PATTERN OF BACTERIAL COLONIZATION AND ANTIMICROBIAL SUSCEPTIBILITY OF TRACHEAL ASPIRATES IN TRACHEOSTOMISED PATIENTS AT THE KENYATTA NATIONAL HOSPITAL

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H58/74944/2014

A dissertation submitted in part fulfilment of the requirements for the Degree of Master of Medicine in Otorhinolaryngology, Head and Neck Surgery, University of Nairobi.

STUDENT'S DECLARATION

This is my original work which has not been presented for a degree award at any other university.

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ABBREVIATIONS

CT SCAN:	Computer Tomography Scan.
CLSI:	Clinical and Laboratory Standards Institute.
EGNB:	Enteric gram-negative bacteria.
ENT:	Ear, Nose and Throat.
ICU:	Intensive Care Unit.
KNH:	Kenyatta National Hospital.
SPSS:	Statistical Packages for the Social Sciences.
UON:	University of Nairobi.

ABSTRACT

Background:
Tracheostomy is an operative procedure that creates a surgical airway in the cervical trachea. Tracheostomy is vital in airway management in both emergency and elective situations. The airway in healthy non tracheotomised individuals is protected from colonization by bacteria through the filtering mechanism of the upper airway. This is bypassed in tracheostomy leading to colonization of the trachea predisposing one to lower respiratory tract infections. Indiscriminate use of antibiotics in treatment of the infections has led to emergence of antibiotic resistance.

Study Objective: To determine the pattern of bacterial colonization and antimicrobial susceptibility in tracheal aspirates of tracheotomised patients at the Kenyatta National Hospital.

Study Design: Cross sectional study

St**udy Duration:** 12 months

Study setting: The Kenyatta National Hospital Operating Theatres, Intensive Care Unit & wards.

Methodology: Eighty eight patients undergoing open tracheostomy were recruited using consecutive sampling technique after obtaining an informed consent/assent. An open surgical tracheostomy was done under strict aseptic conditions. A sterile suction catheter was introduced into the trachea through the tracheostomy tube and suction done to clear the secretions. The secretions within the suction catheter were emptied into a sterile container by pushing 5mls of normal saline and transported to laboratory within 1 hour for bacteriological analysis; the same procedure was repeated on day 7.

Data Analysis and Results: Data analysis was done using SPSS version 22. 88 patients aged between 14-83 years with a mean age of 52.1years were studied. Mechanical obstruction was the commonest indication. The trachea was colonised on day zero in 11.36% and 61.36% in day 7, ($P<0.001$) using independent sample T-test. Gram negative bacteria predominated; *Pseudomonas aeruginosa* 28.6%, *Acinetobacter baumanii17*.9%, *Enterobacterioceae* 12.5%. The commonest gram positive bacterium isolated was *Staphylococcal aureus* at 8.9%. Both gram negative and gram positive bacteria were resistant to amoxicillin, Cefotaxime, Ceftriaxone and Amoxicillin Clavulinic acid. No Vancomycin resistance was found.

Conclusion and Recommendation: Post tracheostomy colonization occurs early in the first week. There is an overwhelming resistance to commonly used antibiotics such as penicillins and cephalosporins at KNH. Serial tracheal aspirate cultures should be incorporated in the care of post tracheostomy patients.

1.0 CHAPTER ONE: BACKGROUND

1.1 Introduction

Tracheostomy is an operative procedure that creates a surgical airway in the cervical trachea[1]. The trachea acts as a conduit between the upper respiratory system and the lungs. The trachea delivers warm, moist filtered air into our lungs while Carbon dioxide and sputum are expelled. Tracheostomy is indicated in several conditions: Mechanical airway obstruction due to: tumours, trauma, vocal cord paralysis and congenital anomalies[2, 3], Protection of tracheobronchial tree in patients at risk of aspiration as these patients have inefficient swallowing and cough gag reflex, Prolonged intubation[4] , Respiratory failure due to reduced respiratory effort and Pulmonary toilet in patients with reduced respiratory effort.

1.2 The Concept of Respiratory Tract Microbiome

The human upper respiratory tract is colonised by complex and dynamic microbial organisms with the oral cavity harbouring the most predominant group. These organisms are thought to be the first line of immune defence by acting as immune primers and occupying the binding sites for pathogenic bacteria. Healthy adult nose is colonised by the *acinetobacteria (corynebacterium, propionibacerium*) and *staphylococcus* species. The orophanynx is predominantly colonised by *streptococcus* and *staphylococcus* species[5].Under normal physiologic conditions a delicate balance is maintained limiting the quantity and dominance of any single organism. The pathogenic community of the upper respiratory tract includes; *streptococcus pneumonia, staphylococcus aureus, maraxella catarrharis* and *hemophilus influenza*[6]. They become opportunistic in the lower airway when the host defences are impaired secondary to local mucosal characteristics such as crusting, change of PH, epithelial cell architecture changes and micro-aspirations. Acquisition of exogenous DNA by the *streptococcus* and *hemophilus* species leads to development of antibiotic resistance and persistent colonization[7].

The lower respiratory tract is considered sterile in healthy people however we are exposed to 10^5 micro-oganisms per day through aerosols. This exposure and subclinical micro-aspirations from the oropharynx predisposes one to colonization. The lower tract harbours a diverse interaction of micro-biomes but at a lower biomass[8]. The lower respiratory tract has been predominately demonstrated to have *enterobacteriociae, hemophilus* and *methyllobacterium* and Obligate anaerobes *prevotella* and *veillonela* which are also

predominant in inflammatory conditions but their role in the lower respiratory tract has not been well understood[9]. These organisms first colonise the trachea before they may cause any disease.

1.3 Prerequisites of Bacterial Colonization

The bacteria adhere to the mucosal surface avoiding clearance by the mucocilliary escalator system[10]. Some bacteria bind to glyco-conjugates on the mucosal surface and use their negatively charged polysaccharide capsule to repel the anionic mucus. Gram positive bacteria use lipoteichoic acids and M proteins for mucosal attachment. Gram negative bacteria (*enterobacteriociae, pseudomonas* and *Hemophilus* species) use pilli to bind host epithelial cells. The pilli also help in extending adhesins beyond the capsule on to the host [11]. After attachment to the host the colonising organisms express a variety of sugar transporters and utilize genes to obtain nutrients for replication.

The indiscriminate use of antibiotics has led to development of antibiotic resistance. Antibiotics eliminate susceptible commensals leading to resistant varieties filling the niche. Depending on the organism's tolerability, local host factors and development of favourable genes these resistant varieties effectively and rapidly multiply. There are several mechanisms of resistance; change of permeability to drugs by increasing efflux in Tetracyclines and impermeability in amino glycosides, development of altered cell target e.g. loss of ribosomal 30s subunit in amino glycosides and 50s subunit for Erythromycin resistance. Micro-organisms such as *staphylococcus* develop drug destroying enzymes e.g. beta lactamase to Penicillin G. Transformation of metabolic pathways enables optimum utilization of local nutrients by the bacteria. Healthcare personnel implicated in the spread due to failure to observe appropriate infection control techniques as they act as vehicles for patient to patient transfer of bacteria[12]

1.4 Host Barriers to Colonization

The respiratory system has natural barrier mechanisms which prevent colonization and infection. The nasal hairs that trap dust and micro particles, the mucociliary escalator system line the mucosa and aid in clearing secretions. The intercellular apical junctional complexes regulate paracellular permeability while airway epithelial cells secrete antimicrobial peptides. The normal flora primes the immunity and occupies binding sites. The cough mechanism expectorates mucus and particulate matter. The phagocytic cells destroy aspirated

microorganisms. The turbulent airflow impacts large particles on the mucosal surfaces. The particles are humidified increasing in size making them difficult to pass to lower airways and enhancing phagocytosis. The airway mucus is continuously secreted by intraepithelial goblet cells and mucus cells of sub-mucosal glands. It contains antimicrobial substances (lysozyme and defensins), cytokines and antioxidants[13]. This respiratory mucus contains large highly charged and cross linked glycoproteins providing a mucous barrier.

1.5 Predisposing Factors in Tracheostomised Patients

Colonization and infection of the airway after tracheostomy predisposes to serious complications as well as morbidity and mortality [14, 15]. The presence of tracheostomy tube bypasses the protective function of the nasopharynx namely; humidification, air filtering and warming. This leads to airway irritation, increased secretions and crusting. The stoma allows direct access of bacteria into the lower respiratory tract[16]. Failure to follow aseptic technique leads to nosocomial colonization during suctioning and tracheostomy tube care. The presence of the tracheostomy tube has a foreign body effect which leads to increased secretions. The splinting effect of the tracheostomy tube impairs the mucocilliary escalator system leading to stagnation of secretions and bacteria multiplication. The suction tube insertion may lead to mucosal trauma offering entry points for bacteria. Leakage of secretions around the tube, inability to clear secretions due to impaired cough reflex and aspiration of organisms from the colonised oropharynx and gastro-esophageal reflux leads to further bacterial dissemination to the lower respiratory tree. This is evidenced by a high number of enteric gram negative organisms colonising the airway of tracheostomised patients[16, 17].

Stoma site infections and pneumonia occur after tracheostomy. A study carried out by Karuga[18] found pneumonia as the commonest post-operative complication followed by stoma site infection at the Kenyatta National Hospital. Biofilms have been identified in more than 90% of tracheostomy tubes as early as seven days post insertion[19]. Evaluation and identification of these organisms is important to standardise the empiric antibiotic regimen[20].

2.0 CHAPTER TWO: LITERATURE REVIEW

Tracheostomy has been associated with lower respiratory tract colonization. Studies conducted in immediate postoperative and the first week have shown growth of both gram positive and gram negative bacteria.

Study by Morar P et al[21] described two groups of potentially pathogenic microorganisms that may colonise the trachea post tracheostomy. First group includes community acquired organisms which are; *Streptococcal pneumoniae, Hemophilus influenza, Moraxella catarrhalis, Escherichia coli,* and *Staphylococcal aureus*. These organisms are found in previously young healthy patients. Second group includes hospital acquired bacteria in patients with underlying acute or chronic conditions and/or infections with the bacteria identified as *pseudomonas, acinetobacter species, klebsiella, enterobacter* and *Methicilin-Resistant S aureus (MRSA*). R. Acharya[22] conducted a study in 30 tracheostomised patients. Tracheal swabs were obtained during tracheostomy and day seven whereby 60 tracheal swabs were analysed out of which 68 isolates were grown. Nine cases had sterile cultures; 6 cases from day 0 and 3 cases from day seven. *Pseudomonas aeruginosa* was the commonest isolate (39.7%) followed by *Acinetobactor anitratus*(27.94%) and *staphylococcus aureus*(19%) . Others *were klebsiella pneumonia* and *enterococcus species*. They reported 5 cases of stoma infections from which 3 developed pneumonia. The colonization during tracheostomy was attributed to nosocomial transmission during the hospital stay. The study showed that *pseudomonas aeruginosa, Acinetobactor anitratus* and *staphylococcus aureus* are the commonest nosocomial colonisers of hospitalised tracheostomised patients.

The colonizing bacterial has been isolated in lower respiratory infections postulated to be as a result of tracheostomy. P. Koirara[23] found that gram negative bacteria were the predominant colonisers in tracheostomised patients leading to tracheobronchitis or pneumonia. He took 50 tracheal aspirates from tracheostomised patients who presented with fever of more than 38^{o}C. 90% of these sample cultures grew 67 pathogens. There were 42 cases with polymicrobial growth. *Pseudomonas aeruginosa* was the commonest at 40 % and the EGNB 40% (*eschelichia coli, klebsiella pneumonia, klebsiella oxytoca, enterobacter cloacae*) followed by *Staphylococcal aureus (*10%). The *pseudomonas aeruginosa* was found to be more sensitive to Amikacin (81%) followed by Ciprofloxacin (70%) and Gentamicin (66%). The 20% resistance to amikacin is attributed to an enzyme N acetyl transferase that hydrolyses amikacin. All *Pseudomonas aeruginosa* isolates were resistant to Cefotaxime. The enteric

gram negative bacteria isolates were sensitive to Chloramphenicol (74%),Amikacin (74%) and Gentamicin (55.55%). *S. Aureus* was 100% sensitive to Vancomycin and resistant to Ampicillin. Methicillin resistant *Staphylococcal aureus* was found in 50% of *staphylococcal aureus* isolates. These results showed that 55% of the EGNB and 55% of gram positive bacteria were multidrug resistant. The high prevalence of *Pseudomonas Aeruginosa* colonization is attributed to mechanical injury to the tracheal mucosa due to tracheostomy tube suctioning that exposes the binding sites. Binding is enhanced by the carbohydrate produced by novel tracheobronchial cell during repair[24].

The lower respiratory colonization has been demonstrated as early as the first week. Aswin M. et al[25] analysed 130 patients whose mean age was 57.2 years with their commonest indication for tracheostomy being upper airway obstruction due to head and neck malignancies. They also had impaired cell mediated immune response. He took samples during tracheostomy procedure (day 0) and day 7. No growth was obtained from 94.6 % of samples analysed (n=123) on day 0. The positive cultures were isolated in 7 patients who had been previously intubated. The organisms grown were; *Acinetobacter* (3.9%) and *klebsiella spp* (1.5%). However on day 7, 86.9% samples (n=113) yielded positive results with *Pseudomonas aeruginosa* (47.7%), *Klebsiella pneumonia* (18.5%), and *S. aureus* (11.5%) ,*Acinetobacter species* (7.7%) were mainly isolated. The others were; enterobacter species and *diptheroids*. He found a high statistical significance in day 0 and day 7 growths ($p<0.001$).

In a retrospective case series study carried out in Hong Kong China between 2010 and 2015 in tracheostomised children with severe neurological impairment, 36 cases were studied and the most prevalent bacteria causing lower respiratory tract infection were found to be *Pseudomonas aeruginosa* 92%, Non typeable *Hemophilus influenza* 75% and *Moraxella catarrhalis* 75%. Others isolated were *S. aureus, acinetobacter spp, diptheroids* and *E. coli*. This was attributed to nosocomial colonization[26].

The exogenous route is implicated as a source of bacteria as opposed to the endogenous route. Hemanth Rao M et al[27] did a comparison of bacterial flora of the oropharynx and lower respiratory tract during tracheostomy and at the first tracheal change (3-5days) in 40 patients, 95% (n=38) were intubated prior to tracheostomy. He found 40% *enterobacter spp* colonizing the oropharynx and 17.5% mainly Acinetobacter spp colonizing the trachea at time of tracheostomy. On first tube change samples done between 3-5 days, 80% of the cultures were positive with *Enterobacter spp* (27.5%), *Acinetobacter spp* (22.5%), and E*scherichia. coli* (20%).In the study, no antibiotics were prescribed by the operating surgeon

before or after the procedure except the ones given by the treating physician which were continued. The most sensitive antibiotic was Imipenem 57.5%, Tazobactum and Piperacilin at 20% and 17.5% of the bacteria were sensitive to Polymyxin B. They found no statistical significance of positive oral growth and those of tracheostomy aspirate during first tube change. They concluded that colonization in tracheostomised patients is from exogenous route and tracheal colonization at the time of tracheostomy does not significantly correlate to post tracheostomy colonization.

3.0 CHAPTER THREE : STUDY JUSTIFICATION & METHODOLOGY

Tracheostomy is a common emergency and elective surgical procedure done by the ENT, head and neck surgeons in KNH. There is an increase in number of tracheotomised patients due to trauma, head and neck malignancies presenting with upper airway obstruction. Post tracheostomy colonization predisposes one to lower respiratory tract infections affecting patient's quality of life. A local study by Karuga[18] found pneumonia and surgical site infections as the commonest postoperative complication. Increase of multiple resistant strains of bacteria due to inappropriate and indiscriminate use of antibiotics is a global problem. Therefore, this study aimed to obtain data on local pattern of bacterial colonization and their antibiotic susceptibility profiles. The findings obtained will guide the Otorhinolaryngologists in care and choice of antibiotics for tracheostomised patients.

3.1 Research Question

What are the micro-organisms found in tracheal aspirates of tracheostomised patients and their antimicrobial susceptibility profile at KNH?

3.2 Aims and Objectives

3.2.1 Broad objective

To determine the pattern of bacterial colonization and antimicrobial susceptibility in tracheal aspirates of tracheostomised patients at the KNH.

3.2.2 Specific Objectives

a) To determine the bacterial colonization in tracheal aspirates during and after seven days of tracheostomy.
b) To determine the bacterial antibiotic sensitivity profile.
c) To correlate the bacterial isolates to the clinical patterns of the study population.
d) To correlate the bacterial isolates to the demographic patterns of the study population.

3.3 Study Design

Hospital based descriptive cross sectional study.

3.4 Study Setting

The study was done at the KNH wards, operating theatres and the Intensive Care Unit.

3.5 Study Population

All patients who underwent emergency and elective open tracheostomy in KNH

3.6 Sample Size

The sample size was calculated using Cochran's formula[28].

$$n_0 = \frac{Z^2 p(1-p)}{d^2}$$

Where n_0 is the calculated sample size assuming infinite population of tracheostomy patients at Kenyatta National Hospital.

Z represents 95% level of confidence interval = 1.96

P is the prevalence of bacterial flora colonisation in tracheostomy patients (80% according to Morar *et al*) [29]

d is the margin of error set at 0.05

$$n_0 = \frac{1.96^2 \times 0.8(1-0.8)}{0.05^2}$$

$$n_0 = 246$$

$$n = \frac{n_0}{1 + \frac{(n_o - 1)}{N}}$$

Where n is the sample size

n_0 is the calculated sample size for infinite population = 246

N was the population of patients undergoing tracheostomy in KNH = 130 (over period of 8months)

Therefore, n = 86

3.7 Sampling Procedure

Consecutive sampling technique.

3.8 Participant Recruitment

3.8.1 Inclusion Criteria

All patients aged between 14-83 years that consented and underwent tracheostomy under sterile conditions in KNH participated in the study. For the patients who were too sick to consent or in I.C.U, consent was obtained from the next of kin at the time of obtaining consent for the tracheostomy.

3.8.2 Exclusion Criteria

a) Patients with lower respiratory tract infection prior to the procedure as confirmed by clinical examination.

b) Patients with Diabetes, immunosuppression (HIV).

c) Patients discharged before 7 days of inpatient care.

d) Patients who did not consent.

e) Tracheostomy in a non-sterile uncontrolled environment.

3.9 Data Collection Procedure

The study team consisted of the principle investigator and a technician from microbiology laboratory. Informed consent was obtained from all adults, guardians and next of kin for children and I.C.U patients. The principle investigator explained the study to the adult patient and the guardians/parents. Once fully informed and in agreement; they were asked to sign the consent form to be able to participate in the study. The children under 18years were informed about the study in presence of their parents or guardian and once everyone was in agreement; they signed an assent form while the parents/guardian signed a consent form.

The study employed consecutive sampling method whereby involving each eligible patient was recruited until the desired sample size was reached. This consisted of patients scheduled for tracheostomy who consented to participate in the study. Those with leucocytosis, neutrophilia from the complete blood count were excluded. Leucocytosis was considered if leucocyte count is above $9.06x10^3$ /cc [30].

The patients' biodata, primary diagnosis, indication for tracheostomy or prior intubation and antibiotics use were recorded.

The principle investigator explained the consent and the details of the study to the patient/guardian. Once fully informed and in agreement; they signed the assent and / or consent form and were recruited to the study.

An open surgical tracheostomy procedure was done in the operating theatre or ICU under strict aseptic conditions. Immediately after the tracheostomy a sterile suction catheter was introduced through the tracheostomy tube and tracheal suctioning done to clear secretions. The size of the suction catheter was half the inner diameter of the tracheostomy tube. The depth of suctioning was the length of the tracheostomy tube or the obturator. The secretions in the suction tube were pushed into a universal sterile screw capped bottle using 5mls of normal saline. It was then labelled and transported to the microbiology laboratory within one hour for analysis marked as day 0 of collection. The specimens that were not delivered to laboratory within one hour were discarded.

In the post-operative period the patient underwent standard tracheostomy care. The tracheostomy tube was changed to non-cuffed after 72 hours using aseptic technique. Post-operative antibiotics included intravenous Ceftriaxone 50 milligrams per kilogram divided doses in children or Amoxicillin clavulinic acid 1.2grams eight hourly in adults. On the 7th post tracheostomy day tracheal suctioning was done using a sterile suction tube and specimen collected, labelled and sent immediately for analysis to the microbiology laboratory.

The specimens were accepted if they met the "acceptance rejection criteria" i.e. transportation within one hour, adequate amount of 5-10mls, use of sterile capped universal container, correctly labelled requisition form. The inoculum was made into culture plates and incubated at 35-37 degrees in Sheep blood agar, Chocolate blood agar (low oxygen tension) and Macconkey for 72hours. Readings were done at 48hours and 72hours. Isolates were characterised by their colony forming characteristics, gram stain and biochemical tests[31]. Antibiotic sensitivity profile was conducted using automated Minimum inhibitory concentration using Vitek 2 machine available in the KNH microbiology laboratory as per current CLSI guidelines[32]. The sensitivities to Amoxicillin/Clavulinic acid, Ciprofloxacin, Levofloxacin, Amikacin, meropenem, Cefepime, Cefuroxime, Ceftazidime, Ceftriaxone, Cotrimoxazole,clindamycin,erythomycin cephazolin,Tetracylin, Cefoperazone/Sulbactam, and Piperacillin/Tazobactum, Gentamicin, were determined. The CLSI has the following definitions in laboratory processes: 'Susceptible' implies isolates inhibited by achievable concentrations of antimicrobial agent when the dosage recommended for treatment of the infection is used. 'Resistant' isolates not inhibited by therapeutic dosage concentrations. 'Empirical therapy' refers to treatment initiated before diagnosis of infection in a patient by identification of a specific organism. 'First isolate' initial microbial isolate of a particular species recovered from a patient. 'Drug resistant' refers to non-susceptibility to at least one antimicrobial agent. 'Multi-drug resistance (MDR) refers to non-susceptibility to at least one

agent in three or more antimicrobial categories. Extensive drug resistance (XDR) denotes non-susceptibility to at least one agent in all but two or fewer categories (i.e. only one or two categories are susceptible). 'Pan-drug resistant' (PDR) denotes non susceptibility to all agents in all antimicrobial categories.

3.10 Quality Control

Quality control was a continuous process for reliable and valid findings of the study. The principal investigator did the patient selection, history taking and clinical examination.
The data collection tools were pre-tested for completeness, missing information and validity of responses. The principle investigator collected the specimen and transported it to the microbiology laboratory under strict aseptic conditions. Specimens were only accepted in the microbiological laboratory if they met the acceptance/rejection criteria. Reporting was reviewed to eliminate clerical and analytical errors. Quality control for drug reagents and media were done at the beginning of each day. Equipment calibration, standardisation and maintenance were done twice per year. The laboratory analysis was done by one designated technician for standardisation.

3.11 Data Management

All retrieved data from completed questionnaires were stored and coded in a database using Microsoft excel so as to maintain confidentiality. The data was compiled and cross checked for errors and corrected as per the questionnaires. The questionnaires were kept in a lockable cabinet with access restricted to the investigator and supervisors; which will be destroyed within two years of publication of the findings in a scientific journal.

3.12 Data Analysis

The data was transferred to SPSS 22.0 statistical package for subsequent analysis. The analysis was guided by the study objectives. Descriptive analysis for continuous variables like age involved mean, standard deviation and range. The analysis of categorical data included calculation of percentages, tables and frequency distribution. The culture isolates from day 0 and day 7 were analysed according to clinical, demographic and antibiotic susceptibility profiles. The independent sample T-test was used to calculate the P value of day 0 and 7 positivity rate. The fisher's exact test was used for analysis of isolated organisms.

A cut off 0.05 was used to determine statistical significance. The results were presented using figures, graphs and frequency tables.

3.13 Ethical Consideration

The study was carried out after approval by the KNH/UON Ethics and Research Committee(P695/112017). Recruitment was by consent/assent. The participants received full disclosure of the nature of the study. No extra cost was encountered by the patient. The cost for specimen collection materials, culture and sensitivity were incurred by the principle researcher. Confidentiality was maintained by anonymising biodata with codes and questionnaires locked and secured. At the end of the study the raw data will be destroyed, the results will be published in scientific journal and presented in medical conferences There were no conflicts of interest or otherwise in this study by the principle investigator, supervisors and the hospital. The patient had a right to withdraw from the study any time without fear of victimization or loss of care.

4.0 CHAPTER FOUR: RESULTS

4.1 Demographic Characteristics

A total of 88 patients were enrolled in the study: n= 26(29.5%) were females and n=62 (70.5%) were males giving a male to female of 2.4:1

The age of the participants ranged from 14 to 83 years with a Mean age of 52.1±17.1 years and median of 57.5 years.

Eight patients (9.1%) patients were aged 25 years or less while three, 3.4%, were above 75 years of age. Majority of the patients were in the age group of 66-75 accounting for 28.4% of our study population. (Figure 1)

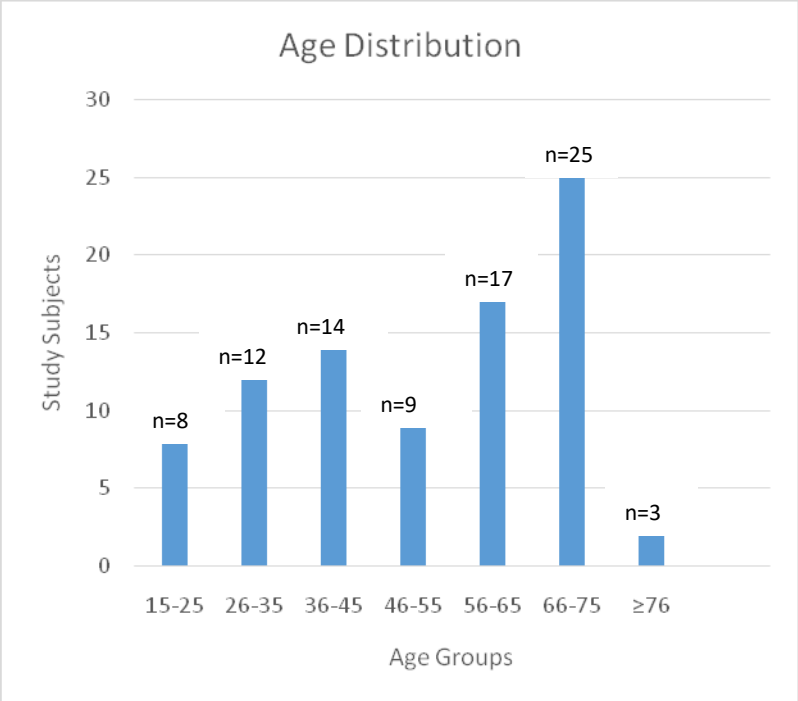

Figure 1 : Age Distribution of the study subjects at KNH

A total of 60(68.2%) tracheostomies were done as emergencies while 28(31.8%) tracheostomies were done as elective procedures. Mechanical obstruction was the commonest indication for tracheostomies n=58(65.9%).(Table1). The highest number of isolated organisms were found in age group 66-75 years n=16(28.6%) followed by age group 26-35years n=11 (19.6%). (Table 5)

Table 1: Indication for tracheostomies

Tracheostomy Indications		
	Frequency(n)	Percentage (%)
Mechanical Obstruction	58	65.9
Prolonged Intubation	19	21.6
Respiratory Failure	5	5.7
Pulmonary Toilet	5	5.7
Protection From Aspiration	1	1.2
Total	88	100.0

4.2 Organisms Isolated from Tracheal Aspirates

One hundred and seventy-six (176) samples were collected. Fifty-six micro-organisms were isolated from the cultures. Two cultures (3.5%) were positive isolates from day zero samples, eight (14%) samples were positive on both days zero and seven while forty-six (82%) were positive from day seven samples. The most common organisms isolated on day 1 were also the commonest on day 7.

Table 2: Isolated Organisms as per the samples collected p=0.859

	Sample		
	A(day 0)	**B (day 7)**	%
Pseudomonas aeruginosa	6	15	**28.6**
Acinetobacter baumannii	2	9	**17.9**
Klebsiella pneumoniae	1	6	**10.7**
Enterobacter Cloacea	1	7	**12.5**
Staphylococcus Aureus	0	5	**8.9**
Escherichia coli	0	4	**7.1**
Streptococcus Pneumoniae	0	3	**5.4**
Enterococcus Feacalis	0	1	**1.8**
Enterococcus species	0	1	**1.8**
Sphingomonas paucimobilis	0	1	**1.8**
Staphylococcus agalactiae	0	1	**1.8**
Streptococcus mitis	0	1	**1.8**
Total	10	54	**100**

There were 10 organisms isolated from the samples on day zero (11.36%) and 54 organisms isolated from samples on day 7 (61.30%) with P value of < 0.001. (Table 3)

Table 3: Difference between day 0 and day 7 colonization

Day	Mean Positivity Rate	P Value
Day1	11.36%	**0.001**
Day7	61.36%	

There was no statistical significance correlation between microorganism isolated in emergency and elective tracheostomy cultures. (Table 4)

Table 4: Isolates in Emergency/Elective Samples

Type	Positive	Negative	P Value
Emergency	35	25	0.12
Elective	21	7	

Most isolates were from the age group 66-75 years (n=16, 28.6%) p=0.03(Table 5). Males had a higher positivity rate than females. Highest isolates were from ENT ward (n=35, 62.5%) followed by main ICU (n=13, 23.3%), Medical ICU (n=8, 14.2%). p=0.09.

Table 5: Isolated organisms as per age groups (p=0.03)

ORGANISMS	AGES (years)							Total
	15-25	26-35	36-45	46-55	56-65	66-75	76	
Pseudomonas aeruginosa	1	3	3	1	0	8	0	**16**
Acinetobacter baumannii	0	1	4	1	0	3	1	**10**
Enterobacter Cloacea	0	3	1	1	2	0	0	**7**
klebsiella pneumoniae	2	2	0	0	0	2	0	**6**
Staphylococcus aureus	0	0	1	0	3	1		**5**
Escherichia coli	1	0	0	1	1	1	0	**4**
Streptococcus Pneumoniae	0	0	0	1	1	1	0	**3**
Enterobacter. Feacalis	0	0	0	0	1	0	0	**1**
Enterococcus species	0	0	0	0	1	0	0	**1**
Sphingomonas caucimobilis	0	1	0	0	0	0	0	**1**
Staphylococcus agalactiae	1	0	0	0	0	0	0	**1**
Streptococcus-mitis	0	1	0	0	0	0	0	**1**
Total	**5**	**11**	**9**	**5**	**9**	**16**	**1**	**56**

Pseudomonas aeruginosa was the commonest micro-organism isolated (28.6%) followed by *Acenotobacter baumanii* 17.9%, *Enterobacter clocaea* 12.5%. *Klebsiella pneumonae* 10.7% and *Staphyloccus Aeurius* 8.9 %.(figure 2). Gram negative organisms were predominant n=44 (78.57%), while gram positive accounted for n=12 (21.43%).

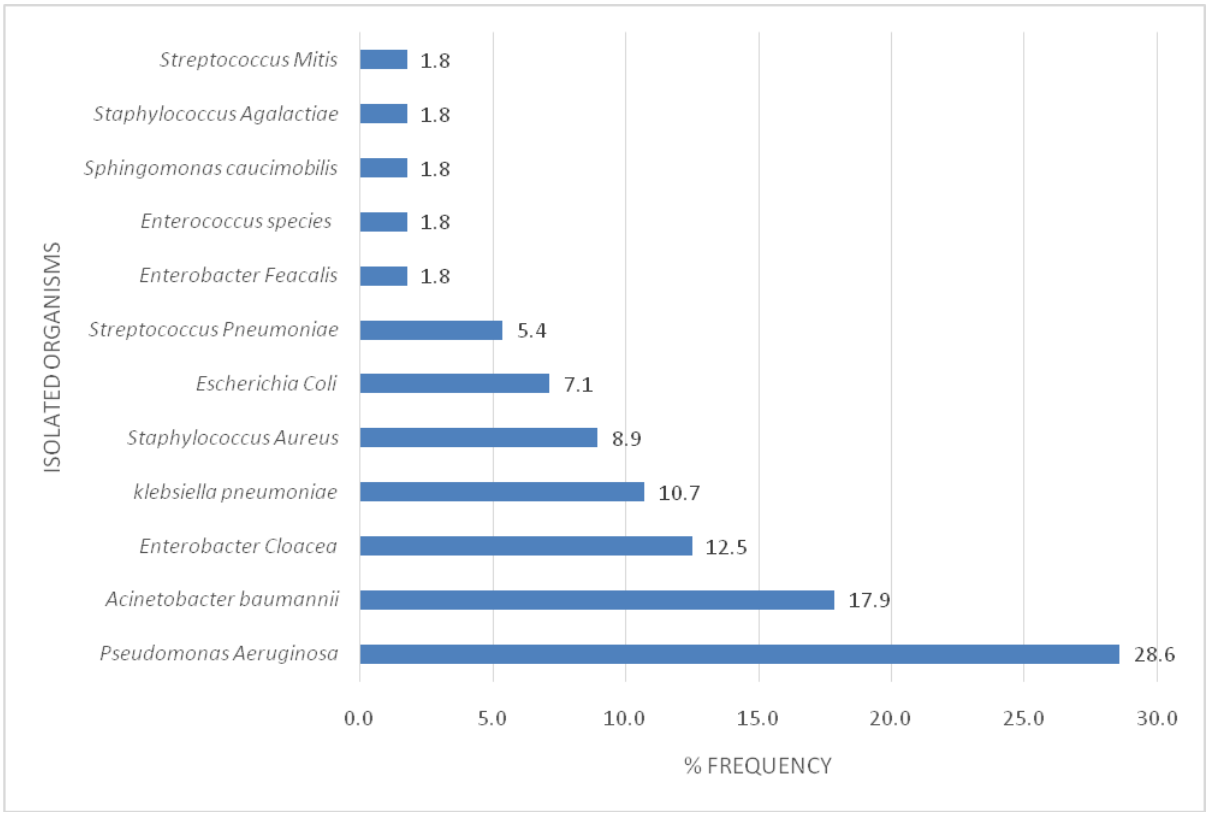

Figure 2:The percentage Frequency of Isolated Organisms

4.3 Sensitivity Profile of Isolated Organisms

Patients on triple antibiotic therapy were more likely to be resistant to Amikacin (P=0.04, Adjusted Odds Ratio=10.1, 95% CI: 1.1-98.9) and Ciprofloxacin (P=0.02, Adjusted Odds Ratio=24.5, 95% CI: 1.8-336)

Antibiotic resistant profiles showed majority of bacterial isolates had multiple antibiotic resistance patterns. However none met the threshold for antibiogram reporting i.e. 30 isolates per species. *Pseudomonas aeruginosa* was highly resistant to Cefotaxime (88%), Cotrimoxazole (77.7%), Amoxi-clavulin (67.2%). However it was highly susceptible to Gentamicin, Meropenem and Amikacin. *Acinetobacter Baumanii* was 100% resistant to amoxicillin and ampicillin and Salbactam It was also 90% resistant to Cefotaxime and Ceftriaxone with average susceptibility to Gentamicin (Table 6).

Table 6: Antimicrobial susceptibility of gram negative organisms to various antibiotics

GRAM NEGATIVE ORGANISMS	No. of strains	PERCENTAGE SENSITIVITY (%S)																						
		PENICILLINS					CEPHALOSPORINS						AMINO			FQ		CPM	MACROLIDES				OTHERS		
		Amoxycillin/ clavulinic acid	AMOXICILLIN	AMPISALBACTAMs	EM	PIPERAZINE/TAAZO BACTAMM	CEFAZOLINE	CEFTRIAXONE	CEFOTAXIME	CEFTAZIDIME	CEFEPIME	CEFUROXIME	GENTAMICIN	AMKACIN	TETRACYCLINE	LEVOFLOXACIN	CIPROFLOXACIN	MEROPENEM	IMIPENEM	ERYTHROMYCIN	CLINDAMYCIN	VANCOMYCIN	CHLORIMOXAZOLE	COTRIMOXAZOLE	LINECID
Sphingomonas. Paucimobilis	1	100	0	_	_	0	0	100	100	100	100	100	100	100	_	_	100	100	_	_	_	0	_	100	0
Pseudomonas Aeruginosa	16	43.8	_	_	_	68.8	0	60	12.5	68.8	75	66.7	81.3	81.3	_	_	73.3	81.3	_	_	_	_	_	33.3	_
Klebsiella Pneumoniae	6	16.7	_	0	_	33.3	0	0	0	0	33.3	33.3	83.3	83.3	0	100	83.3	83.3	_	_	_	_	_	60	_
Enterobacter Cloacae	7	16.7	0	0	_	100	0	85.7	85.7	71.4	85.7	0	100	100	_	_	100	100	_	_	_	_	100	75	_
Escerichia coli	4	0	_	_	_	100	0	25	25	25	25	0	25	100	_	_	25	100	_	_	__	_	_	33.3	_
Acinetobacter Baumani	10	12.5	0	0	_	40	0	10	10	37.5	30	25	50	30	_	_	30	30	_	_	_	_	0	22.2	_

Staphylococcus aureus was 100% resistant to Amoxicillin, 90% resistant to Cefotaxime and Ceftriaxone .80% resistant to Amox-clavulinic acid and Cotrimoxazole. None was resistant to Vancomycin. We also observed moderate resistance to Erythromycin and Clindamycin (60%).(table7).Excellent susceptibility was seen to linecid

GRAM POSITIVE ORGANISMS	No. of strains	PERCENTAGE SENSITIVITY (%S)																					
		PENICILLINS					CEPHALOSPORINS						AMINO			FQ		CPM		MACROLIDES			OTHERS		
		Amoxicillin clavulanic acid	AMOXICILLIN	SALBACTAM	AMPICILLIN	PIPERAZINE IMEPENEM	PIPERAZINETAZOBACTAM	CEFAZOLINE	CEFTRIAXONE	CEFOTAXIME	CEFTAZOLINE	CEFEPIME	CEFUROXIME	GENTAMICIN	AMIKACIN	TETRACYCLINE	LEVOFLOXAIN	CIPROFLOXACIN	MEROPENEM	IMIPENEM	ERYTHROMYCIN	CLINDAMYINE	VANCOMYIN	CHLORAMPHENICOL	COTRIMOXAZOLE	LINECID
Streptococus. pneumonia	3	100	0	0	_	66.7	0	33.3	0	0	0	_	_	_	0	100	33.3	66.7	_	100	100	100	100	0	100
Streptococcus. Mitis	1	100	0	0	_	0	0	100	0	0	0	0	0	0	_	100	0	0	_	100	100	100	100	0	100
Staphylococcus. Aureus	5	20	0	_	_	_	_	_	_	_	_	_	100	_	50	80	75	_	_	60	60	100	_	20	100
Staphylococcus. Agalactiae	1	_	_	_	_	_	_	_	_	_	_	_	_	_	0	100	100	0	_	_	0	100	_	_	100
Enterococcus. Feacalis	2	0	0	_	_	_	_	_	_	_	_	_	0	_	_	100	100	_	_	0	100	100	_	_	100

Table 7 : Antimicrobial susceptibility of gram positive organisms to various antibiotics

4.4 Bacterial Resistance And Patient Clinical Profiles

The 56 resistant isolate were matched with the respective patients the clinical profiles analyzed. Of these 82.2 %(46/56) were classified as drug resistant and 17.8 %(10/56) classified as multi-drug resistant. Due to unavailability of some antibiotic agents for testing all antibiotic classes it was impossible to add the extensively drug resistant (XDR) and pan drug resistant (PDR) profiles .The proportions of resistant bacteria that were isolated and the antimicrobial resistance (co morbidities, empiric therapy) are illustrated (table 8) .All the multidrug resistant isolates were from patients with co morbidities with prior empirical antibiotics. These were all patients in intensive care unit.

Table 8: Bacterial resistance and patient clinical profiles

ISOLATE	Resistance profile	Total isolates (N)	Patient characteristics			
			Sex		Any Comorbidity	Prior antibiotics
			Male n= (n/N)%	Female n= (n/N)%	n= (n/N)%	n= (n/N)%
	Drug resistance	46	30(65.8%)	16(34.8%)	20(43.4%)	15(32.6%)
	Multi drug resistance	10	8(80%)	2(20%)	10(100%)	10(100%)

5.0 CHAPTER FIVE: DISCUSSION

The total number of patients recruited for this study was 88. All the emergency tracheostomies were admitted to ENT ward (n=60) and the elective tracheostomies done (n=28) were from the ICU. The age-sex characteristics were stratified. The age-group 66-75 years accounted for majority of patients (28%). This was similar to the findings in Aswin's study[25].This may be attributed to the fact that this was the age group of patients with mechanical obstruction due to upper aero-digestive cancers. This age group also had the highest number of isolates (n=16, 28.6%) attributed to reduced immunity due to age related factors, malnutrition and malignancy. The age group 26-35 years had the second highest isolates (n=11, 19.7%). This may be due to the fact that this age group comprised of majority of patients who were in the intensive care unit due to head injuries secondary to road traffic accidents. The sex versus isolate stratification was not statistically significant (p=0.8). ENT Ward had 62% of the isolates with *Pseudomonas aeruginosa* being the predominant (n=10, 28.5%) while ICU had the 38% with *Acinetobacter Baumanii* being the highest (n=8, 38%).

The respiratory system has natural barrier mechanisms to prevent colonization in the lower airways. This is impaired in the presence of a tracheostomy. Our study demonstrated this evidence of colonization of trachea by bacteria after tracheostomy. Day zero had 11.36% positive culture of bacteria while day seven had 61.37% positive culture of bacteria growths (p value <0.001). The predominant organism isolated from day zero was *Acinetobacter Baumanii* from prior intubated patients. These were patients who were in intensive care unit with a mean intubation period of 14 days. *Acinetobacter Baumanii* has been described as a hospital acquired bacteria[33]. Endotracheal intubation directly inoculates pathogens to lower airways, impairs muco- cilliary escalator system and causes mucus stasis creating a viable medium for colonization[34]. Even in aseptic techniques; the constant exposure to intensive care unit organisms results in exogenous inoculation of organisms to lower airway. Other studies have found similar predominance of *acinetobacter* species and *klebiella species* in intubated intensive care unit patients[35, 36, 37, and 38]. *Acinetobacter baumanii* has high resistance to antiseptics and disinfectants in the hospital[39]. The risk of colonization is increased by duration of intubation, critical illness, poor immunity, use of broad spectrum antibiotics and multiple drug use[40]. These are favourable conditions for colonization in an intensive care unit setting. The use of multiple drugs leads to multidrug resistant strains and persistent colonization.

The gram negative bacteria were the most common isolates (n=44, 78.5%). There has been a similar finding in other studies[41, 42, and 43]. *Pseudomonas aeruginosa* was the predominant bacteria isolated followed by *Acinetobacter baumanii;* then *Klebsiella species (*n=10.7% and *Enterobactrioceae spp (12.5%). A*swin et al found Klebsiella species to be the second commonest after pseudomonas species[25]. The Enterobacterioceae species is attributed to aspirations from the hypo pharynx and faecal-tracheal contamination. Inoculation to the tracheal stoma is postulated during phonation as the patients occlude the tracheostomy stoma with their thumb.

Pseudomonas aeruginosa has described as an opportunistic infection difficult to eliminate once it colonises the lower airways[14]. It is endemic in hospital environment, forms very hard biofilms in tracheal tubes and calcium alginate capsules[44]. It has affinity to tracheal-bronchial tree[23] binding avidly to tracheal cells[45]. The micro-trauma caused by tracheostomy tube insertion and rigorous suction opens the binding sites for *Pseudomonas aeruginosa* while the inflammation and healing process enhances bacteria-cell intergration[24, 46].

The predominant gram positive bacterium isolated in our study was *Staphylococcus aureus* which has been associated with nosocomial infections such as pneumonia and surgical site infections[47]. In a KNH 2014 study on paediatric surgical patients it was the primary isolate associated with wound sepsis[48]. Karuga et al found pneumonia as the commonest post tracheostomy complication followed by surgical site infections[18]. Mogere et al in 2015 at KNH found *Staphylococcus aureus* average carriage of 34.4% in the nose and hands of health care workers. Highest carriage was found in surgical ward compared to medical wards[49].This may explain its prevalence in our surgical ward setting.

All patients were on antibiotics after the tracheostomy. The patients in intensive care unit were continued on previous antibiotics as prescribed by their primary physician. The most used antibiotics were Penicillins (amoxicillin clavulinic acid) and Cephalosporins (Cefriaxone). *Pseudomonas aeruginosa* as well as *Acinetibacter baumani* had multiple drug resistance to Cefotaxime, Amoxicillin clavulinicacid and Cotrimoxazole. However, they both shared susceptibility to Aminoglycosides such as Gentamicin and Amikacin. The high resistance pattern may be attributed to high usage of Cephalosporins (ceftriaxone,cefotaxime) and Penicillins (amoxicillin, amoxicillin clavulinic acid) in our set-up[50]. Consequently there was alarming rate ceftriaxone and ceftazidime resistance reported for *Escherichia Coli* (75% and75%) and Klebsiella *pneumonia* (77% and 100%). This rates are higher compared to the findings in the private local facility such as Aga Khan University hospital that found 49% *Escherichia Coli* and 61% *Klebsiella Pneumonia* for their surgical inpatients[51]. This

discrepancy could be attributed to patient characteristics, disease burden, clinician prescription practices and antibiotic policies. The global estimates are 50% and 30-60% resistance to *Escherichia Coli* and *Klebsiella Pneumonia* respectively and a regional East Africa range of 0-22% resistance to 3rd generation cephalosporins[52]. Surpassing this range in our findings implies indiscriminate prescription of cephalosporins at KNH. The high antibiotic resistance of *Acinetobacter species* is attributed to its ability to alter penicillin binding proteins, alter cell wall permeability, and targeted site mutations[53].

The *staphylococcal species* was also highly resistant to Penicillins and Cephalosporins however it was 100% susceptible to Vancomycin. Mogere[49] found an overall vancomycin resistance of 53.2% unlike ours which was zero. This may be due to the fact that our patients were mainly from ENT ward where the use of Vancomycin is negligible.

The role of bacterial biofilms in tracheostomy tube may lead to persistent colonization. These have been demonstrated to form within the first week of tracheostomy and persist despite the aseptic techniques used in tracheostomy care[54]. The role of biofilms in our study is highly postulated.

The study found positive lower airway colonization after tracheostomy hence the need to do serial tracheal aspirates cultures. This will identify the colonizing bacteria and resistance patterns in management of post tracheostomy infection and complications. The risk of multidrug resistance is evident hence rational use of antibiotics encouraged as there is limited availability of newer drugs in an exceedingly rising resistant bacterial strains.

5.1 Conclusion

Post tracheostomy colonization occurs early within the first week. There is need to regularly monitor tracheal aspirate cultures. There is an overwhelming resistance to commonly used antibiotics such as penicillins and cephalosporins at KNH. This underscores the need for guided empirical therapy only where indicated and use reserve antibiotics after culture and sensitivity analysis. A combined effort is crucial to ensure proper antibiotic stewardship, surveillance and research to combat antibiotic resistance.

5.2 Recommendations

Serial tracheal aspirate culture should be incorporated in the care of post tracheostomy patients as early as the day seven. This will identify the bacterial colonization patterns and resistance profiles. A larger prospective study spanning a year involving properly trained staff on specimen collection to obtain an adequate number of isolates for antibiogram development.

Elaborate antibiotic stewardship programmes at KNH, to ensure appropriate antibiotic utilization. This will help reduce antibiotic pressure that leads to resistant strains. Strengthen infection control practices in order to reduce microbial carriage, cross contamination and biofilm formation. Empowerment of health care workers on antimicrobial resistance, prescription of appropriate antibiotics through workshops, symposiums, to reduce the knowledge gap in antibiotic resistance.

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TIMELINE

PERIOD	ACTIVITY
April 2017 –September 2017	PROPOSAL WRITING
September 2017	Proposal presentation
October 2017-january 2018	Ethical approval
February 2018 – april 2019	Data collection
May - August	Data analysis
August 2019	Report writing and submission.

BUDGET

ITEM	COST (Kshs)
STATIONERY	30 000
DATA COLLECTION	100 000
LABORATORY	250 000
STATISTICIAN	30 000
DISSEMINATION OF RESULTS	20 000
TOTAL	430 000

APPENDICES

Appendix I: General patient information form (English)

Introduction

I am Dr. Cliffe Omwenga a resident in the ENT Head and Neck surgery department. I kindly request for your permission to participate in a study on bacteriological profile and sensitivity during and after seven days of tracheostomy at the Kenyatta National Hospital. The study is about the bacteria that colonise your respiratory tract and how they respond to various antibiotics.

How will you participate?

Once you consent to your participation, I will take an aspirate sample from your trachea during the tracheostomy procedure and day seven post operation. This will be taken for microbiological analysis.

You will not incur any extra costs or get any monetary benefits. You can withdraw any time voluntarily from the study without any effect on the quality of care you will receive.

How will participation affect you?

The study will not affect you negatively in any way and there are no hidden dangers of participating in the study. There are no risks of participating in this study. The findings from this study will help improve tracheostomy care and treatment of tracheostomy related infections. All information will be confidential and the conclusions from the study will improve current management of tracheostomy related infections.

What will we do with the information obtained?

The information will help improve management of tracheostomy related infections

Like all scientific information we will seek to share our findings with other peoples doing similar studies. Therefore the findings will be presented in scientific meetings and published in scientific journals.

Are you satisfied with the information given?

If you are satisfied with our explanation and you are willing to participate, please sign the consent form below**.**

Appendix II: General Patient Information Form (Swahili)

Kitangulizi

Mimi ni Dr. Cliffe Omwenga anaye endelea na masomo ya juu ya kitengo cha upasuaji wa masikio, mapua, koo, kichwa na shingo katika chuo kikuu cha Nairobi. Ningependa kuomba idhini yako ya kushiriki katika utafiti wenye lengo la kujua viini vinavyotawala koo baada ya upasuaji wa njia ya kupumua na dawa zinazoweza kuviangamiza katika hospitali kuu ya Kenyatta.

Jinsi ya kushiriki

Baada ya kupeana idhini ya kushiriki tutatoa sampuli ya kamasi wakati wa upasuaji na baada ya siku saba kwenye koo yako ambayo itatumwa kwa mahabara kwa utafiti. Hautalipishwa gharama yoyote zaidi na hakuna madhara yoyote ya kushiriki kwa utafiti huu.

Una haki ya kujiondoa kutoka kwa utafiti huu wakati wowote bila adhabu yoyote

Jinsi gani kushiriki kwako kunaweza kuleta madhara

Utafiti huu hautakudhuru kwa njia yoyote; taarifa yote kuhusu mgonjwa itakuwa siri, utambulisho hautatangazwa, na baada ya utimifu wa utafiti maarifa yatakayopatikana tutasaidia kuboresha huduma na matibabu ya wagonjwa waliopasuliwa koo.

Je tutafanyia nini matokeo ya utafiti huu

Maarifa tutakayopata yatasaidia kuboresha huduma ya wagonjwa waliopasuliwa koo

Kuna uwezekano wa kuchapishwa kwa matokeo ya utafiti huu katika majarida ya kisayansi au kuwekwa katika mikutano ya kisayansi.

Je umeridhika?

Ukiridhika na maelezo haya na uko tayari kushiriki, tafadhali weka sahihi yako kwenye fomu ya idhini.

Appendix III: Consent Form patients more than 18 years (English)

Patient number.....

Consent by patient

I Mr/Mrs/Ms.....of.....do hereby give consent to be included in this study on pattern of bacterial colonization and antimicrobial susceptibility in tracheal aspirates of tracheostomised patients at Kenyatta National Hospital.

The nature of the study has been explained by Dr..... My signature is confirmation that I have understood the nature of the study and that whatever information I will give will remain confidential.

I also confirm that no monetary or material gains have been promised or given to me for participating in the study.

Date..... Signed/Thumb print.....

I Dr.....confirm that have explained to the patient the nature of the study.

Date..... Signed.....

Consent Form patients less than 18 years/unconscious patients (English)

I Mr/Mrs/Ms.....the parent /guardian of Mister/Miss..... agree to enrol him/her into the study as explained to me by Dr.....

My signature is confirmation that I have understood the nature of the study and that whatever information that I give will remain confidential.

I also confirm that no monetary or material gains have been promised or given to me for participating in the study.

Date..... Signed/Thumb print.....

I, Dr.....confirm that have explained to the patient the nature of the study.

Date..... Signed.....

Assent Form *for patients 12 to 17 years (*English)

Project title: The pattern of bacterial colonization and antimicrobial susceptibility in tracheal aspirates of tracheostomised patients at the Kenyatta National Hospital.

My name is Dr. Omwenga Cliffe a doctor on training for specialization in Ear nose and Throat surgery in the ENT Head and Neck surgery department. . I am requesting for your permission to participate in a study seeking to find the bacteria that reside in your trachea after tracheostomy and how we can treat infections related to them.

Permission to conduct the study has been granted by KNH/UON ERC protocol No.....

If you decide to be part of this study a sample of mucus will be taken from your tracheostomy tube during surgery and after seven days of surgery. This will be taken for analysis in the laboratory. The study will not harm you in any way. You will not benefit from the study. Benefit means something good will happen to you. We think the findings of this study will help improve care of tracheostomy related infections.

You do not have to be in the study. Nobody will be mad at you if you decide noto participate in this study. Even if you start you can stop later if you want. You may ask questions about the study. The information about you will be secret.

If you have agreed you may sign.

Date..... Signed/ Thumb print.....

I Dr.....confirm that have explained to the patient the nature of the study.

Date..... Signed.....

For any further clarifications contact the following:

Principle Researcher.

Dr. Cliffe Omwenga, Resident in ENT- Head & Neck surgery, University of Nairobi. Telephone number: 0711 117 062. Email: drferongaz@yahoo.com.

Supervisors

Dr. Peter Mugwe, Cunsultant ENT Head & Neck surgery, University of Nairobi.

Dr. Musa Kipingor, Consultant ENT Head & Neck surgery, Kenyatta National Hospital.

The chairman KNH/UON Ethics and Research Committee, Kenyatta National Hospital Nairobi, Tel 2726300 Ext.44355.

Appendix IV: Kibali Cha Utafiti

Watu wazima/Zaidi ya miaka 18

Nambari ya hospitali.....

Mimi Bi/Bwana.....kutoka.....ninakubali kushirikiswa katika utafiti wenye lengo la kujua viini vinavyotawala koo na matibabu yake baada ya upasuaji wa koo katika hospitali kuu ya Kenyatta.

Nimeelezewa na daktari.....

Sahihi yangu ni thibitisho ya kwamba nimeelewa umuhimu wa utafiti huu na kwamba habari yoyote nitakayotoa itawekwa kwa siri. Pia nathibitisha kwamba sijapewa au kuahidiwa pesa au chochote kile kukubali kushiriki kwenye utafiti huu.

Tarehe..... Sahihi.....

Mimi daktari ..... nahakikisha ya kwamba nimeelezea mgonjwa kuhusu utafiti huu.

Tarehe..... Sahihi.....

Watoto walio chini ya miaka 18 au wasiojifahamu

Mimi Bi/Bwana .....mzazi/msimamizi wa.....nimekubali kumushirikisha katika utafiti huu baada ya kuelezewa na daktari .....

Sahihi yangu ni thibitisho ya kwamba nimeelewa umuhimu wa utafiti huu na kwamba habari yoyote nitakayotoa itawekwa kwa siri. Pia nathibitisha kwamba sijapewa au kuahidiwa pesa au chochote kile kukubali kushiriki kwenye utafiti huu.

Tarehe..... Sahihi.....

Mimi daktari ..... nahakikisha ya kwamba nimeelezea mgonjwa kuhusu utafiti huu.

Tarehe..... Sahihi.....

Kibali cha utafiti(Watoto wa miaka kati ya 12-17)

Mimi ni Daktari. Cliffe Omwenga anaye endelea na masomo ya juu ya kitengo cha upasuaji wa masikio, mapua, koo, kichwa na shingo katika chuo kikuu cha Nairobi. Ningependa kuomba idhini yako ya kushiriki katika utafiti wenye lengo la kujua viini vinavyotawala koo baada ya upasuaji wa njia ya kupumua na dawa zinazoweza kuviangamiza katika hospitali kuu ya Kenyatta.

Idhini ya utafiti huu imepeanwa na KNH/UON ERC itifaki nambari.....

Ukikubali kupeana idhini ya kushiriki tutatoa sampuli ya kamasi wakati wa upasuaji na baada ya siku saba kwenye koo yako ambayo itatumwa kwa maaabara kwa utafiti. Hautalipishwa gharama yoyote zaidi na hakuna madhara yoyote ya kushiriki kwa utafiti huu.Taarifa yote kuhusu mgonjwa itawekwa siri.

Una haki ya kujiondoa kutoka kwa utafiti huu wakati wowote bila adhabu yoyote hata baada ya kukubali kuhusika. Hakuna faida au malipo utakayopata Kwa kushiriki. Maarifa tutakayopata yatasaidia kuboresha huduma ya wagonjwa waliopasuliwa koo. Uko na haki ya kuuliza maswali yoyote kuhusu utafiti huu.

Ukiridhika na maelezo haya na uko tayari kushiriki, tafadhali weka sahihi.

Tarehe..... Sahihi.....

Mimi daktari ..... nahakikisha ya kwamba nimeelezea mgonjwa kuhusu utafiti huu.

Tarehe..... Sahihi.....

Mawasiliano

Mtafiti mkuu: Daktari Cliffe O. Omwenga. Mwanafunzi wa upasuaji wa masikio, mapua na koo chuo kikuu cha Nairobi.

Nambari ya simu: 0711 117 062. Barua pepe: drferongaz@yahoo.com

Wasimamizi

Daktari Peter Mugwe Idara ya upasuaji, kitengo cha upasuaji wa masikio, Mapua na koo Chuo kikuu cha Nairobi.

Daktari Musa Kipingor Mshauri upasuaji wa masikio, pua,koo, kichwa na shingo.Hospitali Kuu ya kitaifa ya Kenyatta .

Mwenyekiti, KNH/UON Ethical and Research committe, Hospitali Kuu ya kitaifa ya Kenyatta, simu 2726300 ugani 44355.

Appendix V: Data Collection Form

Study number..... Date.....

Biodata

Study No..... Age.....

Gender.....

Occupation.....

Diagnosis.....

Full blood count

a) Haemoglobin level..... b) white blood cell count..... c) Neutrophil count.....

Type of tracheostomy (tick) Elective ( ) Emergency ( )

ICU tracheostomies 1) Duration of intubation.....

2) Prior antibiotics.....

Smoking (pack years).....

STUDY NO.....

LABORATORY FINDINGS,

1. Pure culture [ ]

   Mixed culture [ ]

   If mixed culture number of isolates.....

2. Name of aerobe isolated (0=no, yes=1)..... and sensitivity.

Aerobe isolated	Sensitive	resistant

2. Name of Anaerobe isolated (0=no, yes=1)

Anaerobe isolated	Sensitive	resistant

Appendix VI: Study Registration Certificate

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KNH/R&P/FORM/01

KENYATTA NATIONAL HOSPITAL
P.O. Box 20723-00202 Nairobi

Tel.: 2726300/2726450/2726565
Research & Programs: Ext. 44705
Fax: 2725272
Email: knhresearch@gmail.com

Study Registration Certificate

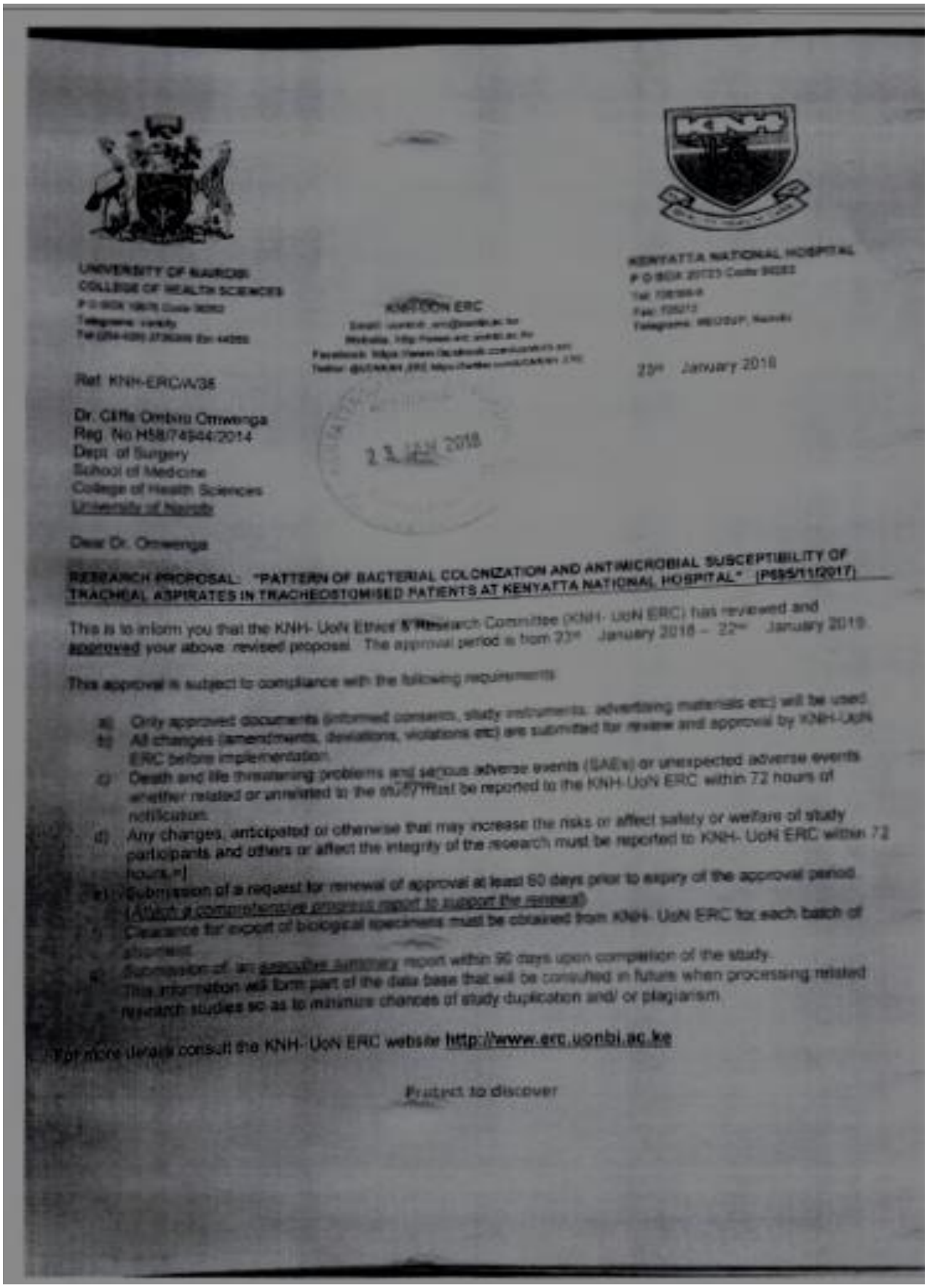
1. Name of the Principal Investigator/Researcher
   DR. OMWENGA CLIFFS
2. Email address: DRFERONGA2 @yahoo.com Tel No. 0711 117 062
3. Contact person (if different from PI).....
4. Email address: ..... Tel No. .....
5. Study Title
   Pattern of Bacterial Colonization and antimicrobial Susceptibility of tracheal aspirates in tracheostomised patients at Kenyatta National Hospital
6. Department where the study will be conducted Ear, Nose, Throat, & Microbiology Laboratory
   (Please attach copy of Abstract)
7. Endorsed by Research Coordinator of the Department where the study will be conducted.
   Name: PRISCILLA OKUMU Signature ..... Date 26-1-2018
8. Endorsed by Head of Department where study will be conducted.
   Name: MARY MUNGANIA Signature ..... Date 30/1/2018
9. KNH UoN Ethics Research Committee approved study number P965/11/2017
   (Please attach copy of ERC approval)
10. I DR. OMWENGA CLIFFS commit to submit a report of my study findings to the Department where the study will be conducted and to the Department of Research and Programs.
    Signature ..... Date 26/01/18
11. Study Registration number (Dept/Number/Year) Lab medicine /59/2018
    (To be completed by Research and Programs Department)
12. Research and Program Stamp

KENYATTA NATIONAL HOSPITAL P.O. Box 20723 - 00202 NAIROBI Research Resource Centre
03 FEB 2018

All studies conducted at Kenyatta National Hospital **must** be registered with the Department of Research and Programs and investigators **must commit** to share results with the hospital.

Version 2: August, 2014

Appendix VII: KNH/UON-ERC Letter of Approval



UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES

KNH-UON ERC

KENYATTA NATIONAL HOSPITAL

Ref: KNH-ERC/A/36

23rd January 2018

Dr. Cliffe Ombiro Omwenga
Reg. No H58/74944/2014
Dept. of Surgery
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Omwenga

RESEARCH PROPOSAL: "PATTERN OF BACTERIAL COLONIZATION AND ANTIMICROBIAL SUSCEPTIBILITY OF TRACHEAL ASPIRATES IN TRACHEOSTOMISED PATIENTS AT KENYATTA NATIONAL HOSPITAL" (P695/11/2017)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above revised proposal. The approval period is from 23rd January 2018 – 22nd January 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (Attach a comprehensive progress report to support the renewal)
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an executive summary report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website http://www.erc.uonbi.ac.ke

Prudent to discover

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
The Chair, Dept. of Surgery, UoN
Supervisors: Dr. Joyce Aswani, Dr. Peter Masinde, Dr. Martin Murage

Protect to discover

Appendix VIII: Certificate of Plagiarism Check

Pattern of Bacterial Colonization And Antimicrobial Susceptibility Of Tracheal Aspirates In Tracheostomised Patients At Kenyatta National Hospital

ORIGINALITY REPORT

7%	2%	4%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Frederick K. Wangai, Moses M. Masika, Godfrey N. Lule, Emma M. Karari et al. "Bridging antimicrobial resistance knowledge gaps: The East African perspective on a global problem", PLOS ONE, 2019 Publication	2%
2	emedicine.medscape.com Internet Source	1%
3	Submitted to University of Nairobi Student Paper	<1%
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