6	13	2.31 (0.57-9.41)	0.242
Primary	11	6	0.87 (0.20-3.90)	0.858
High school	8	5	Ref	
College/University	0	0	1	-
Pregnancy*				
Yes	0	1	1	-
No	11	3		
Diabetes				
Yes	4	6	0.50 (0.12-2.04)	0.334
No	24	18	Ref	
HIV				
Yes	8	4	2.30 (0.99-5.33)	0.052
No	16	10	Ref	
Unknown	4	10		
Immunosuppressive/corticoids treatment				
Yes	1	4	0.19 (0.19-1.79)	0.145
No	27	20	Ref	
Chronic renal diseases				
Yes	2	0	1	-
No	26	24		
Spinal cord injury				
Yes	0	4	1	-
No	28	20		
Prostatic diseases**				
Yes	1	5	0.19 (0.02-1.80)	0.147
No	16	15	Ref	

Urethral stent**				
Yes	0	1	1	-
No	17	19		
McCabe score				
Non-fatal disease	11	3	Ref	
Ultimately fatal disease	4	4	3.67 (0.56-24.13)	0.177
Rapidly fatal disease	5	0	1	-
Unknown	11	17	5.67 (1.28-25.02)	0.022
Patients waiting surgery procedure				
Yes	7	8	0.67 (0.20-2.22)	0.510
No	21	16	Ref	
Patient underwent surgery in the past 30 days				
Yes	15	14	0.82 (0.27-2.48)	0.730
No	13	10	Ref	
Symptomatic patients				
Yes	1	9	0.06 (0.01-054)	0.012
No	27	15	Ref	
Length of stay in hospital, Mean ± SD (IQR)				
≤7	21	3	Ref	
8-14	4	13	22.75 (4.37-118.3)	0.0002
15-21	3	5	11.67 (1.79-76.01)	0.010
≥22	0	3	1 (-)	

Appendix 5: Cox regression analysis of risk factors associated with CAUTI

Variables	HR (95% CI)	p-value
Gender		
Male	Ref	
Female	0.74 (0.21-2.64)	0.642
Age (years)		
<20	Ref	
20-39	1.03 (0.20-5.32)	0.970
40-59	0.49 (0.80-2.94)	0.431
≥60	1.18 (0.19-7.23)	0.860
Marital status		
Single	0.76 (0.21-2.72)	0.674
Married	Ref	
Divorced	5.82e-17 (0)	1.000
Widow	5.13e-17 (0)	1.000
Educational level		
None	5.02e+09 (5.75e+08 – 4.38e+10)	0.000
Primary	5.64e+09 (-)	-
High school	3.45e+09 (1.91e+08 – 6.22e+10)	0.000
College/University		
Pregnancy*		
Yes	0.10 (0.01-1.65)	**0.108**
No	Ref	
Diabetes		
Yes	0.54 (0.14-2.10)	0.371
No	Ref	
HIV		
Yes	1.70 (0.66-4.34)	0.274
No	Ref	
Unknown		
Immunosuppressive/corticoids treatment		
Yes	1.03 (0.22-4.92)	0.967
No	Ref	
S**pinal cord injury**		
Yes	0.41 (0.48-3.48)	0.413
No	Ref	

Prostatic diseases**		
Yes	0.45 (0.09-2.28)	0.332
No		
Urethral stent**		
Yes	9.41e-17 (0)	1.00
No	Ref	
McCabe score		
Non-fatal disease	Ref	
Ultimately fatal disease	2.71 (0.37-19.64)	0.325
Rapidly fatal disease	3.74e-17 (0)	1.000
Unknown	0.51 (0.10-2.67)	0.427
Patients waiting surgery procedure		
Yes	0.15 (0.03-0.78)	**0.024**
No	Ref	
Patient underwent surgery in the past 30 days		
Yes	1.00 (0.28-3.64)	0.997
No	Ref	
Length of stay in hospital	0.34 (0.13-0.92)	**0.034**