INCIDENCE, BACTERIA ETIOLOGY AND FACTORS ASSOCIATED WITH VENTILATOR ASSOCIATED PNEUMONIA AMONG PATIENTS ON MECHANICAL VENTILATOR IN INTENSIVE CARE UNITS AT TERTIARY HOSPITALS, DAR ES SALAAM.

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Mmed (Microbiology and Immunology) Dissertation

Muhimbili University of Health and Allied Sciences

October, 2020

Muhimbili University of Health and Allied Sciences

School of Medicine



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By

Helmut Adrian Nyawale

A Dissertation submitted in (Partial) Fulfilments of the Requirements for the Degree of Master of Medicine in Microbiology and Immunology of Muhimbili University of Health and Allied Sciences

October, 2020

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled "**Incidence, bacterial etiology and factors associated with ventilator associated pneumonia among patients on mechanical ventilator in intensive care units at tertiary hospitals, Dar es salaam''** in partial fulfillment of the requirements for the degree of the of Master of Science (Microbiology/Immunology) degree of Muhimbili University of Health and Allied Sciences.

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Dr Joel Manyahi

(Supervisor)

_____

Date

DECLARATION

AND

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I, HELMUT A NYAWALE, declare that this dissertation is my original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my Almighty God followed by my research supervisors, Dr. Joel Manyahi and Professor Stephen Mshana for their assistance and dedicated support in every step throughout the process. Their guidance helped me in all the time of research and writing of this dissertation. I could not have imagined having the better advisors and mentors for my master's study.

I would also like to thank the rest of the academic members of the department of Microbiology and Immunology who are Prof. M. Matee, Prof.E. Lyamuya, Prof. Said Aboud, Dr. Mtebe Majigo, Dr. Agricola Joachim, Dr. Frank Msafiri, Dr D. Kamori, Ms. Upendo Kibwana and Ms Lilian Nkinda for their encouragement and support during my residency program.

I also acknowledge and extend my appreciation to staff members of the Intensive Care Units (ICUs) from Muhimbili National Hospital (MNH) and Muhimbili Orthopedics Institute (MOI) for their assistance with patient recruitment and sample collection. Also, my thanks are to all staff members of Microbiology laboratory at MUHAS and staff members from the department of the Microbiology, Central Pathology Laboratory in Muhimbili National Hospital for their support in sample processing and bacterial identification.

Finally, I would like to thank my colleagues Dr. Salim Masoud for his good cooperation, advice, and encouragement, throughout my master's Program.

DEDICATION

This dissertation is dedicated to my family and friends who have been very supportive throughout my dissertation work.

ABSTRACT

Introduction

Ventilator Associated Pneumonia (VAP) refers to a nosocomial pneumonia which develops 48-72 hours after endotracheal intubation. VAP remains the common cause of morbidity, mortality and prolonged is the second most common Health care associated Infections (HAI) in the intensive care units (ICU) stay. Multi-drug resistance bacteria account for major cause of VAP. In developing countries, little information is known on VAP. Therefore, investigating the incidence, bacteria etiology and outcomes of the VAP is important in reducing VAP associated the mortality and morbidity.

Methodology: This prospective cohort study was carried out between September 2019 and April 2020 among patients on mechanical ventilator (MV) at the ICU of two tertiary hospitals. All patients with clinical diagnosis of VAP, bronchial secretions were collected for microbiological confirmation of VAP. Quantitative bacterial culture was performed and significant growth was defined as microbiological confirmed VAP. Bacteria were identified by standard bacteriological methods and antimicrobial susceptibility was performed by disk diffusion methods. A study questionnaire was used to collect patient's socio-demographic and clinical data. Data were analysed by statistical package for social sciences (SPSS) version.

Results: A total of 334 patients were enrolled during a study period, 269 met inclusion criteria and were followed up to the end of the study ,65 patients were excluded in the final analysis as did not meet inclusion criteria.Therefore 269 were followed up and included in the final analysis. The median time of mechanical ventilation was 12 IQR[5-19]. The incidence rate of VAP was 40.1/1000 ventilation days.The most common etiology of VAP was *P.aeruginosa* (23.4%) followed by *K.pneumoniae* (19.8%) among the gram negative bacilli , *S.aureus* (19.1%) was the the only gram positive bacteria isolated. Multi-drug resistance bacteria accounted for 70.07% of isolates causing VAP. Antibiotic use in the past 6 months [adjusted odds ratio(aOR) 6.02, Confidence interval(CI) 1.81-20.06 and p-value of 0.002], Cigarette smoking[aOR 3.4,CI 1.22-9.45 and p value 0.019], patients with general surgical conditions [aOR 6.15CI 1.57-24.08 and p value 0.009], neurosurgical conditions [aOR 4.23, CI 1.19 - 14.87 and p value 0.026] and cerebral vascular disease [aOR 6.09, CI 1.60-23.12,

p value 0.008] were independently associated with VAP. VAP [aOR 6.43, CI 1.42-21.23, p value 0.001] and MDR [aOR 5.31, CI 2.12-7.70 p value 0.001] independently predicted mortality among the patients on MV.

Conclusion: The incidence VAP in this study is relatively high among the patients admitted in the four major ICUs at Muhimbili National Hospital and Muhimbili Orthopedic Institute for mechanical ventilation. Majority of the Isolate were Gram negative bacteria and MDR. Mortality among the patients with VAP is higher compared non VAP group. However, the mortality is also higher among the patients infected with MDR isolates. Cigarette smoking, having neurosurgical conditions and general surgical conditions significantly associated with VAP.

These findings point out to the need for performing culture and sensitivity for patients with VAP and change the current prescription among the patients admitted at the tertiary care hospitals in Dar es Salaam.

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ABBREVIATIONS

ARDS	Acute Respiratory Distress Syndrome
BAL	Bronchoalveolar Lavage
BMC	Bugando Medical Centre
CDC	Centre for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
CPSI	Clinical Pulmonary Infection Score
ESBL	Extended Spectrum Beta Lactamase
ETT	Endotracheal Tube
GCP	Good Clinical Practice
GIT	Gastrointestinal Tract
GN	Gram Negative
GP	Gram Positive
HAP	Hospital Acquired Pneumonia
LRT	Lower Respiratory Tract
MBL	Metallo-Beta-Lactamase
MDR	Multi Drug Resistance
MMED	Master of Medicine
MNH	Muhimbili National Hospital
MOI	Muhimbili Orthopaedic Institute
MRSA	Methicillin Resistant *Staphylococcus aureus*
MUHAS	Muhimbili University of Health and Allied Sciences
MV	Mechanical Ventilation
NGT	Nasogastric Tube
NICU	Neonatal Intensive Care Unit
PICU	Paediatric Intensive Care Unit
SOPs	Standard Operating Procedures
SPSS	Statistical Package for Social Sciences
VAP	Ventilator Associated Pneumonia

OPERATIONAL DEFINITIONS

Hospital Acquired Pneumonia (HAP)	Lung infection common of bacterial origin that occurs 48 hours or more post hospital admission, not present at time of admission
Health care associated infections	Also known as Hospital Acquired Infections which occur while receiving health care, develops in a hospital or health care facility after 48 or more hours after admission, or within 30 days after having received health care
Ventilator Associated Pneumonia (VAP)	Nosocomial pneumonia that develops after 48 hours or more in patient on mechanical ventilation through endotracheal tube (ETT) or tracheostomy diagnosed based on the presence of a new persistent infiltrate on chest radiograph, fever of ≥ 38°C; leukopenia (< 4000 white blood cells (WBC)/mm3), leucocytosis (≥ 12,000 WBC/mm3) and confirmed by the presence of a pathogen isolated from bronchoalveolar lavage with quantitative culture methods
Significant Bacteria Growth	Refers to quantitative bacteria growth, whereby each colony was corresponding to 20,000CFU/ml and it was considered significant when the count is greater than $1X10^5$CFU/ml

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Patients admitted in the Intensive Care Units (ICUs) are at a higher risk of morbidity and mortality not only from primary illness but also from secondary complications as well as nosocomial infections, also known as health care associated Infections (HCAI)(1).

Several medical and surgical conditions frequently lead to incapacitation of respiratory functions, necessitating provision of assisted respiratory support through MV making these patients at risk of developing VAP.

The incidence of VAP in developing countries ranges between 7.47 to 21.3 per 1000 ventilation days (2,3). Comparatively, VAP is the second most common HAI among the patients admitted in the ICU in the developed countries with an incidence ranging between 4.8 to 5.8 per 1000 ventilation days (4,5).

Development of VAP has been associated with multiple risk factors such as age, duration of mechanical ventilation, immunosuppressive disorders, patient's previous history of cigarette smoking as well as the frequency of circuit cleaning (6,7).

Diagnosis of VAP is made based on triad of clinical, radiological and microbiological criteria which includes signs of pulmonary infection: fever, purulent secretions, leucocytosis, bacteriologic evidence of pulmonary infection, new persistent pulmonary infiltrates and confirmed by isolation of the causative the microbial aetiology (2)

VAP is usually associated with aerobic bacteria such as *Enterobacteriaceae, Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenza* (8). MDR pathogens are more commonly isolated in late onset VAP, such as *P. aeruginosa, Acinetobacter spp, Klebsiella pneumoniae* and Methicillin Resistant *Staphylococcus aureus* (MRSA) (9,10).

In the United States Mortality was reported to be 12.4% (28) in paediatric population and 18.4% among adults with VAP (49). In a study done in Qatar the mortality of

among adult population with VAP was 23.6% (29). In north Africa, Tunisia, the mortality rate was 77.2% among the patients on mechanical ventilator and 45.6 % among the patients with VAP (26). In a study done in Kenya mortality due to VAP was 54.3 % while among the patient who were on mechanical ventilator with no VAP was 32 % (42).

Several factors have been associated with VAP, such factors include the position of the patients, age and the co morbid conditions of the patients (13). The primary diagnosis has also been reported to be associated with VAP, diagnosis such as Traumatic body injury as well as ischemic heart diseases have been reported to be associated with VAP (46). In a study done in Egypt, factors associated with VAP included advanced age, emergent surgery requirement, underlying chronic lung or heart disease, admission after trauma and state of unconsciousness during hospitalization (6). In two studies conducted in developing countries in Asia, with uncategorized population the factors associated with VAP were co-morbid malignancy, septic shock, and inappropriate empirical antibiotic treatment (26,8).

1.2 Problem Statement

Mechanical Ventilation is essential and lifesaving therapy for patients with respiratory failure and critical illness for basic survival. These patients are at a higher risk of obtaining complications such as ventilator associated pneumonia as well as increased mortality and durations of both ICU and hospital stay (11,12)**.** The incidence of VAP in developing countries ranges between 7.47 to 21.3 per 1000 ventilation days (2,3), with a prevalence of 53% among patients on mechanical ventilator admitted in the ICUs in Tanzania (13,14) contrary to the developed countries whereby the incidence ranges between 4.8 to 5.8 per 1000 ventilation days (15,16)**.** Mortality among the patients on mechanical ventilator has been reported to be high in between 24.4 to 57% and higher among the patient with VAP,77% (22,51,17).

Several factors have also been reported to be associated with VAP, such factors include age of the patients, primary diagnosis, circuit cleaning as well as the mode of intubations (24,19).

The management of patients with VAP in Tanzania is by empirical antibiotics, culture and sensitivity is rarely done hence etiology and antimicrobial sensitivity patterns are not known. In addition, Tanzania has no published data on the incidences and factors associated with development of VAP**.** Therefore, the study will also provide an opportunity for significant improvement of infection prevention and control in the intensive care units.

1.3 Conceptual Framework

The main risk factors involved in acquiring VAP in patients are categorised into two factors: hospital and patient factors, which are defined as independent variables. These risk factors determine the development VAP and the significant outcomes: survival and duration of hospital stay. High level risk factors include Circuit cleaning and co morbid conditions. Low level risk factors include ICU location as well as transferring wards.

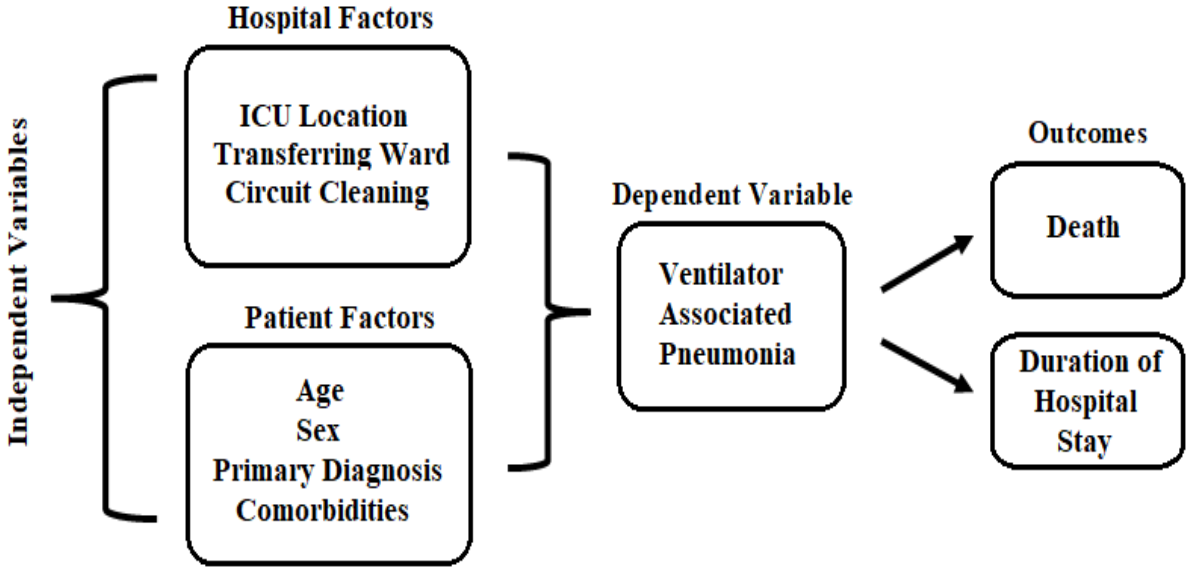


Figure 1: The diagram above shows the contributing risk factors in developing VAP in patients in the ICU and the possible outcomes investigated: dead or not dead.

1.4 Rationale

This study will generate the incidence of VAP, bacterial etiological patterns and the outcomes of VAP in Tertiary hospitals ICU settings. This in turn will provide the opportunity for the improvement of intensive care through recommendation and adoption of evidence-based guidelines for Antibiotic prescription for VAP. Therefore, the study will also provide an opportunity for significant improvement of infection prevention and control in the intensive care units.

1.5 Research Questions

1. What is the incidence of VAP in Tertiary hospitals in Dar es Salaam?
2. What is the bacterial etiology of VAP in Tertiary hospitals in Dar es Salaam?
3. What are the risk factors associated with VAP in Tertiary hospitals in Tanzania?
4. What factors are associated with poor outcome among patients with VAP?

1.6 Broad Objective

To determine incidence, bacterial etiology, risk factors and outcome of ventilator associated pneumonia in Tertiary hospitals, Tanzania

1.6.1 Specific Objectives

1. To determine the incidence of VAP among adult patients admitted in the ICUs at MNH and MOI
2. To determine bacteria etiology patterns of Ventilator Associated Pneumonia among adult patients admitted in the ICUs at MNH and MOI
3. To determine the risk factors associated of VAP among adult patients admitted in the ICUs at MNH and MOI.
4. To determine the outcomes of patients with Ventilator Associated Pneumonia among adult patients admitted in the ICUs at MNH and MOI

1.7 Literature Review

1.7.1 Incidence of VAP

Incidence of VAP in developed world

In a retrospective study done in Italy, the incidence rate of VAP was 5.82/1000 ventilation days in adults (17), with the retrospective study the accuracy of the data could not be certain thus with a prospective study the incidence can be better determined. In a prospective surveillance study in France, incidence was 4.8 per 1000 ventilation days however the incidence was only for the early onset VAP and the incidence late onset VAP was not assessed (18), thus, the overall incidence was not be assessed however this study will account the Incidences in both categories.

Incidences of VAP in Africa.

Table 1:Incidences of VAP in different populations in different countries of Africa.

Country	Incidence	Population	Sample size
South Africa	7.9 per 1000 ventilator days	Adults, Six hospitals	762(19)
Egypt ICU	7.47 per 1000 ventilator days	Adults11 hospitals	1666(3)
Egypt PICU	21.3 per 1000 ventilator days	Pediatric, single Centre.	427(2)

In a study done in South Africa showed the incidence of VAP was 7.9 per 100 ventilation days in adults. However, in a similar study done in Egypt, north Africa, in Adult ICU showed the incidence of VAP was 7.47 per 1000 days (3,19). The incidences of VAP are almost similar, therefore, the incidence calculated from this study will give a comparative advantage of the incidence of VAP in the eastern part of Africa. In a prospective study done in pediatric ICU in Egypt the incidence was 21.3 per 1000 ventilator days (2), however, the incidence calculated is based on pediatric population while in this study will determine the incidence of VAP in adult population.

Incidences in Sub Saharan Africa

A retrospective study done by Farhat et al (36) in Ethiopia, reported the incidence of VAP to be 73 per 1000days (36). However, this study was a retrospective and had no age stratification and therefore could not necessarily give the similar findings. WHO-Africa report on HAIs (2008) reported the incidence of VAP in Senegal was 50 cases/1000 days (41). However, in the report the design could not be documented and therefore this incidence could be different from this study**.**

Incidence of VAP in East Africa

There limited studies on incidence of VAP in East Africa, however a cross sectional study done in Kenya reported the incidence of VAP to be 53% (36). This study was a cross sectional design which is different from our study design which is a prospective cohort and therefore the reported incidence could be different due to different study designs.

1.7.2 Bacterial aetiological patterns in VAP

In a prospective study in Poland showed antibiotic resistance was great a concern in *Acinetobacter baumanni*i with resistant to carbapenems: 70.6% Imipenem and 52.9% Meropenem, *Klebsiella pneumonia* showed resistant: ampicillin and ceftazidime (20), however, in
this study they did not report on the ESBL and MRSA isolates which will be reported in this study.

In a surveillance study done in German most frequent bacteria were *Staphylococcus aureus,* followed *Pseudomonas*
aeruginosa, K.pneumoniae, Escherichiacoli, Haemophilus influenzae, Acinetobacter spp, however, there were similarity in aetiology between early and late onset VAP (21). Thus, this study will also make a comparative aetiological difference between the two categories of VAP.

In a systemic review and meta-analysis study of VAP in adults in Asia *Acinetobacter baumannii* (26%), *Pseudomonas aeruginosa* (22%), *Klebsiella pneumonia (*14%) and

Staphylococcus aureus (14%) were the leading cause of VAP (22). In a prospective study done in South Africa, in a high HIV paediatric population, *Acinetobacter baumannii* was the most common VAP pathogen, followed by *Klebsiella pneumonia* and *Staphylococcus aureus*, however this study was done on pediatrics population (23).

In study conducted in South Africa, *Haemophilus influenzae* and *Acinetobacter baumannii* were the most frequent bacteria found in early and late VAP respectively. MDR *Acinetobacter baumannii* was resistant to all drugs except colistin and amikacin while Non ESBL *Haemophilus influenza* was susceptible to Amoxicillin/Clavulanic acid and the ESBL were sensitive to ciprofloxacin (24). However, the study included patients admitted in surgical ICUs only.

1.7.3 Factors associated with Ventilators Associated Pneumonia

In a prospective study done in Brazil, MDR bacteria isolates and previous use of antibiotic were associated with increased frequency of VAP, however, the analysis included only 33 patients. In a study done in Egypt, factors associated with VAP included advanced age, emergent surgery requirement, underlying chronic lung or heart disease, admission after trauma and state of unconsciousness during hospitalisation. However, this was due to MRSA VAP as a sole aetiology (6). In two studies conducted in developing countries in Asia, with uncategorised population the factors associated with VAP wereco-morbid malignancy, septic shock, and inappropriate empirical antibiotic treatment (26,8), however, the study was done on the pediatric populations. In a study conducted in a pediatric intensive care positive blood cultures were reported as being one of the risk factors for VAP (27). However, the study was done in a paediatric population. A meta-analysis study done in China included 196 studies concluded that old age, tracheostomy and prolonged ventilation were significantly associated with occurrence of VAP (7) . However, the study was done in a paediatric population. A meta-analysis study done in China included 196 studies. concluded that old age, tracheostomy and prolonged ventilation were significantly associated with occurrence of VAP (7). Patients admitted in the ICUs often die from secondary causes e.g. nosocomial infections, deep vein thrombosis, Acute Respiratory Distress Syndrome (ARDS) in addition to the reasons related to the primary diagnoses and respiratory infections including HAP and VAP [36]. Ranjit et al, in a study in Nepal reports the

association of development of VAP with re-intubation, invasive lines, use of H_2 blockers and low arterial SPO_2/FiO_2. In this study, patients with VAP had longer duration of hospitalization without altered mortality [10]. However, these findings were not replicated in another similar study conducted elsewhere [39]. Both the development of VAP and mortality are associated with a number of risk factors e.g. prolonged mechanical ventilation, longer stay at intensive care unit and chest injury [40]. Some of these factors are the immune status of the patient, route of intubation or placement of tubes, mode of feeding, positioning of the body, prevention of stress-related bleeding and role of personnel hand washing to avoid cross-contamination and maintenance of patient's oral hygiene through use of antiseptics and antibiotics for prevention of bacterial colonization of the LRT. Lack of specific hospital bundles for infections control in the ICU and hospital in general is an important risk factor. VAP carries a substantial morbidity and mortality among patients admitted in the ICUs. Therefore, multiple strategies are currently advocated for prevention of VAP. The preventive strategies include measures prior to intubation, during intubation and post-intubation. In order to prevent VAP, hospitals ought to have specific measures for prompt management of reversible causes of respiratory failure and where necessary to employ non-invasive mechanical ventilation [36]. When the patient requires mechanical ventilation efforts should be made to avoid gastric over- distension and the use of oro-endotracheal route. The strategies for the post-intubation period include measures to minimize the risk of VAP. These strategies includes insertion of gastric tubes via the oral route, elevation of the head of bed at $30^{\circ} - 45^{\circ}$, good hand hygiene, the use of HME, change of respiratory circuit only when necessary and under good respiratory care hygiene, subglottic suctioning, use of rotational beds, antiseptic oral rinse in post cardiac surgery patients, minimal use of sedation to shorten periods of mechanical ventilation and use of weaning protocols [41-46].The concerns over the choice of open suctioning system versus closed suctioning system remain inconclusive. Findings from a recent systematic reviews and meta-analysis did not find firm evidence for superiority and recommended further studies [47]. Intermittent suctioning of oral secretions before changing positions of the patients is a simple and cost-effective technique for prevention of VAP. A study by Tsai revealed significant reductions of the incidence

rate of VAP following intermittent suctioning of oral secretion before each. positional change [48].

1.7.4 Outcome of the patients on Mechanical Ventilator

In a multicentered prospective observational cohort study in Children's hospitals in the United States Mortality was 12.4% (28) however the outcomes were from a developed country. In a study done in Qatar the mortality of the patients with VAP was 23.6% and was significantly higher in patients above the age of 60 years, female gender, patients with Diabetes Mellitus and hypertension (29). In a retrospective study done in north Africa Tunisia showed the mortality rate was 77.2 among the patients on the MV, however, MDR *Acinetobacter baumannii* was considered as the sole bacterial aetiology that cause of VAP (30), however, the study was a prospective and focused on a single bacterial aetiology for VAP. In a systematic review and meta-analysis observational studies found no evidence of mortality attributable to VAP in Trauma patients with (31).

CHAPTER TWO

2.0 Materials and Methodology

2.1 Study Design and duration

This was a prospective cohort ICUs based study which was conducted for duration of 7 months from September 2019 to April 2020.

2.2 Study Setting

The Study was conducted in the adults' ICUs at Muhimbili National Hopital(MNH) and Muhimbili Orthopaedic Institute(MOI).

1. MNH has 3 adults ICUs which are located in Mwaisela and maternity blocks. Mwaisela block has 2 ICUs, Medical and surgical ICUs. The medical ICU admitted patients with medical related conditions and surgical ICU admitted patients with different surgical conditions as well as post-surgical procedures. Maternity block has 1 ICU which admits patients with obstetrics and gynaecological conditions. Surgical ICU has 13 MV, Medical has 14 and Maternity has 5 MV. MNH is the largest tertiary health care facility in Tanzania with 1500 bed capacity, which serves as a teaching and referral hospital to the population of Dar es Salaam and Tanzania at large. The hospital attends to 1000 to 1500 outpatients per day, admitting 1000 to 1200 inpatients per week with nearly 3000 employees.

2. MOI is a specialized tertiary care hospital located within the Muhimbili National Hospital with one ICU. MOI has 21 Ventilators with an average admission of 120 patients per months. The ICU admits patients with neurosurgical conditions as well as patients with other forms of trauma. Majority of the patients required Mechanical ventilation had Neurosurgical conditions due to traumatic head injury both severe and moderate head injury.

2.3 Study Population and sampling

Study population included ICU admitted patients on mechanical ventilation. All adult patients on mechanical ventilation admitted in the ICU were conveniently enrolled and closely followed up. Patients were followed up throughout their duration of ICU stay. Participants were followed up daily, each day the vitals were recorded from the monitors (temperature, oxygen saturation, heart rate and oxygen and oxygen saturation). The baseline chest X rays' radiographs were taken for all the participant. The full blood counts were taken for the patients present with fever and the neutrophil counts were recorded. The patients with fever, increased tracheal secretions and/ or worsening of oxygen saturation, X rays were taken to look for increased pulmonary infiltrate. The patients were then followed(daily) until they develop VAP, discharged or die from the ICUs.

2.4 Sample Size

Sample size estimate (Computed using open epi): (32)
The prevalence used is 24 (14)

Sample Size for Frequency in a Population

Population size (for finite population correction factor or fpc) (*N*):	500
Hypothesized % frequency of outcome factor in the population (*p*):	16%+/-3
Confidence limits as % of 100(absolute +/- %) (*d*):	3%
Design effect (for cluster surveys-*DEFF*):	1

Sample Size(*n*) for Various Confidence Levels

Confidence	**Level (%)**	**Sample Size**
95%		**334**

The minimum required sample size was 334.

Justification of the sample size:

1 MNH (Mwaisela and Maternity ICUs) with an average of more than 160 admissions per month, about 80 out of them were on the Mechanical ventilations.

2 MOI has 21 Mechanical ventilators with an average admission of 120 patients in a month, about 70 patients were on mechanical ventilations.

2.5 Sample Selection

2.5.1 Inclusion Criteria

All ICU patients aged 15 years and above admitted in the ICUs for mechanical ventilation support due to diverse primary conditions were included.

2.5.2 Exclusion Criteria

- Admitted in the ICU due to any type of pneumonia or other respiratory distress conditions
- Patients developed pneumonia within 48 hours of Mechanical ventilation.
- Patients with in situ chest tube
- Patients on mechanical ventilation for less than 48 hours

2.6 Study Variable

2.6.1 Independent Variables

Age, sex, co-morbidities, medical history, prior ICU admission, prior history of cigarette smoking and prior use of Antibiotics

2.6.2 Dependent Variables

Incidence of VAP, survival status, duration of ICU stays, and duration of ICU stay.

2.7 Case Definition of VAP

VAP defined by means of a modified CDC criterion of clinical and radiological findings as follows (33):

i. A new and persistent infiltrate in the chest X-ray in patients mechanically ventilated for more than 48 hours

ii. Fever: ≥38.0°C
iii. White cell counts above 12,000/µl or below 4000/µl.
iv. Purulent tracheobronchial secretion (TBS).
v. Confirmed by the presence of a pathogen isolated from bronchial aspirate with quantitative culture methods

2.8 Criteria for specimen collection for culture

Clinical definition of VAP (33)
Patients on MV and report the following:

- New pulmonary infiltrates on the chest X ray

In addition, report at least *two* of the following:

- Fever: ≥38.0°C
- Leukopenia (≤4000 WBC/mm3) or Leucocytosis (≥12000 WBC/mm3)
- Purulent tracheobronchial secretion (TBS)
- Worsening gas exchange: O_2 desaturations.

2.9 Data Collection Methods

Structured standard questionnaire was used to collect socio-demographic data and clinical data. The data will be collected by the PI together with other clinical research assistants. For the followed-up patients, the baseline chest X rays radiographs were taken for all the participants enrolled. The follow daily data were collected, the collected data include the date of participant entry in the study, daily vitals were recorded from the monitors (temperature, oxygen saturation, heart rate and oxygen and oxygen saturation). The full blood counts were taken for the patients present with fever and the results were documented (neutrophil counts). The patients with fever, increased tracheal secretions and/ or worsening of oxygen saturation, X rays were taken to look for increased pulmonary infiltrate. The dates when clinical diagnosis of VAP were recorded the patients were then followed(daily) until they develop VAP, discharged or die from the ICUs.

2.10 Specimen collection

Patient meeting clinical definition of VAP

Bronchial aspirates were collected by using mucus extractors (polymed mucus extractor, Boulevand General, Wahis 531030 Brussels, Belgium) under sterile conditions. Two health care workers were involved in sample collection, one assisting the other. One of the mucus extractor tube was inserted into the endo tracheal tube to the level just below the carina and the other end was inserted to the extraction machine and the negative pressure was created by suction machine the aspirates were collected by the mucous extractors. The specimens were sent to the laboratory in cooling boxes within 1 hour of collection. Specimen were then processed and analyzed at the MUHAS microbiology Laboratory and Central pathology laboratory.

2.11 Laboratory Procedures

2.11.1 Specimen quality control

Barry method for specimen screening for bacterial culture to ensure quality was used (34).Therefore gram stain was done on tracheal aspirates and the gram stained smear were examined under low power field. The number of neutrophils and squamous epithelia cell were counted. The neutrophils were grouped into 1-74, 76-150 and above 150, these were equated to +1, +2 and +3 respectively. In case of squamous epithelia cells, they were grouped into 1-15,16-25 and above 25, these were equated to -1, -2 and -3 respectively. The equated values of neutrophils and squamous epithelia cell were added together. Any positive summation for a specimen was considered a specimen of good quality and passed the quality control test for further processing.

Specimen	Method	Minimum criteria for specimen acceptance
Bronchial aspirate	Microscopic examination of Gram stained smear, and then calculate the sum of neutrophils/Low power field (LPF) (1–75, +1; 76–150, +2; >150, +3) and Squamous epithelia cells/LPF (1-15, -1; 16–25, -2;>25, -3)	Positive summation score

2.11.2 Bacterial Isolation

Bronchial samples

The quantitative culture with standard loop of 1.0µL, bronchial samples collected were inoculated onto Blood agar (BA) and Mac Conkey agar (MCA). The media were incubated aerobically at 37 ºC for 24 to 48 hours (14).In the interpretation of results, each colony was corresponding to 20,000CFU/ml and it was considered significant when the count is greater than $1X10^5$CFU/ml. (30).

2.11.3 Bacterial identification

Preliminary identification of bacterial isolates was performed based on microscopic characteristics, colonial characteristics, and biochemical tests.

2.11.3.1 Conventional Biochemical Tests

Isolates will be sub cultured on NA for biochemical tests to assist in final identification of the isolates. Gram-negative rods and cocci will be identified by the following biochemical test; carbohydrate utilization tests, indole production, urease test, citrate utilization, oxidase test and Triple sugar Iron. Analytical Profile Index (API 20 E) was used for isolates with ambiguous results. Gram-positive cocci were identified based on their gram reaction, Catalase, Coagulase, DNase test, Pyrrolidonyl Aminopeptidase (PYR), Bacitracin disc sensitivity and bile solubility test (35).

2.11.4 Antibiotic Susceptibility Test

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI 19) (36). Selected 3 to 5 colonies of bacteria from a pure culture were transferred to a tube containing 5mL Normal saline mixed gently to form a homogenous suspension. Turbidity of the culture suspension was matched to 0.5 McFarland standards. A sterile cotton swab was used, and the excess suspension was removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. The swab was used to distribute the bacteria evenly over the entire surface

of Mueller-Hinton Agar (MHA). The plates were incubated at 37 ºC for 20-24 hours and the diameters of the zone of inhibition around the disk will be measured using Vernier callipers in millimetres and interpreted according to CLS1 2019 criteria; sensitive, intermediate, and resistant. *S. aureus* ATCC25923, *E. coli* ATCC 25922, *P.aureginosa* ATCC 27853 and *A. baumanii ATCC 19606* were used as control organisms.

The antibiotic disks selection for each bacteria species will be used based on CLSI 19 guidelines (36).

Gram-positive organism: discs tested were amoxicillin/clavulanate (20/10µg), gentamicin (10µg), erythromycin (15µg), tetracycline (30µg), ciprofloxacin (5µg), clindamycin (2µg), trimethoprim/ sulphamethaxazole (1.25/23.75µg) and chloramphenicol (30µg).

Gram-positive organism: discs tested were amoxicillin/clavulanate (20/10µg), gentamicin (10µg), erythromycin (15µg), tetracycline (30µg), ciprofloxacin (5µg), clindamycin (2µg), trimethoprim/ sulphamethaxazole (1.25/23.75µg) and chloramphenicol (30µg).

Gram-negative organism: discs tested were amoxicillin/clavulanate (20/10µg), ceftriaxone (30µg), ceftazidime (30µg), cefotaxime (30µg) gentamicin (10µg), ciprofloxacin (5µg), trimethoprim/sulphamethaxazole (1.25/23.75µg), imipenem (10µg) and meropenem (10µg).

For *P. aeruginosa*: discs which were used included ceftazidime (30µg), gentamicin (10µg), ciprofloxacin (5µg), piperacin-clavulanic acid (75/10µg), piperacillin (100µg), aztreonam (30µg) and meropenem (10µg).

For *Acinetobacter spp*: discs tested were ceftazidime (30µg), gentamicin (10µg), ciprofloxacin (5µg), tetracycline (30µg), ticarcillin-clavulanic acid (75/10µg), piperacillin (100µg), aztreonam (30µg) and meropenem (10µg).

Antibiotic disks will be applied on the surface of the plates at least 15mm apart from the edges of the plates to prevent overlapping of inhibition zones.

The *Staphylococcus* isolates were tested for methicillin resistance using cefoxitin as outlined by CLSI, 2019(37). All isolates with zone of inhibition ≤21 mm will be reported as methicillin resistant, ≥22mm will be considered as methicillin susceptible.

For extended spectrum β-lactamases (ESBL) detection, the initial screening and phenotypic confirmatory tests was done as recommended by the CLSI (36), which was used out to assess the ESBL of the significantly isolated isolates. For screening Inoculation on the MCA media containing ceftazidime 1.0 mg ⁄ L was done and incubated aerobically at 37 ºC for 24 hours. For confirmatory, which was done for the significantly isolated isolates, Ceftazidime (30µg) and ceftazidime + clavulanic acid (30/10µg) and Cefotaxime (30µg) and cefotaxime+ clavulanic acid (30/10µg) was placed on lawn culture of the organism on MHA and incubated overnight. Organism was considered as ESBL producer if there is a ≥5 mm increase in diameter of ceftazidime plus clavulanic disk and that of ceftazidime disk alone. *Klebsiella pneumoniae* ATCC 700603 was used as ESBL positive control strain.

2.11.5 Quality Control

All stains and reagents were clearly labelled, dated, and stored correctly. The operating temperature of the refrigerator and incubator were also be monitored and documented. All culture media was prepared according to the directions of the manufacturers and was tested for performance and sterility. To standardise the inoculums density of bacterial suspension for the susceptibility test, a 0.5 McFarland standard was used and standard reference strain *S. Aureus* (ATCC-25923), *S. Aureus* (ATCC 29213) for MRSA, sensitive, *E. coli* (ATCC-25922), *K. pneumoniae* (ATCC -700603) for ESBL and *P. Aeruginosa* (ATCC-27853) were used as control bacteria strains.

Multidrug resistant (MDR) was defined as acquired resistance to at least one agent in three or more antimicrobial categories. Antimicrobial categories tested were Cephalosporin class (ceftazidime, cefotaxime, ceftriaxone); Aminoglycosides class (gentamicin); Tetracycline class (Tetracycline); fluoroquinolones class (ciprofloxacin); folate pathway inhibitors class (co-trimoxazole); phenicols class (chloramphenicol); macrolides class (erythromycin) and lincosamides class (clindamycin) and Carbapenems (imipenem/meropenem).

2.12 Data Analysis

2.12.1. Data Management

Socio-demographic, clinical information and laboratory results of participants were crosschecked and coded before being entered into computer software. Data were edited, entered and analysed using statistical package for social science (SPSS) version 20.0.

2.12.2 Statistical Analysis

VAP incidence rate was calculated by: (Number of new cases with VAP/Number of ventilator days) x 1000.Nominal variables expressed as percentages, whereas quantitative variables were expressed as means, standard deviations or medians.

Frequency distribution and two-way tables were used to summarize the data on bacteria etiology and analysis using either Fisher´s exact or the chi square test was done.
Logistic regression was used to determine the risk factors associated with VAP. The fisher's exact test was employed to determine the association between VAP and patient outcome. P-value of < 0.05 was considered as statistically significant on the odds ratio within 95 Confidence Interval (CI). The result was presented in suitable tables and charts.

2.13 Ethical Consideration

Ethical clearance was obtained from MUHAS Ethical Review Board (MUHAS Senate Research and Publication Committee). Permission to conduct the study was obtained from MNH and MOI administration. The written informed consent to participate into the study will be sought from the parent or guardian of every participant since the patients were unconscious. Before the participants are asked to be recruited into the study, information sheet written in Swahili or translated to a language best understood by the guardian of the participant were provided. The informed consent was provided to briefly inform the participants about the aims, potential risks and benefits of the study. The

parents or guardians of each participant were given time to understand the study and discuss about the decision. If the participant chooses not to participate or to withdraw at any time, there was no subsequent complaints or consequences. If the participant chose for the patient to participate, the participant was asked to further sign the Informed Consent Form administered by the interviewer. The use of the data for future studies were also requested from the guardian's participants for their consent. All information from participants were handled with confidentiality.

2.14 Participants benefits

This study offered opportunity for the study participants to a series of clinical tests that helped to identify the type and sensitivity of the microbial pathogen responsible for VAP. This information was shared with the attending clinicians to help modify antibiotic therapy.

CHAPTER THREE

3.1 RESULTS

3.1.1 Basic demographic data and clinical characteristics of study participants.

Total number of patients recruited and kept in the Mechanical ventilator during study period was 334.In the first 48 hours 65 patients dropped out of the study due to various clinical and radiological outcomes. Therefore, a total 269 patients were included in the final analysis. The median age of the participants was 41 years IQR [35-48]. Majority were males 150 (55.8%) and MOI accounted for most of the participant (51.3%). The median duration of ventilation and hospital stay were 8 IQR [5-18] and 13 IQR [9-24] days respectively. The leading causes of ICU admissions were neurosurgical (Severe and moderate) and Cerebrovascular diseases (Hemorrhagic and Ischemic strokes), which accounted for about 36.8% and 16% respectively (Table 2). Majority of the patients reported no history of cigarette smoking 72.5% and most of them had no history of hospital admission in the past 6 months (69.14%).

Table 2: Basic demographic data and clinical characteristics of study participants

VARIABLES		
Variable	**Frequency**	**Percentage (%)**
Sex		
Female	119	44.24
Male	150	55.76
Age group		
15 to 30	58	21.56
31 to 60	152	56.51
60 and above	59	21.93
Location of the ICUs		
MNH	131	48.7
MOI	138	51.3
Smoking status		
NO	195	72.49

YES	74	27.51
Previous hospitalization within 6 months		
No	186	69.14
Yes	83	30.86
Primary diagnosis		
Cerebral vascular diseases	44	16.36
Endocrine diseases	14	5.2
Infectious diseases	32	11.9
Obstetric conditions	4	1.49
Other medical conditions	11	4.09
Renal diseases	16	5.95
General Surgical Conditions	36	13.38
Neuro Surgical Conditions	104	36.8
Malignant	13	3.35
Patients outcome from ICU		
Died	158	58.74
Discharged	111	41.26
Mode of intubation		
ETT	264	98.14
Tracheostomy	5	1.86
recent antibiotic used within 6months day		
No	124	46.1
Don't known	57	21.19
Yes	88	32.71
Median duration of Ventilation stays (IQR)	12	5 - 18
Mean duration of ICU stay	10 days	
Mean duration of Hospital stay before ICU	3 days	

3.2 Incidence of VAP among adults' patients admitted in the ICUs at MNH and MOI.

Out of 269 participants. A total of 112(41.6%) patients fulfilled the clinical criteria for the diagnosis of VAP. Out of 112 with clinically VAP, 82 met microbiological criteria of VAP.

Incidence of clinical VAP=New cases of clinical VAP/Number of ventilator days x 1000= 54.7/1000 ventilation days.

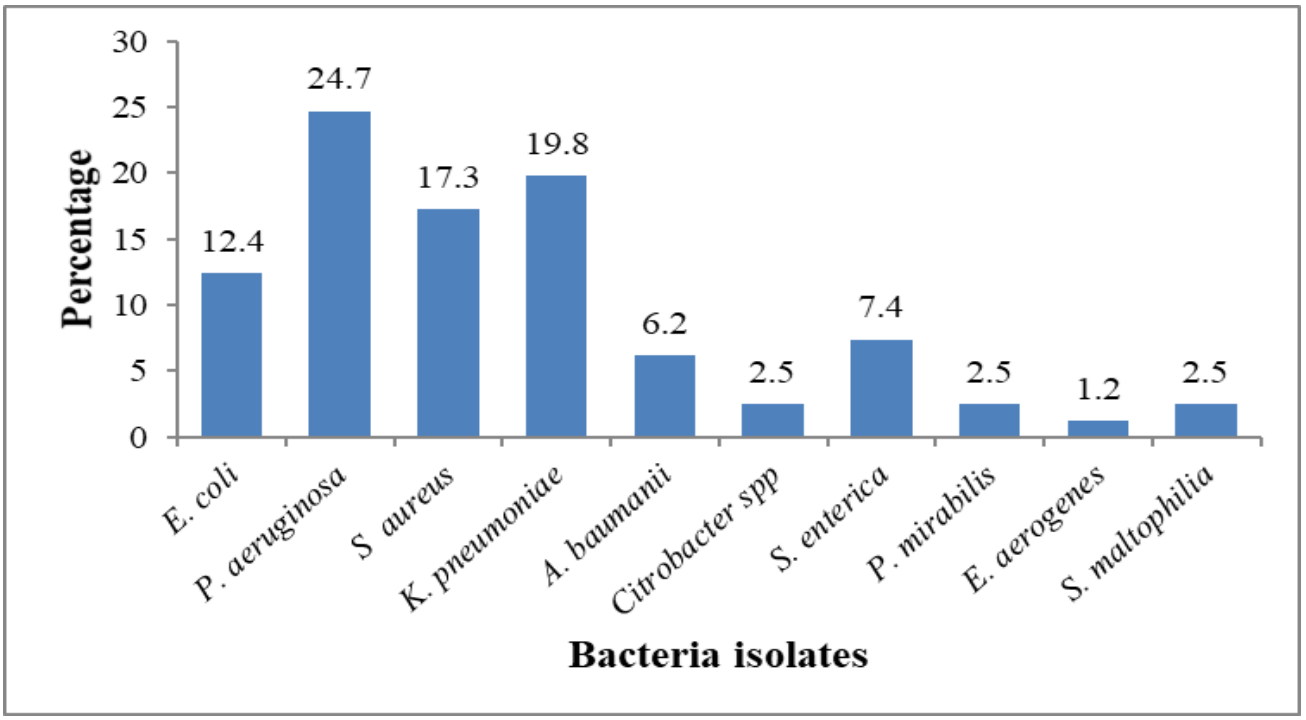
Incidence rate for microbiological confirmed VAP: -

Incidence rate (Incidence density) = New cases/ Number of ventilator days x 1000=40.1 / 1000 ventilation days

3.3.1 Bacteria aetiology of Ventilator Associated Pneumonia

A total of 85 bacteria were isolated from 82 patients. Most of the patients had monomicrobial isolates 79(96.3%). The common bacterial isolates identified were Gram-negative bacilli 68(80.1%), *Pseudomonas aeruginosa* accounted for 23(24.7%) of VAP cases followed by *K.pneumoniae* which was responsible for 17(19.8%) of all cases. Other Gram-negative bacilli isolated were *E.coli*(12.4%), *Acinetobacter baumanii(6.2%), P. mirabilis(2.5%), Enterobacter spp(1.2%),* and *S.maltophilia(2.5)* [Fig 3] . *S.aureus* (19.1%) was the only gram positive isolates identified.

Figure 2: Isolation frequency of pathogenic bacterial isolates causing VAP



3.3.2 Antimicrobial susceptibility patterns

A number of antimicrobial agents were used to test for sensitivity of the bacterial isolates from patients with VAP. *Pseudomonas aeruginosa* was least resistance to Imipenem (20.2%) as well to Piperazine-tazobactam (37.4%) but high resistance to cefepime (72.2%). *Klebsiella pneumoniae* showed low resistance the resistance to Imipenem (30.1%) but high resistant to Ceftriaxone (88.2%) and Other gram-negative bacteria were least resistant to Imipenem. Gram positive bacteria (*S.aureus*) was least resistant to (Cefoxitin 21.4%) but high resistant Doxycycline(70.2%).

Table 3: Bacteria isolates and their resistance patterns

Bacterial isolate (N)	Antimicrobial agents resisted (%)															
	CN	**CAZ**	**FEF**	**PIP**	**TAZ**	**IMP**	**ATM**	**CRO**	**AMC**	**DOX**	**FOX**	**SXT**	**CIP**	**MRP**	**E**	**CL**
***E. coli* (10)**	75.6	62.2	NA	NA	NA	NA	NA	71	61	NA	NA	NA	66.1	30.2	NA	NA
***K.pneumoniae(*17)**		40.7	43.5	68.5	NA	NA	NA	88.2	80.6	NA	NA	NA	71.4	30.1	NA	NA
***P. aeruginosa (*23)**	62.1	68.1	72.2	62.1	37.4	20.2	63.4	NA	NA	NA	NA	NA	NA	NA	NA	NA
***A. baumanii* (5)**	80.5	100.0	100.	NA	NA	50.0	100	NA	NA	NA	NA	NA	100	NA	NA	NA
***S.enterica*(6)**	68.4	67.6	NA	NA	NA	NA	NA	76	68	NA	NA	NA	65.9	46.2	NA	NA
OGNB (7)	61	63	NA	NA	NA	NA	NA	72	75.7	NA	NA	NA	73	33.3	NA	NA
S. aureus (17)	60.0	NA	NA	NA	NA	NA	NA	NA	60	71	24.6	66.5	70.2	NA	75	69

KEY: OGNB= Other Gram-Negative Bacteria, AMC = Amoxycillin/clavulanic acid, CRO = Ceftriaxone, CAZ = Ceftazidime, CTX = Cefotaxime, SXT = Co-trimoxazole, E = Erythromycin, CD = Clindamycin, CN = Gentamicin, CIP = Ciprofloxacin, MRP= Meropenem., TAZ piperacine tazobactam, PIP=Piperacine

3.3.4 Multidrugs Resistance Isolates

A.baumanii had highest MDR(100%) followed by *K. pneumoniae* and *P aeruginosa* among the gram negative. S. aureus had MDR (73.4%). Overall magnitude of MDR was at 71.8%. among the isolates.

Table 4: Multi-drug resistant bacteria isolated from ventilator associated pneumonia

Bacterial isolate (N)	Classes of antimicrobial resisted (%)			N	Average (%)
	R3	**R4**	**R5**	**R6**	
***E. coli* (10)**	5(71.4)	2(28.6)	-	-	7(70)
***K.pneumoniae*(17)**	5(38.4)	6(61.6)			13(76.4)
***P. aeruginosa* (23)**	8(47.1)	6(35.3)	1(5.8)	2(11.8)	17(73.9)
***A. baumanii* (5)**	1(20)	2(40)	2(40)	-	5(100)
***S.enterica*(6)**	-	4(100)	-	-	4(66.6)
OGNR (7)	2(50)	1(25)	-	1(25)	4(57.1)
***S. aureus* (17)**	4(30.7)	7(53.8)	2(11.5).	-	13((73.4)
Total	25(40.9)	28(45.9)	5(8.3)	3(4.9)	61(71.8)

Key: R3- R6 = resistant to 3, 4, 5, 6 classes of antimicrobials tested. Other GNR

3.4. Risk factors associated with VAP

A number of factors for developing VAP were assessed in the study. The anticipated predicting factors included age, sex, transfer ward, ICU location, ICU admission criteria, primary diagnosis, previous history of hospital admission and history of cigarette smoking. Univariate analysis revealed significant association between VAP and age above 60 (crude odds ratio(cOR 3.02,95% CI 1.33 to 6.84 p=0.008), smoking cigarette (cOR 1.88,95% CI 1.07 to 3.29, p = 0.028), previous hospitalization (cOR 95%,CI 3.87 to 19.28, p=0.001), primary diagnosis:-Cerebral vascular diseases(cOR 9.51,95% CI 1.42 to 63.73,0.02), neuro surgical conditions(cOR 5.23,95% CI 0.94-29.13,p=0.049) and malignancy (cOR 2.43,95% CI 2.02-667.92, p=0.028). However, on multivariate analysis only previous hospitalization(adjusted odd ratio 6.02,95% CI ,1.81-20.06, p=0.003)) smoking

cigarette(aOR 3.4 , 95% CI,1.22-9.45), cerebral vascular disease (aOR 6.09, 95% CI,2.16-62.22,p=0.004) and patients admitted due to neurosurgical conditions (aO 6.15,R95% CI 1.19-14.87,p=0.026) independently predicted VAP [table 4].Confounders were controlled in the analysis , the confounding factors controlled were age, sex , history of hospital admission in the previous six months and the transferring wards**.**

Table 5: Risk factors of Ventilator Associated Pneumonia

Variable				Bivariate analysis			Multivariate analysis		
	VAP	**Total (n)**	**P-value**	**cOR**	**95% CI**	**Pvalue**	**aOR**	**95% CL**	**P-value**
Sex			0.544						
Female	34 (28.6)	119		1					
Male	48(32.0)	150		0.97	0.37-2.52	0.66			
Age group			0.019						
<30	12 (20.7)	58		1					
31-60	44(28.9)	152		1.56	0.76-3.23	0.229	0.87	0.28-2.77	0.819
60 and above	26(44.1)	59		3.02	1.33-6.84	0.008	1.81	0.45-7.21	0.402
ICU location			0.777						
MNH	41(31.3)	131		1					
MOI	41(29.7)	138		1.9	0.15-22.88	0.622			
Smoking Status			0.027						
Non smoker	52(26.7)	195		1					
Smoker	30(29.7)	74		1.88	1.07-3.29	0.028	3.4	1.22-9.45	0.019
Hospitalization(6 months prior)			0.001						
No	32 (17.2)	186		1					
Yes	50 (60.2)	83		8.64	3.87-19.28	0.001	6.02	1.81-20.06	
Primary diagnosis			0.038						
Infectious diseases	3 (9.4)	32		1					
Endocrine diseases	1 (7.1)	14		0.07	0.01-1.02	0.073	0.74	0.07-7.84	0.805
CVDs	17(38.6)	44		9.51	1.42-63.73	0.020	6.09	1.60-23.12	0.008
Obstetrics	1 (25.0)	4		3.44	0.09-	0.494	3.22	0.25-41.25	0.370

conditions				18.67				
Renal diseases	4(25.0)	16	0.53	0.15-1.19	0.332	3.22	0.62 - 16.63	0.162
GSCs	4 (38.9)	36	6.71	0.94-29.13	0.050	6.15	1.57-24.08	0.009
NSCs	32 (31.1)	103	5.23	0.94-29.13	0.049	4.23	1.19 - 14.87	0.026
Malignancy	4 (44.4)	9	2.43	2.02-66.92	0.015	7.73	1.31 -45.51	0.024
Recent antibiotic (<6months)								
No	17 (9.4)	181	1					
Yes	65 (73.9)	88	13.2	3.85-47.63	<0.001			

Key: GSCs = General surgical conditions, NSCs=Neuro surgical conditions, CVDS= Cerebral vascular diseases

3.5.1 Factors associated with mortality among the patients on Mechanical ventilator

The overall mortality for patients on Mechanical Ventilator was 58.7%. Mortality was significantly higher among the patients with VAP (73.17%,) than among the patients who had no VAP, 52.4%. However, mortality was also higher among patients infected with MDR isolates (81.1%) than patients infected with non MDR isolates (45.8%). As these two groups were not similar in other aspects, so the excess mortality could not be attributed entirely to VAP. Bivariate analysis of showed there is a significant association between outcome with VAP (aOR 6.43 95% CI 1.67 to 4.4, P=<00.1) and as well as MDR (aOR 5.31, 95%, CI 2.59-23.69, p=<0.001) isolates.

Table 6: Factors associated with mortality.

				Bivariate analysis			Multivariate analysis		
Variable	**Mortality**	**Total (n)**	**P-value**	**cOR**	**95% CI**	**P-value**	**aOR**	**95% CL**	**P-value**
VAP			0.001						
Yes	60(73.2)	82		1					
No	98(52.4)	187		4.03	1.67 – 4.4	0.001	6.43	1.42-2.13	**0.001**
MDR Status			0.001						
MDR	47(81.1)	58		1					
Non-MDR	11(45.8)	24		7.88	2.59-23.69	0.001	5.31	2.12-7.70	**0.001**
Sex			0.496						
Female	65(54.6)	119		1					
Male	93(62.0)	150		1.91	2.14-6.411	0.621		0.12-8.65	0.671
Age group (Years)			0.230						
<30	20(20.2)	99		1					
31-60	31(27.4)	113							
≥60	44(77.2)	57		3.71	1.13-8.34	0.051	2.69	1.91-6.31	0.072
ICU location			0.148						
MNH	81(61.8)	131		1					
MOI	77(55.8)	138		0.83	0.31-2.24	0.725	0.91	0.01-3.21	0.561
Smoking Status			0.526						
Non smoker	47(67.1)	70		1					
Smoker	111(55.8)	199		3.51	1.09-11.12	0.210	3.12	1.23-8.12	0.101
Hospitalization (6 months prior)			0.216						
No	34(19.1)	178		1					
Yes	57(62.6)	91		4.9	1.39-2.13	0.113	1.23	1.21-3.11	0.312
Primary diagnosis			0.004						
Infectious diseases	13(65)	20		1					
Endocrine diseases	7(53.8)	13		1.4	0.10-18.11	0.399	1.9	1.02-21.21	0.221
CVDs	25(45.5)	55							
Obstetrics conditions	1(25)	4		0.61	0.21-83.33	0.512	0.8	0.11-8,42	0392
Renal diseases	9(56.25)	16		1.2	1.1-16.21	0.472	1.1	0.2-14.20	0.312
GSCs	24(64.9)	37		2	0.17-24.51	0.555	0.6	0.11-19.10	0.421
NSCs	69(60.0)	115		1.4	0.33-5.92	0.008	6.1	1.27-4.90	0.110
Malignancy	8(88.9)	9		3.38	2.13-66.21	0.023	5.1	1.21-68.11	0.212

Key: GSCs = General surgical conditions, NSCs=Neuro surgical conditions, CVDS= Cerebral vascular diseases

CHAPTER FOUR

4.0 DISCUSSION

4.0.1 Incidence

Overall incidence density in our study was 40.1/1000 ventilation days. The incidence in this study is relatively higher to global and most of regional incidences, compared this incidence to other studies which used similar methodology, the incidences are quoted between 9.5 to 29.5/1000 ventilation days in high income countries to low-mid income countries respectively[1,6,12].However, in a surveillance study by Kollel *et al*[3] on the epidemiology of VAP, the global incidence of VAP was reported to be 5.7/1000 with the regional incidence rate of 3/ 1000 ventilation days in the USA, 5/ 1000 ventilation days in Europe, 14/1000 ventilator days in Latin America and 7/ 1000 ventilation days in Asia Pacific.. Two studies done in Cairo Egypt and Durban, South Africa with similar methodology reported the incidence of VAP to be 22.1/ 1000 ventilation days and 27.4/ 1000 ventilation days respectively. Therefore, the incidence in our study still is relatively high compared to the studies done in Africa. Divergence of incidence can be attributed to several factors such as differences in the study population, differences in the definition of VAP, for examples clinical versus microbiological and possibly the use of preventive strategies in other studies. However, this high incidence provides and highlights on the need of adding other parameters on VAP prevention bundles currently not practiced our in the ICUs.

4.0.2 Bacterial isolates

In our study the most common bacteria isolated were gram negative bacteria. *Psudomonas aeruginosa* and *Klebsiella pneumoniae* accounted for almost half of all isolated cases (48.8%). A study done in S. Africa by J.Amel et also reported gram negative bacteria causing VAP, *A baumanii* being the leading cause[18]. Other studies done in the Northern Africa, Tunisia and Egypt, also reported gram negative bacteria, particularly *A.baumanii* and *P aeruginona* causing Ventilator Associated pneumonia in the Northern part of Africa [2,3].Some studies in Developed countries reported *S aureus*(MRSA) as the leading cause of VAP in developed countries however the change in pattern from gram positive bacteria

to gram negative bacteria has also been reported in several studies[23,17,21,18]. In a meta-analysis study done in Asia on bacteria isolates causing VAP majority at 95.7% of bacterial isolates were found to be Gram-negative bacilli, *Acinetobacter* spp. Accounted for 34.28% of VAP cases followed by *Pseudomonas aeruginosa* which was responsible for 25.71% cases [35]. In our study the magnitude of MRSA was 21%, thus most of the findings in other studies tally to the findings in our study.

4.0.3 Antimicrobial resistances

In our study the most of the isolates were multidrug resistant (MDR) to commonly prescribed drugs. The magnitude of MDR was around 71.8%. A number of studies reported the MDR gram negative and gram positive bacteria causing VAP, F.Fellal et all, reported around 47.3% of the isolates were MDR and majority of the MDR were gram negative[22]. In other review papers the reported MDR due to gram negative bacteria was about 22 to 58%, *P. aeroginosa* and *A baumanii* reported very high levels of MDR [33,42]. Thus, in our study the total MDR was 71.8%, this is higher compared to most of the reported studies on MDR isolates causing VAP in different settings. This could due hospital settings and levels, our study was conducted in tertiary referral hospitals and most of the patients are referral cases, and most of the patients with VAP had history antibiotics use prior to at least 6 months prior to hospital admission.

4.0.4 Factors associated with VAP

A panel of factors for determining of Ventilator Associated Pneumonia were assessed in the study. However, on multivariate analysis only previous hospitalization (adjusted odd ratio 6.02,95% CI ,1.81-20.06, p=0.003)) smoking cigarette (aOR 3.4, 95% CI,1.22-9.45), cerebral vascular disease (aOR 6.09, 95% CI,2.16-62.22, p=0.004) and patients admitted due to neurosurgical conditions (aOR 6.15, R95% CI 1.19-14.87, p=0.026) independently predicted VAP. The recent studies showed increased risk of developing Ventilator Associated Pneumonia with increased age, type of the ICUs, medical conditions, traumatic brain injury, previous antibiotics use, the type of endo tracheal tubes used, history of antibiotics use as well patients with COPDs [37, 23]. In many studies report COPDs as the predictors of VAP, cigarette smoking in this study could give a clue on how cigarette

smokers could be associated with VAP. The differences in other factors could be to population differences between the study populations.

4.0.5 Factors associated with outcomes of the patients on mechanical ventilator

The overall mortality for patients on Mechanical Ventilator is 58.7%. Mortality on the patients who developed VAP was observed to be at 78% while Mortality in non VAP group was comparatively low at 52.4%, mortality was higher compared to studies done in other developing countries which report mortality range between 22 to 48.3% [11,2,44] The overall mortality associated with VAP was observed to be 48.33%. Mortality in non VAP group was significantly low at 20%. [2]. High mortality in our study could be due to population in which our study was conducted, included both medical and surgical (general surgical and neurosurgical) cases thus mortality could be contributed to other causes.

However, mortality was also higher among patients infected with MDR isolates (81.1%) than patients infected with non MDR isolates (45.8%). The mortality is higher compared to other studies where mortality was reported at 47% and 51% [3,22]. The high mortality in our study could be due to the fact that our study was conducted at the tertiary and national level hospitals, thus the patients were brought in critical states and the patients had history of use of antibiotics prior which could cause such high levels of MDR.

Several factors have been reported to predict the outcome of the patients in the mechanical ventilator such factors included age, primary diagnosis, MDR and VAP [19,43]. In our study MDR and VAP independently predicted mortality among the patients on MV.T Puskin N et al

[41] reported the MDR and VAP as factors predicting mortality among the patients admitted in the ICU in a study done in Saudi Arabia. A. A Behali et all reported in South Africa that VAP significantly worsened the outcomes of patients on the MV in the ICUs [40]. This could be due the prior use of antibiotics to most patients who were infected with MDR in our study population.

CHAPTER FIVE

5.0 CONCLUSIONS

The study revealed High incidence among the patients with VAP admitted in in four major ICUs setting at MNH and MOI for mechanical ventilation due to various conditions.
Majority of the Isolate are Gram negative bacteria and MDR. Cigarette smoking, having neurosurgical conditions and general surgical conditions have shown to be significantly associated with VAP. The overall mortality for patients on Mechanical Ventilator was 58.7%. Mortality was significantly higher among the patients with VAP (73.17%).

5.1 RECOMMENDATIONS

1. Due to high incidence of VAP, there should be an established standard protocol (Algorithms) to identify patients at risk of Ventilator associated pneumonia
2. Majority of the isolates were the MDR, therefore the is a need to review the antibiotics practices in the ICUs and establish an evidence based empirical antibiotics
3. A couple of risk factors have been associated with VAP, such factors include traumatic and general surgical conditions. Thus, the VAP prevention bundles should be instituted and monitored closely among the patients with traumatic and general surgical conditions
4. The mortality among the patients with VAP in this study is very high and therefore, there is a need for both increase in parameters in VAP prevention bundles currently missing in practices in the ICUs.

5.2 LIMITATIONS

5.2.1 Study Limitations

1. Portable Chest X rays' machines were not always available and some patients had no base line Chest X rays' radiographs.
2. The patients were randomly selected due to my study design and therefore some of the patients may be missed out.
3. The VAP caused by other microbial organisms such as viruses and fungi were missed out.
4. The tertiary hospital settings and therefore the results could not be generalized in other health care settings.

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APPENDICES

Appendix I: Consent Form – English Version

Introduction

I am Dr Helmut Nyawale, a researcher from Muhimbili University of Health and Allied Sciences (MUHAS). I am conducting a study titled '**Incidence and bacterial atiology of ventilator associated pneumonia among the patients on Mechanical Ventilator in ICU at the Tertiary hospitals, Tanzania**'. The aim of this research seeks to estimate the incidence, bacteria aetiology and sensitivity patterns among the patients with ventilator-associated pneumonia in order to provide evidence for improved and standardized intensive care for patients on mechanical ventilation in ICU settings in Tanzania.

Participation in the study

You are kindly requested to participate in this study. If you accept to participate in this study your particulars/information will be taken and used for the purpose of this research and this will certainly not bother you or cause any discomfort to you. Your participation in this study will involve the following: Taking your/patients' records from clinical notes, being directly observed and investigated for the course of disease development during and after ICU stay to their respective wards.

Confidentiality

You are strongly assured of the confidentiality of the information obtained that will only

be used for the purpose of this research and anonymity will highly be observed when collecting data and compiling report. To assure you, even your name will not be required to appear in the questionnaire.

Risk to participants

The study will involve taking the specimens from the patients and therefore the risks such as pain during blood drawing as well as during BAL will be expected.

Benefit to the participants

This study will offer the microbiological tests whereby the results for bacterial aetiologies and drug sensitivities will be shared back and used in the management of the patients.

Right of participation in the study.

Your participation is absolutely voluntary and there is no penalty for refusing to participate. You are free to ask any question and you may stop to participate in this study any time.

Contact Person

Dr. Helmut Nyawale (071794485) is a key contact person with regard to any queries about this study. If you have any questions/concerns about your rights as a participant you may contact the chairman of the university senate research and publications, MUHAS P.O.BOX 65001, Dar es Salaam. Telephone; 2150302-6

Signing of the consent

If you agree to participate in this study, please sign in this consent form.

I (initials)……have read and understood the contents of this form and I have been given satisfactory explanation with all my questions answered. I therefore consent to participate in this study.

Signature of interviewee .....……Date.....[**Guardian/next of kin**]

Signature of interviewer .....Date ……

Appendix II: Consent Form – Swahili Version

Utangulizi

Mimi naitwa Dkt. Helmut Nyawale, mtafiti kutoka Chuo Kikuu cha Sayansi ya Tiba Muhimbili. Ninafanya utafiti kuhusu wagonjwa walioko kwenye machine za upumuaji "Ukubwa, maambukizi ya, pamoja na aina za usugu wa dawa ambazo zinaweza kutibu kwa wagonjwa walioko kwenye mashine za upumuaji katika wodi za wagonjwa mahututi, katika hospitali za rufaa Tanzania". Lengo la utafiti huu ni kubaini ukubwa wa tatizo, aina ya maambukizi ya bacteria na usugu wa dawa kwa tatizo la nimonia kwa wagonjwa walioko kwenye mashine za upumuaji katika hospitali za rufaa, Tanzania.

Kushiriki katika utafiti huu

Tafadhali unaombwa kushiriki katika utafiti huu, na mara tu utakaporidhia, una hakikishiwa kuwa habari zako na maelezo utakayotoa yatatumika kwa makusudio na malengo ya utafiti huu tu na kuwa hii haitakuletea usumbufu wowote.

Usiri wataarifa za mshiriki

Unahakikishiwa tena kuwa taarifa zozote zitakazopatikana kutoka kwako wakati wa utafiti huu zitapewa usiri mkubwa sana na hazitatumika kwa malengo mengine yeyote tofauti na utafiti husika. Kuhakikisha hilo dodoso litakalohusika halitakuwa na jina lako wakati wote wa utafiti na hata baada ya utafiti.

Athari za utafiti huu kwa mshiriki

Kwasababu katika utahusisha uchukuaji wa sampuli kutoa kwa mshiriki kama vile damu na Bronchoalveolar lavage, mshiriki atapata athari mbalimbali kama vile maumivu wakati wa kuchukua sampuli.

Faida za utafiti kwa mshiriki

Majibu ya aina ya bakteria na usugu wa dawa yatarudishwa kwenye vyumba mahututi husika kusaidia matibabu ya mshiriki**.**

Haki ya kushiriki au kutoshiriki katika utafiti huu

Ushiriki wako katika utafiti huu ni wa hiari kabisa, unayohaki ya kushiriki au kutoshiriki bila kulazimika. Pia unayohaki ya kukataa kuendelea kushiriki/kuacha Kujibu maswali wakati wowote utakapojisikia kufanya hivyo na hakutakuwa na hatua yeyote itakayochukuliwa dhidi yako au kulaumiwa kwa kufanya hivyo.

Mawasiliano

Wasiliana na mtafiti mkuu, Dkt. Helmut Adrian Nyawale, kwa simu namba 0717944855

Wakati wowote utakapokuwa na maswali au jambo lolote lenye kuhitaji ufafanuzi kuhusu utafiti huu.

Kukubali kushiriki

Ukikubali kushiriki tafadhali thibitisha kwa kujaza na kusaini sehemu ya fomu hii hapa chini.

Mimi (Herufi za mwanzo za majina yako) ……nimesomewa na kuelewa yaliyomo kwenye fomu hii na maswali yangu yote yamejibiwa vizuri. Hivyo ninakubali mwenyewe kwa hiari yangu bila kushurutishwa au kushawishiwa kushiriki

katika utafiti huu.

Sahihi ya mhojiwa …… Tarehe
…….....……[Mzazi(mlezi)/ Ndugu wa karibu]

Sahihi ya mhoji ……..... Tarehe
……

Appendix III: Questionnaire – English Version

Part 1: Patients Particulars and ICU Care

1.Date of hospital admission

2.Date of ICU admission and transfer

3. Patient ID:

4. Age of the patient (year)……

5. Sex a) Male b) Female

6. Transferring ward (a) EMD (b) Theatre (c) Medical (d) Surgical (e) Obstetrics(f) Gynaecology (g) Others

7. Location of ICU: a) Mwaisela b) Maternity c) JKCI, d) Bugando Adult ICU e) MOI

8.Primary Diagnosis……...

9.Other Comorbid Conditions…….

10. Recent antibiotics therapy 3 months prior to admission a) Yes b) No

11. Place of intubation a) ICU b) Theatre c) EMD d) Ward e) Others

12. Mode of intubation: a) Orotracheal b) Nasotracheal c) Tracheostomy

13.Date of intubation .....……

14Date of MA removal…….

15. Antibiotics given in ICU, dosage, frequency: ……

Antibiotics after culture and sensitivity results if any, dosage, frequency

16.Previous hospitalization…. a) yes b) no

17. Patients previous history of cigarette smoking

18. Date of transfer from ICU to the wards…….

19. Patient outcomes from ICU……. a) discharged b) death,

20. Patient outcomes from wards…… a) discharged b) death,

21. The date of Discharge or death from the ward…….

Appendix IV: Dodosa la Kiswahili

1. Tarehe ambayo mgonjwa alilazwa hospitali:……

2.Tarehe aliyolazwa chumba cha wagonjwa mahututi……

3.Namba ya mgonjwa

4. Umri wa mgonjwa

5. Jinsia a) Mwanamke b) Mwanaume

6. Wodi ambayo ametokaa) EMD b) Theatre c) Medical d) Surgical e) Obstrestrics f) Gynaecology f) Nyinginezo

7. Jina la wodi ya wagonjwa mahututi: a) Mwaisela b) Maternity c) JKCI d) Bugando Adult e) MOI

8. Ugonjwa uliosababisha kuwekewa mashine za kupumulia……...

9. Magonjwa mengine aliyonayo……..

10. Matumizi ya antibiotics hivi karibuni (miezi mitatu iliyopita) a) Yes b) No

11. Eneo alilofanyiwa intubation a) ICU b) Theatre c) EMD d) Ward e) Others

12. Namna ya intubation: a) Orotracheal b) Nasotracheal c) Tracheostomy

13. Tarehe ya kuwekewa mashine y akupumulia……

14. Tarehe ya kutoa mashine ya kupumulia…….

15. Aina ya antibiotiki aliyopewa, kiasi, mara ngapi:

……

Aina ya antibiotiki aliyopewa baada ya majibu ya Culture and sensitivity, kama mabadiliko yalifanyika, kiasi, mara ngapi…….

16. Histolia ya kulazwa hapo nyuma….....…

17 Historia y auvutaji wa sigara kwa mgonjwa……..

18. Tarehe ya kuamishwa kutoka Chumba cha wagonjwa mahututi kwenda wodini.....

19. Matokeo ya mgonjwa katika chumba cha wagonjwa mahututi a) Ameruhusiwa kurudi wodini b) Amefariki

20. Matokeo ya mgonjwa katika wodi husika a) Ameruhusiwa b) Amefariki

21. Tarehe yamatokeo ya mgonjwa katika wodi……

Appendix V: Ethical letter

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
OFFICE OF THE DIRECTOR OF POSTGRADUATE STUDIES

P.O. Box 65001
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Ref. No. DA.287/298/01A/

7th August, 2019

Dr. Helmut Adrian Nyawale,
MMed. Microbiology/Immunology,
School of Medicine,
MUHAS.

RE: APPROVAL OF ETHICAL CLEARANCE FOR A STUDY TITLED: "INCIDENCE AND BACTERIAL AETIOLOGICAL PATTERNS OF VENTILATOR ASSOCIATED PNEUMONIA AMONG A PATIENTS ON MECHANICAL VENTILATOR IN INTENSIVE CARE UNITS AT TERTIARY HOSPITAL"

Reference is made to the above heading.

I am pleased to inform you that, the Chairman has, on behalf of the Senate, approved ethical clearance for the above-mentioned study. Hence you may proceed with the planned study.

The ethical clearance is valid for one year only, from **06th August, 2019 to 05th August 2020.** In case you do not complete data analysis and dissertation report writing by **05th August, 2020,** you will have to apply for renewal of ethical clearance prior to the expiry date.

Dr. Emmanuel Balandya
ACTING: DIRECTOR OF POSTGRADUATE STUDIES

cc: Director of Research and Publications, **MUHAS**
cc: Dean, School of Medicine, **MUHAS**

Appendix VI: Introduction letter

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
OFFICE OF THE DIRECTOR OF POSTGRADUATE STUDIES

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Ref. No. HD/MUH/T.298/2017

09th August, 2019

The Executive Director,
Muhimbili National Hospital,
P.O. Box 65000,
DAR ES SALAAM.

Re: INTRODUCTION LETTER

The bearer of this letter Dr. Helmut Adrian Nyawale, is a student at Muhimbili University of Health and Allied Sciences (MUHAS) pursuing MMed. Microbiology/Immunology.

As part of his studies he intends to do a study titled: " ***Incidence and Bacterial Aetiological Patterns of Ventilator Associated Pneumonia Among A Patients on Mechanical Ventilator In Intensive Care Units At Tertiary Hospital.*** The research has been approved by the Chairman of University Senate.

Kindly provide him the necessary assistance to facilitate the conduct of his research.

We thank you for your cooperation.

Ms. Sharifa Kamby
For: DIRECTOR, POSTGRADUATE STUDIES

cc: Dean, School Medicine, **MUHAS**
c.c. Dr. Helmut Adrian Nyawale

Appendix VII: Permission letter

MUHIMBILI NATIONAL HOSPITAL

Cables: "MUHIMBILI"
Telephones: +255-22-2151367-9
FAX: +255-22-2150534
Web: www.mnh.or.tz

Postal Address:
P.O. Box 65000
DAR ES SALAAM
Tanzania

In reply please quote:

MNH/TRCU/IRB/Permission/ 2019/161 **30th August, 2019**

Head of Department
- Internal Medicine ✓
- Obstetrics and Gynaecology

Muhimbili National Hospital

HOD. Critical Care & Pulmonology. Kindly facilitate 16/9/19

RE: PERMISSION TO COLLECT DATA AT MNH.

Name of Student	**Dr. Helmut Adrian Nyawale**
Title	**INCIDENCE AND BACTERIAL AETIOLOGICAL PATTERNS OF VENTILATOR ASSOCIATED PNEUMONIA AMONG ON MECHANICAL VENTILATOR IN INTENSIVE CARE".**
Institution	Muhimbili University of Health and Allied Sciences
Supervisors	**Dr. Helmut Adrian Nyawale** **Dr. Mabula Kasubi**
Period	30th August 2019 to 30th February 2019

Approval has been granted to the above mentioned student to collect data at MNH.

Named MNH based supervisor must ensure that the student abide to the ethical principles and other conditions of the research approval.

Sincerely,

Dr. Faraja Chiwanga
Head of Teaching, Research and Consultancy Unit

HEAD, TEACHING, RESEARCH & CONSULTANCY UNIT
P. O. Box 65000
DAR-ES-SALAAM
MUHIMBILI NATIONAL HOSPITAL

c.c DMS
c.c DSS
c. c Dr. Helmut Adrian Nyawale
c.c Dr. Mabula Kasubi

26/9/19.

All correspondence to be addressed to the Executive Director

Dear incharge of ICU 1, 2 & 3S, please accept this Doctor who is doing Theses on VAP, give necessary assistance. Dr Chiwa

Appendix VIII: Approval for permission

MUHIMBILI ORTHOPAEDIC INSTITUTE

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OFFERING SERVICES IN ORTHOPAEDICS, NEUROSURGERY AND TRAUMATOLOGY

AB.145/146/01A/129 20th August, 2019

Director, Postgraduate studies
MUHAS
P.O.BOX 65001
DAR ES SALAAM

RE: APPROVAL FOR PERMISSION TO CONDUCT RESEARCH.

Reference is made to your letter dated 9th August 2019 with reference NO:HD/MUH/T.298/2017 captioned the above subject matter.

I am pleased to officially inform you that, your request for Dr. Helmut Adrian Nyawale to conduct a research heading "**Incidence and bacterial A etiological Patterns of Ventilator Associated Preneumonia Among A Patients on Mechanical Ventilator in Intensive Care Unit At Tertiary Hospital** " has been approved. Therefore you are requested to inform the student to start his research.

It's my hope that you will extend enough cooperation regarding this matter.

With regards

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Abdallah Mbuguni
For: Executive Director

TRAINING OFFICER
MUHIMBILI ORTHOPAEDIC INSTITUTE
P. O. Box 65474
DAR-ES-SALAAM

Cc: MD-MOI
Cc: Dean school of Medicine-MUHAS
Cc: Dr.Helmut Adrian Nyawale

Kindly allow him to proceed
7/10/19

All correspondences to be addressed to the Executive Director