ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES, SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

PREVALENCE AND ANTIMICROBIAL-RESISTANCE PATTERNS OF *PSEUDOMONAS AERUGINOSA* AMONG BURN PATIENTS ATTENDING YEKATIT 12 HOSPITAL MEDICAL COLLEGE, ADDIS ABABA ETHIOPIA

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AUGUST 2021

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Abbreviations/Acronyms

AIDS	Acquired Immunodeficiency Syndrome
AST	Antibiotic Sensitivity Test
BAP	Blood Agar Plates
CLSI	Clinical & Laboratory Standards Institute
CNS	Coagulase-Negative Staphylococci
DALYs	Disability-Adjusted Life-Years
DMIP	Department of Microbiology, Immunology, and Parasitology
DST	Drug susceptibility test
HMIS	Health Management Information Systems
HIV	Human Immunodeficiency Virus
LMICs	Low and Middle-income countries
LOS	Length of Hospital Stay
MDR	Multidrug Resistant
SOPs	Standard operating procedures
TBSA	Total Body Surface Area
WHO	World Health Organization
Y12HMC	Yekatit 12 Hospital Medical College

ABSTRACT

Background:-Burns are one of the most common and devastating forms of trauma globally. Patients with burn wounds are at higher risk for infections due to multiple factors. *P. aeruginosa* plays a prominent role as an etiological agent involved in serious infections among burn patients, which varies by geographical location. There is a paucity of information about the specific prevalence and antimicrobial resistance patterns of *P. aeruginosa* among burn patients in Ethiopia.

Objective:-This study was designed to assess the prevalence and antimicrobial-resistant patterns of *P. aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia

Methods:-Hospital-based cross-sectional study was conducted at Yekatit 12 Hospital Medical College among patients attending burn clinic from November, 2020 to April, 2021. Detection and identification of *P. aeruginosa* were performed using conventional culture, Biochemical tests, and Gram staining. Antimicrobial resistance testing was done using Kirby-Bauer disc diffusion method. Logistic regression analysis was computed to determine the associated factors for *P. aeruginosa* infection.

Results:-From the total of 210 burn wound cultures, 27 (12.86%) were found positive for *P. aeruginosa*. All the isolates showed greater than 70% susceptibility for the tested antibiotics except Gentamycin (29.63%), Ceftazidime (59.3%) and Ciprofloxacin (66.67%). Imipenem was the most effective drug (89%) in inhibiting the growth of *P. aeruginosa* isolates. In addition, 33.33% of *P. aeruginosa* isolates were Multidrug-resistant (MDR). Admission type, Hospital stay time and total body surface area (TBSA) had a statistically significant positive association with *P. aeruginosa* infection (all with P-value >0.05).

Conclusion:-The overall, prevalence of *P. aeruginosa* among burn patients in the current study is almost 13%. *P. aeruginosa* isolates were most sensitive to Imipenem, while they were most resistant to Gentamycin. One-third of *P. aeruginosa* isolates were multidrug-resistant. This suggests the need to improve and monitor the treatment of infection with the pathogen to limit the possibility of the emergence of multidrug-resistant isolates in burn treatment centers.

Keyword: Burn infections, Antimicrobial resistance, *Pseudomonas aeruginosa*

1. INTRODUCTION

1.1 Background

Burns are one of the most common and devastating forms of trauma mostly caused by heat, radiation, electricity, friction, or contact with chemicals. It removes the protective skin layer, which results in disruption of the normal skin barrier accompanied by depression of immune responses as the result of which the body is generally exposed to numerous potential pathogens. Microbial infection after burns, where a large part of the skin is damaged, is a very serious complication that is often the principal cause of patients' death [**1** and **2**].

The burn wound surfaces (in deep partial-thickness and in all full-thickness burns) contain a large amount of necrotic tissue and a protein-rich environment that provides a favorable niche for microbial colonization and proliferation [**3**]. Although burn wound area is sterile immediately following thermal/burn injury, a complex and changing microbial ecology rapidly develops thereafter within an average of 5 to 7 days [**4**]. This microbial colonization takes place due to various groups of microorganisms including gram-positive bacteria, gram-negative bacteria, and yeasts derived from the host's normal gastrointestinal and upper respiratory flora and/or from the hospital, environment or that are transferred via a health care worker's hands [**3** and **4**].

Infection in Burn wound is the major cause of disability and mortality affecting all ages groups in both developed and developing countries. Organ failures and burn shocks are the major cause of death in the first 48 hours of hospitalization, which are dependent upon the type of burn and total body surface area (TBSA). However, nosocomial infections, wound infections, and septicemia are the major cause of death after 48hours of hospitalization. Disappearance of the first line defenses, provides favorable conditions for implantation and invasion of opportunistic pathogens that can cause infection in a short time. *P. aeruginosa* is the most common infectious bacteria that causes septicemia in burn patients [**5**]. Burn patients are also at high risk of line sepsis, and ventilatory-associated pneumonia because of reduced immune responses relative to non-burned patients [**6**]. Gram-negative bacteria cause most burn wound infections, with similar prevalence, incidence, and pathogens regardless of institution or geography [**7**]. Burn wound infection caused due to *P. aeruginosa, E. coli, K. pneumoniae,* and *S. aureus* are independent predictors of mortality [**6** and **7**].

P. aeruginosa is a commonly known opportunistic pathogen frequently causing serious infection and complications in burned patients throughout the world, which accounts for about 45% of mortality among these patients [**8** and **9**]. The presence of dead, denatured tissues and moist environment makes the burn wound vulnerable to infection by *P. aeruginosa* [**10**]. Additionally, a breach in the protective skin barrier, reduced immunity, and prolonged hospital stay are important factors responsible for infection of burn wound with such opportunistic pathogens especially multi-drug resistant (MDR) *P. aeruginosa* [**11**].

Different studies have reported *P. aeruginosa* isolation rates ranging between 22–75% from infections of burn patients [**8** and **9**]. This pathogen mostly causes burn wound infections from the patient's endogenous gastrointestinal flora and/or an environmental source [**4** and **12**]. It has been associated with sporadic or clustered cases of infection generally confined to single hospitalization units [**13**].

Infections caused due to *P. aeruginosa* are difficult to cure and often require combination therapy because high rates of resistance to antibiotics are associated with *P. aeruginosa* strains. One important factor for the success of *P. aeruginosa* antibiotic resistance is that it forms biofilm, which allows it to adhere to any surface, living or non-living, and thus its infections can involve any part of the body. Additionally, the genetic changes and adaptive behavior of the microorganisms within the biofilm make them resistant to all known antimicrobial agents making the *P. aeruginosa* infections more complicated and life threatening [**13** and **14**].

Furthermore, highly problematic in burn situation is both the spread of *P. aeruginosa* from one patient to another and the persistence of this strain in patients throughout several courses of antibiotic treatment, which were administered to treat *P. aeruginosa* and non-*Pseudomonas* infections [**13** and **15**]. Therefore, currently *P. aeruginosa*, antibiotic resistance is an increasing problem globally and raises serious concerns [**15** and **16**].

1.2 Statement of the Problem

Burns are a major public health issue globally, ranking among the top 15 leading causes of the global disease burden [**17**]. It results in an estimated 265,000 deaths and 19 million disability-adjusted life-Years (DALYs) lost annually but approximately 90 up to 95 percent of burns occur in low- and middle-income countries (LMICs), mostly in the WHO African and South-East Asia regions, which are least equipped to provide timely and comprehensive care [**18** and **19**].

In Sub-Saharan Africa, burns are an important cause of morbidity and mortality, which accounted for an average of 17% mortality rate [**20**]. In Ethiopia, it remains a significant source of morbidity and mortality with average of 11.6%. Even though, specific data concerning burden of *P. aeruginosa* among burn patients has not been known to-date, some studies showed that burn is an important health problem in our country, and it is also additional burden on the existing poor health care system [**21**].

Burns are serious injuries often complicated by colonization with bacteria, particularly *P. aeruginosa*. Several bacterial species are also commonly encountered in burns: *Staphylococcus aureus* is the most common gram-positive pathogen while *P. aeruginosa* is the commonest gram-negative species isolated from burn patients. Infection in the burn patient is an important cause of morbidity and mortality, which accounts for 50% - 75% of mortality due to burns after initial care [**9** and **22**]. In fact, *P. aeruginosa* is an epitome of all opportunistic nosocomial pathogen and despite advances in therapy, the mortality to nosocomial *P. aeruginosa* is approximately 70% in immunocompromised patients [**12** and **23**].

P. aeruginosa is a leading cause of healthcare associated infection especially in patients admitted to critical care units such as intensive care units and burn care centers [**24**]. According to one study involving periodic swabs taken from burn wound, nasal, axillary, inguinal, and umbilical regions of the patients on admission and 7th, 14th, and 21st days of hospitalization, *S. aureus* and *Coagulase-negative staphylococci* (CNS) were the most prevalent isolates in admission cultures [**4**]. However, there was a gradual decrease in the number of isolates of CNS and a marked increase in the numbers of *S. aureus* and *P. aeruginosa* from admission 14th to 21st day. In other studies, *P. aeruginosa* has been identified as the second most common cause of infections in Burn Patients [**6**].

The currently increasing incidence of MDR isolates of *P. aeruginosa*, severely compromises the selection of appropriate treatments, and therefore is associated with significant morbidity and mortality [**6**]. It is an emerging cause of mortality and morbidity in burn patients, which causes 4-60% nosocomial infections in different parts of the world [**25**]. It was also identified as the cause of outbreak of serious wound infections that results in mortality among burn patients [**26**].

P. aeruginosa is naturally resistant to many antibiotics, high concentrations of salts, dyes, weak antiseptics and it is increasingly becoming resistant to many anti-pseudomonal agents. These properties of *P. aeruginosa* are the main factors for its ecological success, which also help explain the ubiquitous nature of the organism and its prominence as a nosocomial pathogen. Therefore, treatment of burn patients infected with *P. aeruginosa* becomes very difficult due to limited treatment options [**10**]. Current therapies focus only on the use of antibiotics. However, the development of multi-antibiotic resistance and expression of multiple virulence factors has led to the ineffectiveness of current therapies, thereby jeopardizing the selection of appropriate treatments [**12** and **23**].

In most developing countries like Ethiopia, antibiotics purchase without prescription is a common practice. This practice leads to misuse of antibiotics by the public thereby contributing to the emergence and spread of antimicrobial resistance [**27**]. The current spread of MDR bacteria pathogens has added a new dimension to the problem of burn wound infections. Regular microbiological tests are needed for treatment of infected burn if affected patients must receive quality health care, particularly when blind treatment is a necessity, as in underdeveloped and developing nations [**28**].

In Ethiopia, there are paucity of information about the specific prevalence and antimicrobial resistance patterns of *P. aeruginosa* among burn patients even though some studies were done on a general bacterial profile among burns patients. Therefore, this study was initiated to provide comprehensive information on burns in general and updated information about prevalence and antimicrobial resistance patterns of *P. aeruginosa* and its associated factors in particular among burn patients.

1.3 Significance of Study

Assessing the prevalence of *P .aeruginosa* among burn patients with bacteriological examination is critical to prevent drug resistance, like MDR and considered among superbugs. Infection due to *P. aeruginosa* is an important indicator for burn patient's mortality. Due to the gradually increase of this bacterium in burn treatment centers it becomes a major cause of nosocomial infections in burn patients. In countries with high burden of burn and less equipped medical services, rapid diagnosis of burn wounds, detection and regular monitoring of antibiotic resistance, and continuous surveillance in burn patients are essential measures for disease management and earlier treatment initiation. These in turn improve burn patients' treatment outcomes, enable to know the magnitude of problem and allow taking effective public health measures.

Since there is no enough study done on this problem in Ethiopia, the data generated from the present study are hoped to provide important information regarding the magnitude of *P. aeruginosa* and its antibiotic resistant patterns among burn wound patients. Therefore, this study was intended to fill this gap of information by specifically assessing the prevalence of *P. aeruginosa* and its antibiotic resistant patterns and associated factors among burn patients.

Furthermore, the result of this study can be used as an input for Federal Ministry of health, Policy planning bodies, Clinicians, Pharmacist, and other responsible bodies in guiding for more appropriate empirical antibiotic therapy and consequently reducing the death and disability associated with burn wound infection due to *P. aeruginosa*. Finally, the study produced a baseline data that may be used for other future studies.

2. LITERATURE REVIEW

2.1 Microbiology and virulence of *P. aeruginosa*

P. aeruginosa was first isolated in 1882 from green pus by Gessard who called it *Bacillus pyocyaneus* [**29**]. It is a highly adaptable bacterium primarily to soil habitat, although it also survives in aquatic environments. Its nutritional diversity allows *P. aeruginosa* to survive in toxic waste degradation. Therefore, *P. aeruginosa* has ubiquitous life-style, which allows this bacterium to involve in frequent infections in humans and animals [**30**].

P. aeruginosa is a rod shaped, Gram-negative bacteria, which belongs to the family of *Pseudomonadaceae.* These opportunistic pathogens are widespread in nature, inhabiting soil, water, plants, and animals including humans. It rarely causes disease in healthy persons, but can multiply easily in immunocompromised patients [**31**]. Surface factors of bacteria such as Pili, flagella and lipopolysaccharide as well as active processes such as the secretion of toxins, biofilm formation, and quorum sensing are virulence determinants that impact the outcome of *P. aeruginosa* infections [**32**].

P. aeruginosa is involved in a variety of human infections ranging from neonatal sepsis, to burn sepsis, acute and chronic lung infections. This organism is a common opportunistic pathogen commonly responsible for infections in patients with defects in host defenses, such as chronic neutropenia and defects of neutrophil function, hematologic cancers, human immunodeficiency Virus (HIV)/ acquired immunodeficiency syndrome (AIDS), cystic fibrosis and diabetes mellitus [**30**].

Colonization of *P. aeruginosa* in a patient with severe burn wounds is acquired when normal skin flora is replaced by hospital flora; typical infections from *P. aeruginosa* occur several weeks after the initial burn. Burn wound culture alone may not differentiate wound colonization from true invasive disease, necessitating biopsy and quantitative cultures to differentiate infection from colonization. The fruity or grape-like odor associated with *P. aeruginosa* can be observed in wounds heavily colonized with the organism. Burn sepsis is typically associated with positive blood cultures and significant morbidity and mortality [**30** and **33**].

Numerous *P. aeruginosa* virulence factors, especially such as pili and flagella, contribute to the pathogenesis of burn wound infection. The ability to colonize a burn wound depends upon the

concerted impairment of several host immune mechanisms. The importance of *P. aeruginosa* in burn wound infections is due to its ability to take advantage of the host's immune compromisation and secretion of a variety of important virulence factors [**30**].

2.2 Epidemiology and Global Burden of *P. aeruginosa* Infection

P. aeruginosa has become a major cause of nosocomial infections worldwide which accounts for about 10% of all infections and so a serious threat to Public Health [**34**].McManus in 1985 suggested that 10% of all burn patients develop *P. aeruginosa* bacteremia which carries a mortality rate of 80%, and the risk of *P. aeruginosa* infection increases substantially in burns to >30% [**35**].

Burn infection caused due to *P. aeruginosa* poses a significant challenge in terms of systemic sepsis, graft loss, prolonged hospital stays, and even increased mortality [**6**]. However, *P. aeruginosa* infections are not only associated with considerable morbidity and mortality, they also present an economic burden, as they are associated with high treatment costs and longer duration of hospital stay, due to their enormous drug resistance capability, when compared to those associated with their drug-susceptible counterparts [**36**].

Types of bacteria that colonize and infect burn patients and their antibiotic sensitivity are highly variable between burn units and highly influenced by topical antimicrobials and wound care policies as well as systemic antibiotic use [**37**]. Gram-negative bacteria continue to cause the most severe infections in burn patients. Among these organisms, *P. aeruginosa* is the most commonly encountered source of chronic or acute burn wound infection in the United States [**33** and **38**].

In a recent survey of 104 U.S. burn units, 44% of the respondents identified *P. aeruginosa* as the most prevalent gram-negative pathogen, followed by *Acinetobacter baumannii* and *Enterococcus* species [**38**]. In Europe, *P. aeruginosa* and *E.coli* are the two most common pathogens, with a frequency for each at 13% of all gram-negative infections [**39**]. The picture is slightly different in Asian countries such as China, where *Proteus mirabilis* and *A. baumannii* are the most common causes of burn infection, with *P. aeruginosa* in third place [**40**].

A Study conducted in India among burn patients reported that the most common bacteria isolated were *P. aeruginosa* (55.0%) [**41**]. Similar studies were conducted in many countries in the world all showing the predominance of *P. aeruginosa* accounting for over 50% of the cause of burn wound infections: in Iran, 57% among 145 burn patients admitted at Tohid Hospital burn unit [**42**];

in Palestine, 50% among 118 burn patients [**43**] and in Nigeria, 62.7% among 62 burn patients at the University of Benin Teaching Hospital [**44**]. Contrary to these reports, other studies observed Prevalence of *P. aeruginosa* burn infection lower than 50% but maintained its predominance: 27% among 985 burn patients in Iraq [**45**]; 24.91% among 109 burn patients in Pakistan [**46**]; 30.2% among 86 burn patients in Ghana [**47**]; 46.46% among 90 war-related burn wound infections in Yemen [**48**], (22.6%) among 475 burn patients in Lebanon[**49**] and 39.5 % among 406 burn patients in Kenya [**50**].Some other studies report different prevalence as shown below(Table 1).

Table 1: Some report of Prevalence of *P. aeruginosa* among burn patients

Continent	Country	Prevalence of *P. aeruginosa (%)*	References
Asia	Indonesia	12	[**51**]
	China	9.3	[**52**]
	Turkey	12	[**53**]
	Nepal	14.6	[**54**]
Africa	South Africa	14.5	[**55**]
	Kenya	13.7	[**56**]
	Tanzania	12.6	[**57**]

In Sub-Saharan Africa, there is a lack of quality data on burns in spite of the fact that it is continuing to plague the continent significantly resulting in a high number of burns, high morbidity, and mortality. As the result, management of burns remains a challenge, [**20**]. A cross-sectional survey of nearly 1400 households in Tigray, Ethiopia, showed that 1.2 percent of the population suffer from burn each year. Over 80 percent of these burns occurred at home, and 90 percent healed without any complications. Only 1 percent of the burn victims died [**58**]. Recently done Meta-analysis in Ethiopia estimates that burn victims account for about 1.5% to 9% of injuries in all age groups [**59**].According to cross-sectional study conducted from April to July 2010 on 50 burn patients, by accessing its bacteriological profile admitted at Yekatit 12 Hospital burn unit, the rate of *P. aeruginosa* isolation was 1/21(4.8%) [**60**]. Another study from Yekatit 12 Hospital burn unit showed that *P. aeruginosa* was isolated in 40 (39.6%) of all burn wounds [**61**]. However, this study was done on small number of burn patients. In addition to this, there is no any report on the specific prevalence and antibiotic resistance patterns of *P. aeruginosa* among burn patients in our country.

2.3 Antimicrobial Resistance Patterns of *P. aeruginosa*

Conventional antibiotic therapies against *P. aeruginosa* infections have become increasingly ineffective due to the rise of multidrug-resistant strains [**62**]. MDR is defined as non-susceptibility to at least one antibiotic in three or more antimicrobial classes, [**63**].

The capability of *P. aeruginosa* to generate resistance against commonly used broad-spectrum antibiotics contributes greatly in its notorious fame. Infections caused by *P. aeruginosa* are difficult to treat as the majority of isolates exhibit varying degrees of innate resistance such as outer membrane impermeability, target site modification, multidrug efflux pumps, production of several antibiotic inactivating enzymes and biofilm formation [**15**].

P. aeruginosa is frequently resistant to multiple antibiotics and consequently has joined the ranks of 'Superbugs' due to its enormous capacity to engender resistance [**64**&**65**]. This opportunistic pathogen is also a leading cause of nosocomial infections. Compared with other pathogens, *P. aeruginosa* is very difficult to eradicate as it displays high intrinsic resistance to a wide variety of antibiotics (including Aminoglycosides, Fluoroquinolones and B-lactams), acquired resistance and adaptable resistance as indicated below (Table 2 and Fig.1) [**42**& **66**].

Table 2. Overview of the different types of resistance exhibited by *P. aeruginosa* [**66**]

Class of resistance	Stable[a]	Inheritable	Dependency on environment	Mechanisms	Examples of genes involved
Intrinsic	+	+	–	Low outer membrane permeability, β-lactamase production and efflux pump overexpression	*crc, lon, psrA*
Acquired	+	+	–	Horizontal transfer, mutations leading to reduced uptake and efflux pump overexpression	*ampD, gyrA, nalA, nfxB, cbrA,* MBLs
Adaptive	–	–	+	Gene expression changes including β-lactam and efflux pump overexpression owing to factors triggering expression of regulatory genes	*ampC, mexZ, phoQ*

[a]+, property applies; –, not a property of this form of resistance.

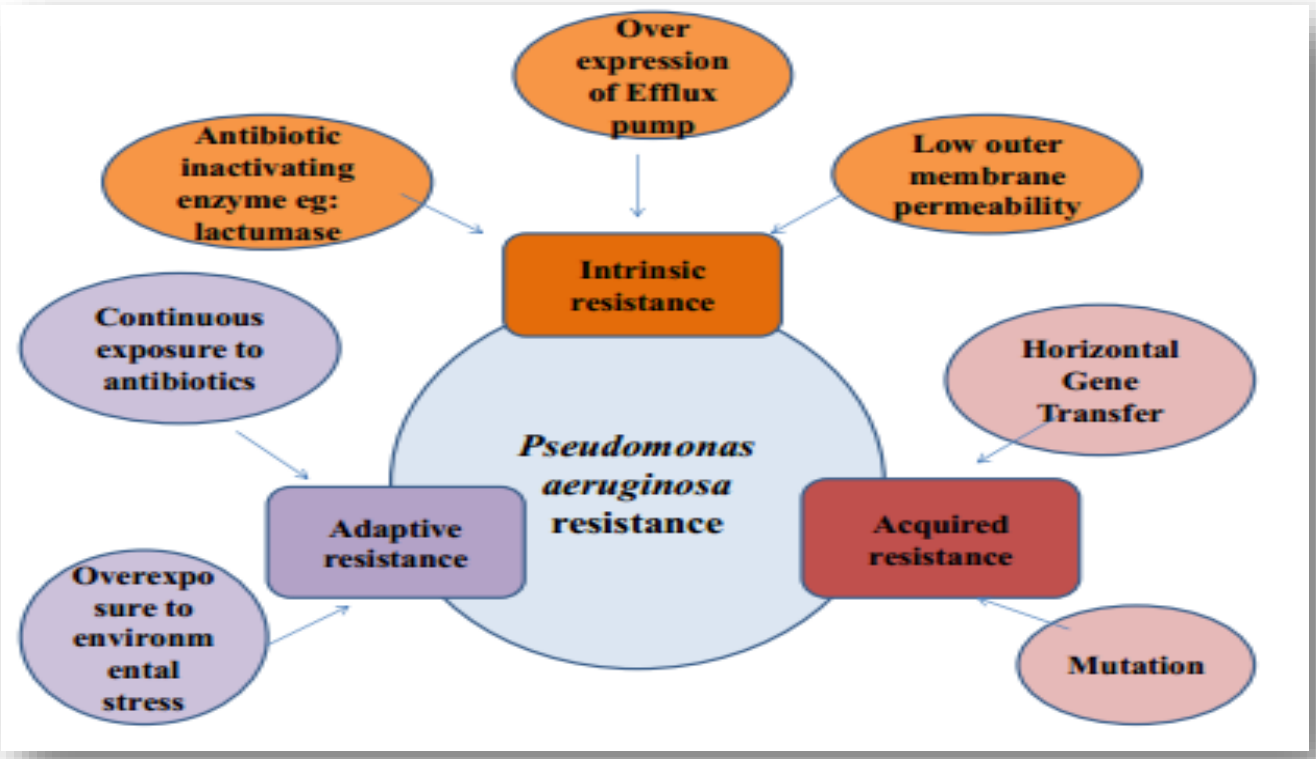

Figure 1: Possible mechanism of antibiotic resistance in *P. aeruginosa* [**66**].

Multidrug resistance in bacterial population is a great challenge in the treatment of burn patients with *P. aeruginosa* infections [**64**].Studies done in Iraq showed that most effective antibiotic was Imipenem with 32% resistance followed by Meropenem (37%) in all specimens. Gentamicin was the least effective antibiotic with 86% resistance [**45**].

One study from Turkey indicates various level of *P. aeruginosa* resistance against different antibiotic, including 32% to Ceftazidime, 31% to Piperacillin/tazobactam,46% to Imipenem, 19% to Meropenem, 36% to Gentamicin, 25% to Cefepime, 25% to Ciprofloxacin, 21% to Amikacin. However, (43%) of *P. aeruginosa* isolates were multidrug-resistant while Meropenem, Amikacin, Ciprofloxacin and Cefepime were found to be most active antimicrobial agents [**53**]. However, 43% of *P. aeruginosa* isolates from that study were multidrug-resistant. Similarly, many antimicrobial sensitivity studies from different countries against a variety of antimicrobial agents have documented alarmingly very high resistance to the majority of drugs tested, as shown in the following table below (Table 3).

Table 3: **Some reports of Antimicrobial resistance patterns of *P. aeruginosa* among burns**

Antimicrobials		Country [References]					
Class of antimicrobials	Name of antimicrobials	Yemen (%)	Nepal (%)	India (%)	Malaysia (%)	Ghana (%)	Kenya (%)
		[**48**](R)	[**54**](S)	[**67**](R)	[**16**](R)	[**47**](S)	[**50**](S)
Aminoglycosides	Amikacin	82.6	50	36.1	50	80	68.1
	Gentamycin	87	20	59	94.3	87	67.6
	Tobramycin	78.2	NA	NA	NA	NA	NA
Cephalosporins	Ceftazidime	76.1	NA	70.5	89.8	75	68.1
	Cefepime	71.7	33.3	39.4	NA	NA	67.6
Carbapenems	Imipenem	NA	50	3.3	73.9	NA	NA
	Meropenem	NA	50	11.5	NA	100	65.9
Beta-lactamase inhibitors	Piperacillin-tazobactam	52.2	33.3	13.1	61.4	NA	55.1
Monobactams	Aztreonam	58.7	NA	NA	52.3	NA	57.8
Fluoroquinolones	Ciprofloxacin	65.2	50	49.2	92	70	68.1
	Levofloxacin	NA	50	NA	NA	NA	NA

Key:-R=Resistant, S=Sensitive and NA=Not Applicable

2.4 Associated factors for *P. aeruginosa* infections

There are many factors which lead to infections in burn patients, such as exposed body surface, exposure to antimicrobial therapy, immunocompromised state such as being HIV/AIDS positive, invasive procedures carried out in the health care facility, and prolonged hospital stay. Factors related to a patients (such as age, total body surface area of burn wound (TBSA), and depth of burn wound, presence or absence of concomitant inhalation injury) [**27** and **68**], and factors related to microbiological organisms (such as type and number, toxin or enzyme production, and motility of organisms), both determine invasive infection. Superficial bacterial contamination can cause sepsis in burn patients and both are directly correlated with one another [**9**, **22** and **69**].

Several studies conducted in different parts of the globe have shown that the length of hospitalization after a burn injury is associated with the types of bacterial species that are isolated from patients. Although length of hospital stays in patients admitted for burn injury is obviously associated with various clinical characteristics such as burn size and the presence of inhalational injury, burn center length of stay is also a major risk factor for infection with bacteria [**70**]. Studies from Lebanon showed that Length of stay (LOS), sepsis, blood transfusion and female sex independently and positively predicted infection in burn patient, [**49**].The prolonged hospitalization of patients resulted in high emergence of resistant strains of *P. aeruginosa* [**44**].

The most important associated factors for *P. aeruginosa* were delayed burn wound, burn size, high percentage TBSA and having an underlying disease, [**23**].

However, other studies indicate that risk of *P. aeruginosa* infection increases substantially in burns >30% with the age of the patient, the total area of the burn wound (TBSA), and the presence or absence of concomitant inhalation injury). Burn size and depth are generally known to increase the risk of colonization and infection, probably due to longer delay between injury and hospitalization, increasing the likelihood of colonization, [**37**&**45**].

2.5 Control and Prevention of *P. aeruginosa* infections

P. aeruginosa are ubiquitous bacteria, i.e., being present commonly in the home, hospital and clinic environments. The bacterium is a difficult organism to eradicate completely from areas that become contaminated such as hospital rooms, clinics, operating rooms, and medical equipment. If a patient comes up with a known infection from *P. aeruginosa* in a hospital room, the same strain of the bacteria can be found in the toilet, sink drain, the shower stall and on surfaces in the hospital room within a matter of days. Nosocomial spread of bacteria including *P. aeruginosa* is frequently by hands [**30**&**33**].

Education of hospital and all medical personnel on proper hand hygiene is vital for successful infection control of *P. aeruginosa*. Those who enter the room of a patient colonized with a MDR organism should wear gloves and gowns and practice strict hand hygiene on leaving the patient's room and removal of the protective apparel is a very important feature in preventing and controlling the transmission of antibiotic resistant organisms. However, patient-to-patient transmission of multiply drug resistant *P. aeruginosa* may also occur in the hospital environment [**30**].

Ongoing surveillance of infections in the burn unit is very important so that new resistant organisms can be quickly isolated, confined, and continuous maintenance of the environment can be achieved. To this end, implementing effective infection control measures to reduce nosocomial transmission of the bacteria within the burn care setting is critical for cost-effective and successful burn care [**37**].

2.6 Challenges of *P. aeruginosa* among burn patients

P. aeruginosa has a predilection for moist and warm wound environments, thus posing a major challenge for burn patients [**33**].Use of conventional antibiotics has resulted into an increase in multi-drug resistant strains of *P. aeruginosa*, which is one of the most worrying opportunistic factors in nosocomial infections [**71**].

The increasing threat posed by *P. aeruginosa* infections and the accelerating development of multidrug resistance in this organism is one of the greatest diagnostic and therapeutic challenges to modern medicine. Because diagnosis of resistance before antibiotic treatment is essential to guide clinicians in their choice of anti-infective therapy, rapid results on antibiotic susceptibility are mandatory. To be reliable, molecular tests must assess all the genetic determinants playing a direct or indirect role in resistance or adaptation to every anti-pseudomonal antibiotic [**34**].

Bacterial population that develops multidrug resistance is a great challenge in the treatment of patients with *Pseudomonas* infections. These problems call for monitoring and optimization of antimicrobial use. Additionally, strengthening of laboratory services at national and international levels will ensure effective surveillance of antimicrobial resistance. Further studies should focus on better administration of the existing antibiotic armamentarium, along with antibiotic stewardship programme [**36**&**71**].

3. OBJECTIVES

3.1 General objective

- To determine the prevalence and antimicrobial-resistant patterns of *Pseudomonas aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia.

3.2 Specific Objectives

- To determine the prevalence of *P. aeruginosa* among patients with burn wound infections
- To determine the antimicrobial resistance pattern of the *P. aeruginosa* isolates from burn wound infections
- To identify factors associated with *P. aeruginosa* among burn wound infections

4. METHODS AND MATERIALS

4.1. Study design and period

Hospital based cross-sectional study design was conducted from November 2020 to April, 2021.

4.2. Study Area

This study was conducted in Yekatit 12 Hospital Medical College, Addis Ababa City, Ethiopia. It is a teaching hospital under Addis Ababa City Administration Health Bureau. It is located at Arada Sub-City in Addis Ababa. It was established in 1915 with a total of 25 beds and 37 health professionals. According to the data obtained from the hospital, currently the hospital has 725 health professionals and 375 administrative staff with around 272 beds. It provides different medical services for around 5million people. It is the only hospital under the city Administrative Health Bureau of Addis Ababa with high number of burn beds with a dedicated burn center that provide services for high number of adult and pediatric burn patients. According to current Health Management Information Systems/HIMS; the burn center has totally 26 beds that serve for around 60 admissions per month.

4.3. Target Population

4.3.1. Study population

This study was conducted on all burn patients attending/visiting Burn unit (Pediatric and Adult IPD wards) and Burn OPD and who developed wound infections during the study period.

4.4 Selection of study participants

4.4.1 Inclusion criteria

- All age group patients with burn wound infection during the study period
- Patients who agreed to participate and gave informed consent

4.4.2 Exclusion criteria

- Patients who completely healed from burn trauma
- Patients under critical condition and unconscious

4.5 Sample Size and Sampling Technique

4.5.1 Sample Size Calculation

The sample size was calculated based on single sample size estimation by considering the prevalence of *P. aeruginosa* 14.5 % (P= 0.145) from a previous study done in South Africa [**55**], 95% confidence interval, 5% margin of error, the sample size was calculated using the following standard formula

$n=Z^2P\ (1-P)/d^2$

n=sample size

z=level of confidence, 1.96

p=prevalence, 14.5%

d=margin of error (precision), 0.05

Inserting the prevalence into the formula and computing, the sample size was determined to be **191**. Adding 10% of contingency, it became **210**.

4.5.2 Sampling technique

Non-probability convenient sampling technique was applied to recruit the study participants with the order of their consecutive arrival until the achievement of the expected sample size.

4.6 Variables of the Study

4.6.1. Independent variables:

- Age
- Sex
- Educational status
- Occupation status
- Causes of burn
- Anatomical site
- Burn levels
- Co-morbidity
- Total Body Surface Area/TBSA
- Hospital stay time

4.6.2. Dependent variables:

- *P. aeruginosa* isolates
- Antibiotic Susceptibility Test/AST

4.7 Operational Definitions

Burn is an injury to the skin or other tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals.

Infection is invasion and multiplication of microorganisms such as bacteria, viruses and others.
Total body surface area (TBSA) is an estimate of the percentage of total body surface area involved in burn exposure and injury.
First-degree (superficial) burns only the epidermis or outer layer of skin is affected. The burn site is red, painful, dry, and with no blisters.
Second-degree (partial thickness) burns involve the epidermis and part of the dermis layer of skin. The burn site appears red, blistered, and may be swollen and painful.
Third-degree (full thickness) burns destroy the epidermis, dermis and sometimes other tissue as well. The burn site appears white or charred.

Fourth-degree burns go through both layers of the skin and underlying tissue as well as deeper tissue, possibly involving muscle and bone**.**
Scald is a type of burn injury caused by hot liquids or gases.

Multidrug resistant (**MDR**) is those non-susceptible to at least one drug from 3 or more of the antimicrobial classes.

4.8 Data and sample collection procedures

4.8.1 Demographic characteristics and exposure to risk factors

Data collectors (3 Nurses from IPD &OPD burn unit) were identified and trained on how to collect data as per the pre-structured questionnaire. The purpose of the study, any harm and benefit related to the research activities was explained to the study participants accordingly. Each eligible patient was providing a swab of burn wound specimen once during the study period. Demographic data and potential associated factors for burn wound infection including burn wound area /TBSA, prolonged hospital stay and others were recorded as presented in Annex 2.

4.8.2 Specimen collection

Burn wound swabs were collected using sterile cotton swab after cleansing the wounds with normal saline prior to obtaining swab specimens to avoid contaminating them with skin commensals (Annex 1). Sample collection continued until the expected sample size from burn wound patients was achieved. Following collection, the swabs were placed in to sterile test tube with screw cup and were transported to the microbiology laboratory within 30 minutes [**72**].

4.8.3 Sample Processing and culture identification

In the Microbiology laboratory at Yekatit 12 Hospital Medical College, all the burn swab samples were cultured on differential and selective media for bacterial cultivation by two laboratory personnel alongside with principal investigator. The swabs were inoculated onto MacConkey agar and blood agar plates (BAP).The plates were incubated at 35°C-37°C for 24-48 hours and examined for bacterial growth. When growth was observed, colonies were examined morphologically for size, shape, fruity odor and lack of sugar (lactose) fermentation. Further identification was done by sub culturing the isolate from a single colony of pure culture. Definitive diagnosis was done based on colony characteristic appearance on their respective media, gram-staining reaction (annex 1) and biochemical reactions using the standard method as briefly described below [**72**].

4.8.4 Biochemical tests

Biochemical tests were performed on colonies from primary cultures for final identification of the isolates. *P. aeruginosa* was identified by different test such as: - positive catalase test, positive oxidase test, positive motility test, positive citrate test, negative triple sugar iron test, negative urea test, negative lysine iron agar test and negative indole test (annex 1).

4.8.5 Antibiotic susceptibility test (AST)

Antimicrobial susceptibility testing was done using the Kirby Bauer disk diffusion method on Mueller-Hinton agar according to standard operational procedures [**73**].

Antibiotics regularly available and frequently prescribed in the study area were considered for the study. These include: - Ceftazidime (30μg), Ciprofloxacin (5 μg), Gentamicin (10 μg), Tobramycin (10μg), Cefepime (30μg), Meropenem (10μg), Imipenem (10μg), Amikacin (30 μg) and Piperacillin-tazobactam (30 μg).

Pure colony of isolated bacterial organism was picked by sterile wire loop and added to normal saline then mixed thoroughly too adjusted 0.5McFarland standards for susceptibility testing. After 5-10 minutes, sterile swab dipped into the prepared suspension of the isolate in broth, and speeded uniformly over Muller-Hinton agar plate (Oxoid, LTD), and then the above mentioned antibiotics were placed on MHA of *P. aeruginosa* isolates by using sterile forceps placing each disk within equal distances from other disks. After that, the medium was incubated at 35^0C for 18-24 hours [**73**]. After incubation, the plates were examined for zone of inhibition and the diameter of each inhibition zone was measured with a pair of calipers, and recorded in mm. The results were then interpreted as sensitive (S), intermediate (I) or resistance(R) according to CLSI guidelines (2019) (Annex 1) [**73**&**74**].

4.9 Data Management and Quality Assurance

Data quality was ensured through use of standardized data collection materials, pretesting of the questionnaires, proper training of data collectors before the start of data collection and intensive supervision during data collection by the principal investigator.

4.9.1 Pre-analytical

Before the actual data collection data collectors were trained, study participants were informed about the study and were asked for their consent. The swab collection containers were labelled with patient identifier code and date of collection accordingly. The specimens were collected and transported as quickly as possible to the laboratory, and stored according to Standard operating procedures (SOPs). All patient information collected during the study period was checked for its clarity and completeness in a regular basis. Each lot of the culture medium was checked for expiration dates prior to use as part of quality control.

4.9.2 Analytical

Well-trained laboratory personnel performed all the laboratory tests alongside with the investigator. The already established SOP of the host laboratory ensured the reliability and validity of test results. All samples brought to the testing bench were analyzed as soon as possible. The performance of the media and antibiotic discs were evaluated by using positive controls; i.e. American Type Culture Collection (ATCC) 27853 reference strain of *P. aeruginosa*.

4.9.3 Post-Analytical

All laboratory results were recorded on a Laboratory result form and logbook during the study period. Each specimen was stored in a refrigerator for any kind of checkup and future use.

4.10 Data processing and analysis

Data was entered and analyzed using SPSS version 25.0. Descriptive statistics, frequency and percentage, were used to describe the study participants. Univariate, bivariate and multivariate logistic analysis were performed to assess the association of the factors associated with *P. aeruginosa* infection. In all cases P-value, less than 0.05 was considered as statistically significant. Results were presented by text, figures and tables.

4.11. Ethical Considerations

The current study was conducted after obtaining full approval from Departmental Research Ethics and Review Committee (DRERC) of the Department of microbiology, Immunology & Parasitology (DMIP), College of Health Sciences, Addis Ababa University. In addition, the Department wrote a letter to Yekatit 12 Hospital Medical College asking for cooperation. Accordingly, permission was obtained from Yekatit 12 Hospital Medical College to collect data from study participants and for Laboratory analysis. Inclusion of patients for participation was on voluntary basis. Participants were informed of the purpose of the study, risks associated with the study, confidentiality of personal data, and their right when taking part in the study. Study participants were recruited after, they provided the informed written consent and/or assent. Laboratory results of study participants were communicated with their respective physicians for better management.

4.12 Dissemination of the Result findings

The study result will be presented to the Department of Medical Microbiology, Immunology and Parasitology, School of Medicine, College of Health Sciences, Addis Ababa University as MSc thesis. In addition, the finding of this study will be submitted to Yekatit 12 Hospital Medical College, to stakeholders and other concerned bodies interested, and presented in different scientific conferences. Finally, manuscript be prepared and will submitted to different international journal for publication.

CHAPTER FIVE: RESULTS

5.1. Sociodemographic and Clinical data Characteristics of burn patients

During the five months of study period, 210 study participants with burn wound infections were included. Burn wound swabs were collected from study participant admitted to burn IPDs (110(52%)) and burn OPDs (100(48%)) of the hospital. Among these, (98 (46.7%)) were males and (112 (53.3 %)) were females with the age range of 1 to 85 years (Mean 20 years and Median 18years). The majority of the study participants (43.8%) were in the age groups of 0–15 years and among the study participants (50(23.8%)) were students, while majority of participants (130 (61.9%)) lived in urban areas and (154 (73.3%)) had family size <4 (Table 4).

Table 4: Summary of Socio-demographic Characteristics of burn patients at Yekatit 12 Hospital Medical College, Addis Ababa- Ethiopia, 2021.

Variables	Category	Frequency	Percent (%)
Age	0-15	92	43.8
	16-40	90	42.8
	41-60	22	10.5
	>60	6	2.9
Sex	Male	98	46.7
	Female	112	53.3
Educational level	Illiterate	80	38.1
	Elementary	78	37.1
	High school	31	14.8
	College and above	21	10
Residence	Urban	130	61.9
	Rural	80	38.1
Occupation	Government employed	18	8.6
	Self employed	37	17.6
	Daily laborer	35	16.7
	Student	50	23.8
	House wife	38	18.1
	Others	32	15.2
Family size	2−3	154	73.3
	4─7	50	23.8
	>7	6	2.9

Pertaining to cause of burn injury, majority (98(46.7%)) had sustained burn through scald followed by open flame (79 (37.6%)).Anatomical site of burn injury was mostly Extremities (141(67.1%)) followed by Head and Neck (20(9.5%)). Regarding to the depth of burn wounds, majority (118 (56.2%)) were 2nd degree burn followed by 1st degree burn level (62(29.5%)).

Regarding the comorbidity among burn patients, only 35 cases (16.7%) had different related diseases, while Epilepsy accounted for(14(40%)) and Diabetic Mellitus(DM) accounted for (12(34.29%)).Concerning the TBSA, the percentage of burns ranged from 3% to 45% medium value being 13%. Almost half of the patients had TBSA <10% accounted for (86(41%)) followed by 10-19% TBSA accounted for (70(33.33%)).Concerning the Hospital Stay time, almost all of the patients (192 (91.4%)) had total hospital stay between 2-30days, and only 18 cases (8.6%) had Hospital Stay time above one months. Majority of the patients (144(68.6%)) had used antibiotics for their burn wound treatment (Table 5).

Table 5: Summary of Clinical data Characteristics of burn patients at Yekatit 12 Hospital Medical College, Addis Ababa- Ethiopia, 2021.

Variables	Category	Frequency	Percent (%)
Admission type	Burn OPD	100	48
	Burn IPD	110	52
Cause of burns	Scalds	98	46.7
	Open flame	79	37.7
	Chemical	8	3.8
	Electrical	19	9
	Others	6	3.8
Anatomical site	Extremities	141	67.1
	Trunk	5	2.4
	Head & Neck	20	9.5
	Head, Neck &Extremities	15	7.1
	Head, Neck, Perineum &Extremities	11	5.3
	Extremities and Perineum	15	7.1
	Whole body Parts	3	1.5
Level of burn	1st degree	62	29.5
	2nd degree	118	56.2
	3rd degree	22	10.5
	4th degree	8	3.8
Co-morbidity	Mental problem/Epilepsy	14	40
	DM	12	34.29
	HIV	5	14.29
	Others	4	11.42
Total body surface area(TBSA) involved in the burn	<10%	86	41
	10−19%	70	33.3
	20−29%	38	18.1
	≥30%	16	7.6
Hospital stay Time	2-10days	86	41
	11-20days	58	27.6
	21-30days	48	22.8
	>30days	18	8.6
Antibiotic usage	Yes	144	68.6
	No	66	31.4

5.2 Distribution and Prevalence of *P. aeruginosa* among burn patients

Among the 210 burn wound swabs, 27 (12.86%) yielded positive result for *P. aeruginosa.* The *P. aeruginosa* isolation rate was higher (66.7%) among 0-15 age category (Table 6) than the rest age groups. From the total isolates 13(48.15%) were from males and 14 (51.85%) from females.

Table 6: Prevalence of *P. aeruginosa* among burn patients with respect to Sociodemographic variables at Yekatit 12 Hospital Medical College burn center, Addis Ababa- Ethiopia, 2021

Variables	Category	*P. aeruginosa* Isolated					
		Yes		No		Total	
		No	%	No	%	No	%
Age	0-15	18	19.6	74	80.4	92	43.8
	16-40	7	7.8	83	92.2	90	42.9
	41-60	1	4.5	21	95.5	22	10.5
	>60	1	16.7	5	83.3	6	2.9
Sex	Male	13	13.3	85	87.6	98	46.7
	Female	14	12.5	98	87.5	112	53.3
Educational level	Illiterate	9	11.3	71	88.7	80	38.1
	Elementary	13	16.7	65	83.3	78	37.1
	High school	3	9.7	28	90.3	31	14.8
	College and above	2	9.5	19	90.5	21	10
Residence	Urban	15	11.5	115	88.5	130	61.9
	Rural	12	15	68	85	80	38.1
Occupation	Government employed	3	16.7	15	83.3	18	8.6
	Self employed	4	10.8	33	89.2	37	17.6
	Daily laborer	2	5.7	33	94.3	35	16.7
	Student	9	18	41	82	50	23.8
	House wife	6	15.8	32	84.2	38	18.1
	Others	3	9.4	29	90.6	32	15.2
Family size	2−3	20	13	134	87	154	73.3
	4─7	6	12	44	88	50	23.8
	>7	1	16.7	5	83.3	6	2.9

Most of the *P. aeruginosa* isolates were obtained from burn IPD (24(89%)), and only 3 isolates were from burn OPD (11%). Concerning to *P. aeruginosa* isolation rate with respect to cause of burn injury, majority (15/27) were from sustained burn through scalds (56%), followed by open flame 7/27 (26 %), (Figure 2).

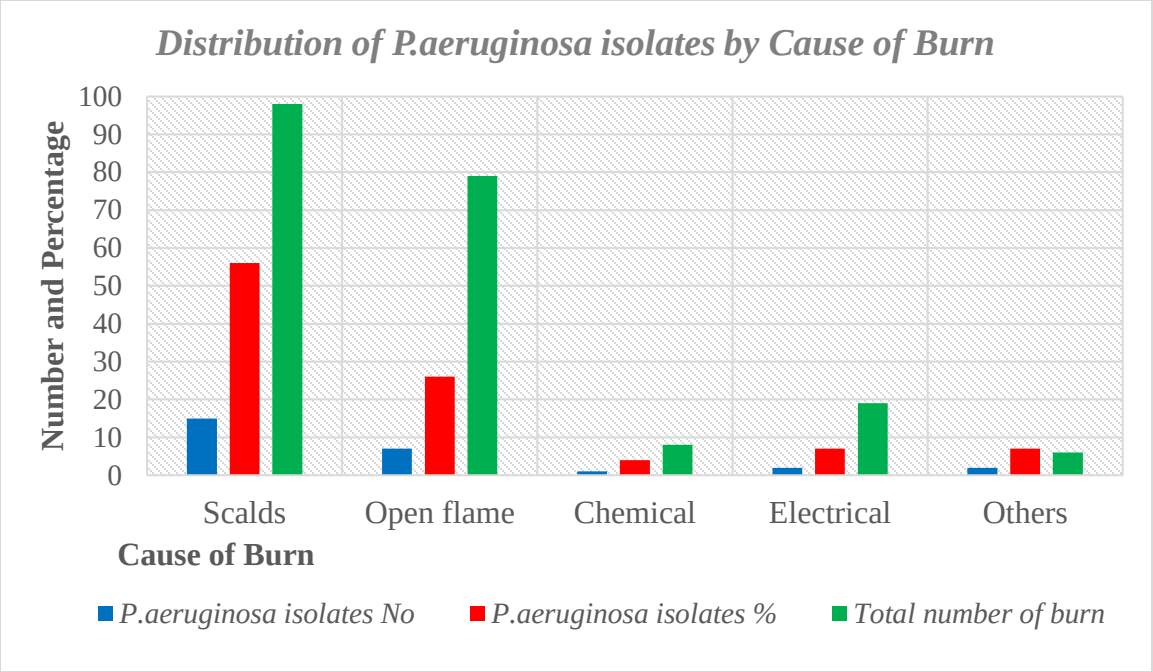

Figure 2: Distribution of *P. aeruginosa* isolates by cause of burns among burn patients at Yekatit 12 Hospital Medical College burn center, Addis Ababa- Ethiopia, 2021.

The proportion of *P. aeruginosa* was the highest in Extremities anatomical site of burn (14/27; 52%) followed by Extremities and Perineum (4/27; 15%) (Figure 3).

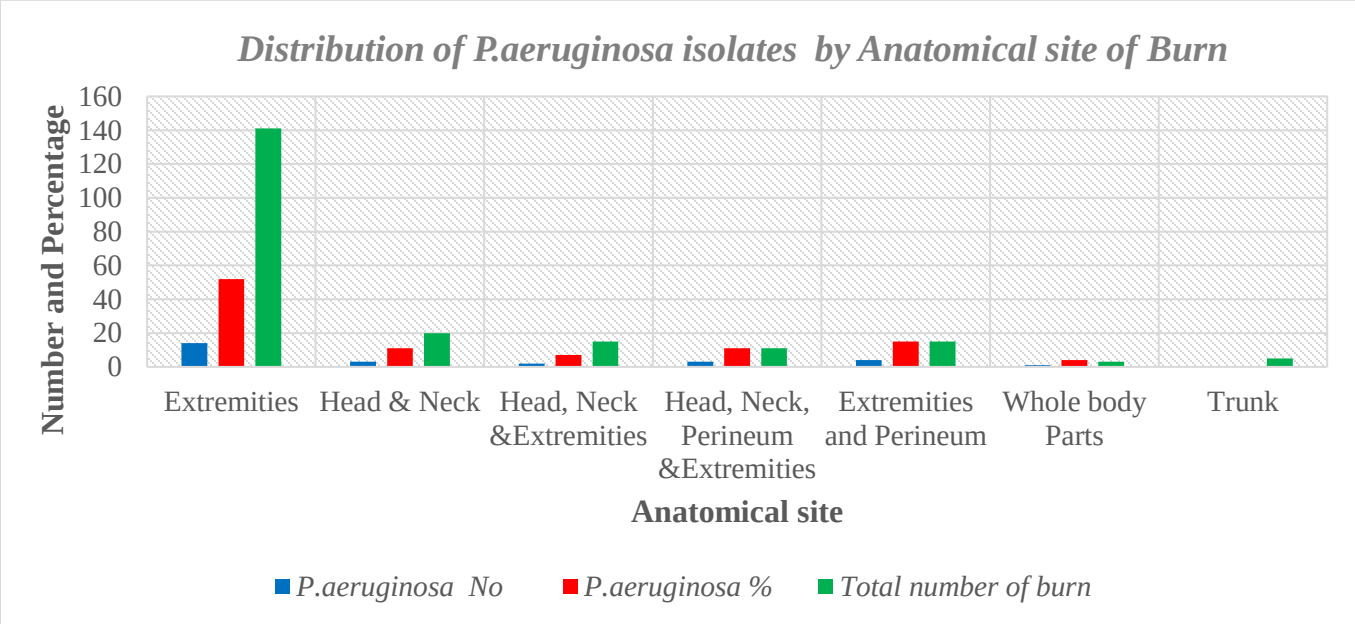

Figure 3: Distribution of *P. aeruginosa* isolates by Anatomical site of Burns among burn patients at Yekatit 12 Hospital Medical College burn center, Addis Ababa- Ethiopia, 2021.

Regarding the depth of burn wounds, the proportion of *P. aeruginosa* was the highest in 2nd degree burn (16/27; 59.3%) followed by 4th degree burn (5/27; 18.5). (Figure 4). However, if the computation of isolation rate is made within the same category (same degree of burn), the highest rate was obtained from the 4th degree burn where 5/8 (62.5%) 4th degree burn patients developed *P. aeruginosa*. This was followed by 3rd and 2nd degree burn patients where isolation rate from each of the two groups of burn patients was 13.6%.

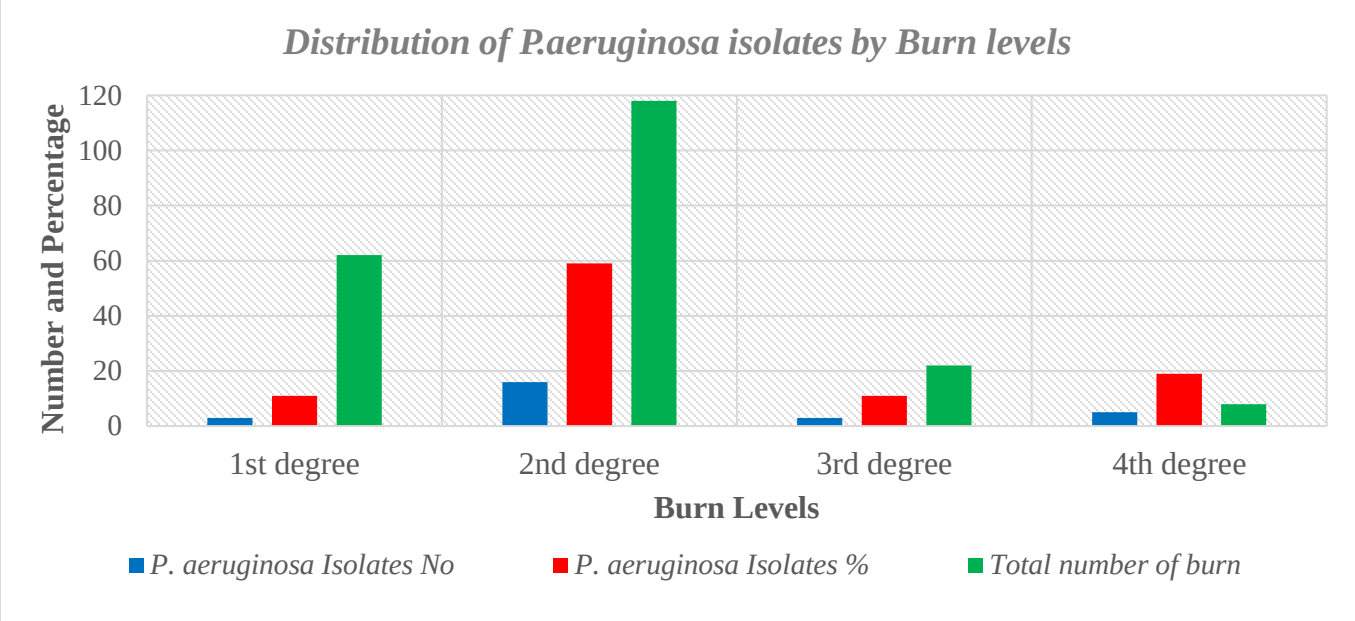

Figure 4: Distribution of *P. aeruginosa* isolates by Level of Burns among burn patients at Yekatit 12 Hospital Medical College burn center, Addis Ababa- Ethiopia, 2021.

The proportion of *P. aeruginosa* was the highest in TBSA greater than or equal to 30% (11/27; 40.7%) followed by 20-29% TBSA category group (9/27; 33.3%) (Figure 5). In terms of isolation rate within the same category of Total Body Surface Area, still TBSA of ≥30% had the highest isolation rate (11/16; 68.8%) followed by TBSA of 20-29 (23.7%).

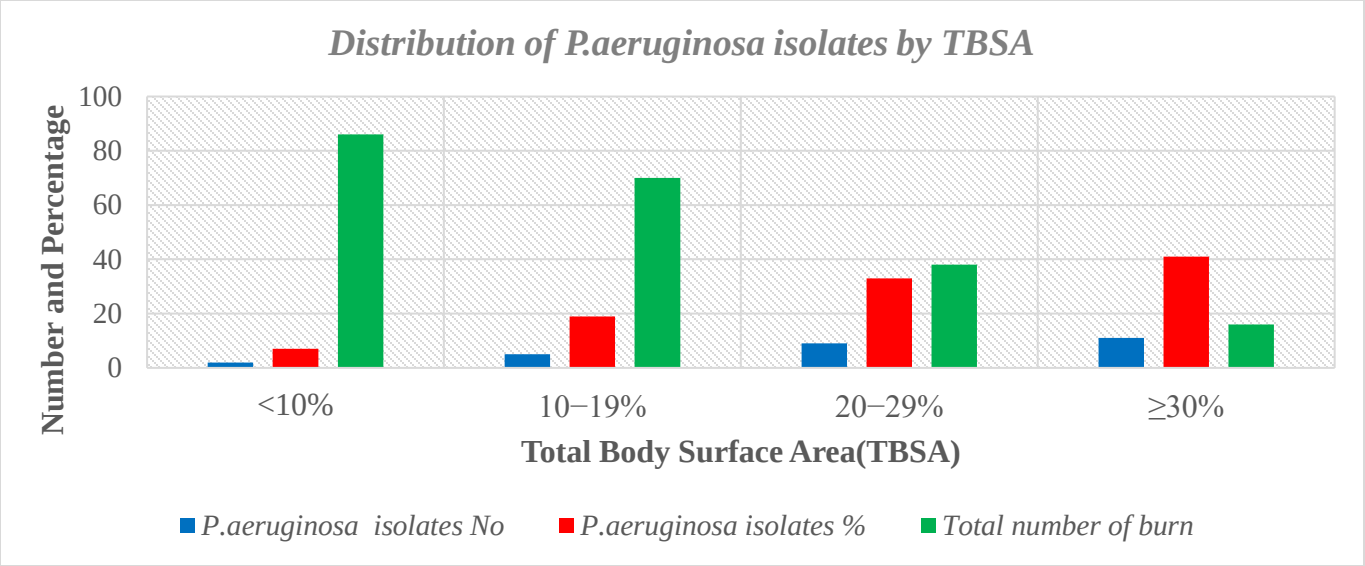

Figure 5: Distribution of *P. aeruginosa* isolates by TBSA of Burns among burn patients at Yekatit 12 Hospital Medical College burn center, Addis Ababa- Ethiopia, 2021.

Concerning *P. aeruginosa* isolation rate among the different Hospital Stay time, most of the isolates were obtained from study participants who stayed in the hospital between 21-30 days (22/27; 81.5), followed by > 1 month (3/27; 11.1%). (Figure 6). On the other hand, 45.8% (22/48) of patients who stayed in the hospital for 21-30 days developed *P. aeruginosa* infection while 16.7% (3/18) patients with hospital stay time of > 1 month developed the infection.

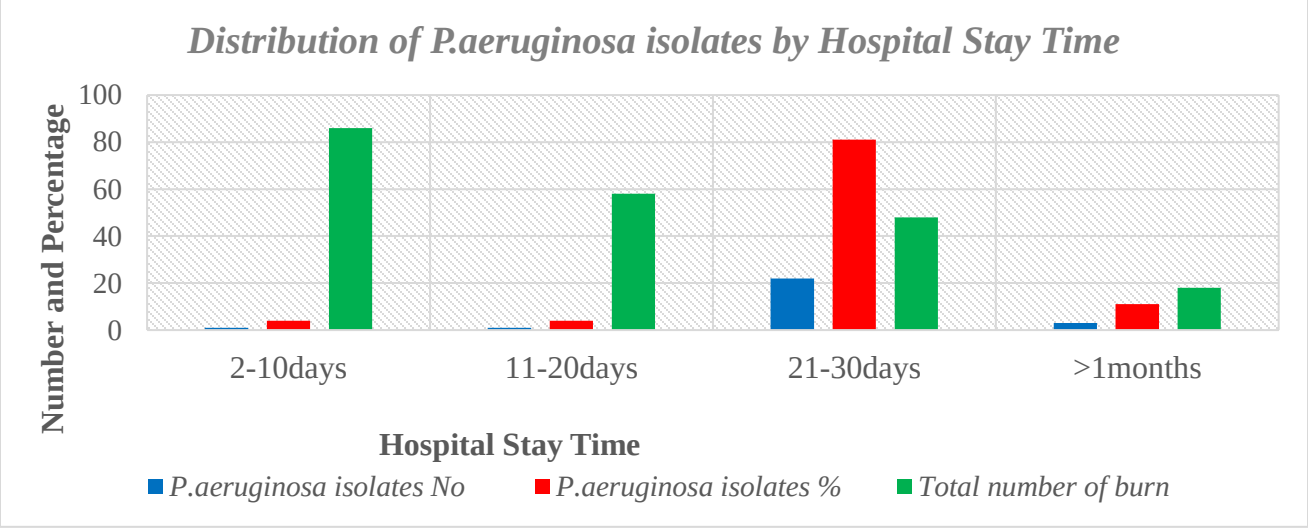

Figure 6: Distribution of *P. aeruginosa* isolates by Hospital stay time among burn patients at Yekatit 12 Hospital Medical College burn center, Addis Ababa- Ethiopia, 2021.

5.3 Antimicrobial resistance patterns of *P. aeruginosa* isolates from burn patients

Antimicrobial susceptibility testing was carried out for all 27 *P. aeruginosa* isolates using Kirby-Bauer disk diffusion method. Antibiotic sensitivity tests were undertaken for Cefepime, Ciprofloxacin, Imipenem, Meropenem, Ceftazidime, Amikacin, Gentamicin, Piperacillin-tazobactam and Tobramycin. The most effective antibiotic was found to be Imipenem with 88.90% sensitivity followed by Amikacin (81.50%). Gentamycin was the least effective antibiotic with 62.97% resistance, (Figure 7).

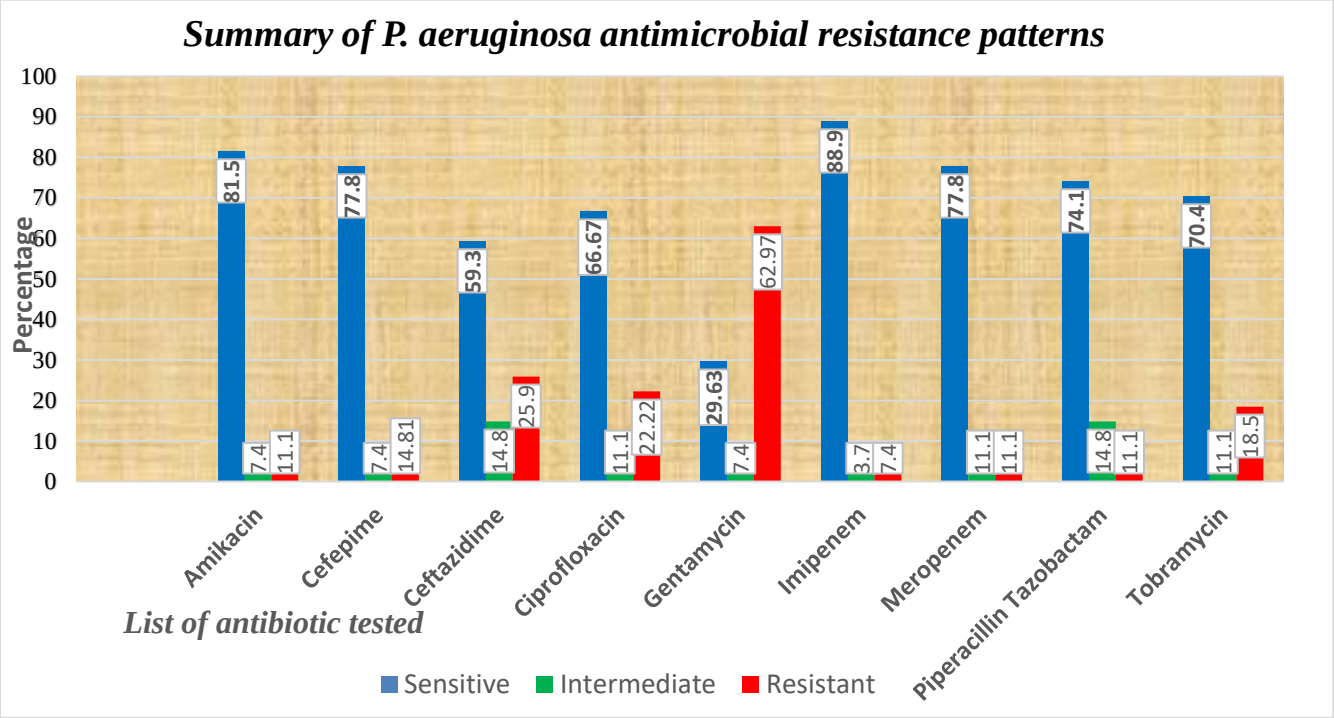

Figure 7:Summary of antibiotic resistance patterns of *P. aeruginosa isolates* among burn patients at Yekatit 12 Hospital Medical College burn center, Addis Ababa- Ethiopia, 2021.

A total of 9 (33.33%) *P. aeruginosa* isolates were multidrug-resistant, i.e., resistance to at least one drug from three or more antibiotic classes, (Figure 8).From total 27 isolates of *P. aeruginosa*, only 4 isolates (15%) were 100% sensitive to all drug tested and no isolate was found resistant to all the antibiotics(Figure 8).

Antibiogram profile of *P. aeruginosa* isolates (N=27)

1. Resistant to at least one antibiotic tested was 11 (41%)
2. Resistant to Aminoglycosides and Cephalosporin class was 2 (7.4%)
3. Resistant to Aminoglycosides, Cephalosporin and Fluoroquinoles class was 5 (18.5%)
4. Resistant to Aminoglycosides, Fluoroquinoles and Carbapenems was 1(3.7%)
5. Resistant to Aminoglycosides, Carbapenems, Cephalosporin and Beta lactam inhibitors class was 1 (3.7%)
6. Resistant to Carbapenems, Cephalosporin and Beta lactam inhibitors class was 1 (3.7%)
7. Resistance to all Aminoglycosides class of antibiotic was 1 (3.7%)

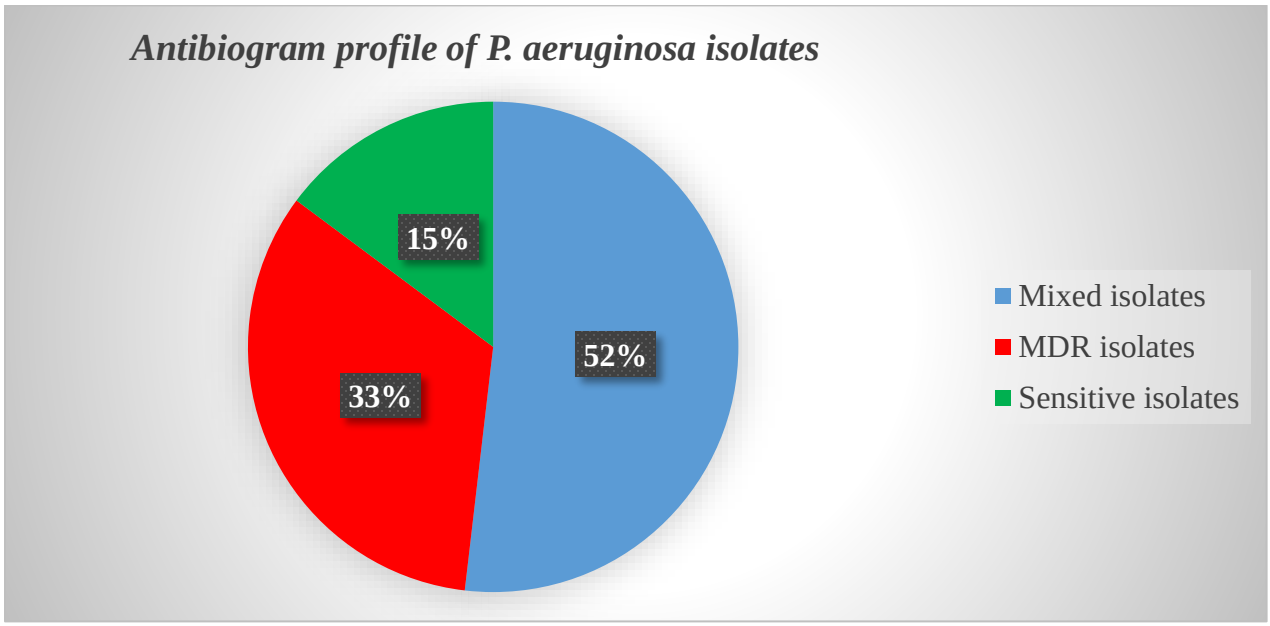

Figure 8: Antibiogram profile of *P. aeruginosa* isolates among burn patients at Yekatit 12 Hospital Medical College burn center, Addis Ababa- Ethiopia, 2021.

Key:-**Mixed isolates**=different isolates with sensitive, intermediate and resistant antibiotic tested.

Sensitive isolates= isolates sensitive to all antibiotic tested.

MDR isolates=isolates resistant to at least one drug from three class of antibiotics or more.

5.4 Possible factors associated with *P. aeruginosa* among burn patients

A binary and multiple logistic regression was performed to determine the effect of Age, Admission type, Cause of burn, Anatomical site, Level of burn, TBSA, Comorbidity and Hospital stay time of burn patient with *P. aeruginosa* as the outcomes. From Binary logistic regression analysis Admission type, Level of burn, TBSA and Hospital stay time had positive association with *P. aeruginosa* infection, while the results of multiple logistic regression showed that only Admission type (positive association with Inpatient department (IPD) wards; P= 0.031), Total body surface area of ≥30% (P= 0.005) and Hospital stay time >1month (0.011) had statistically significant association.

The correlation between the total body surface area (TBSA) with the isolation of *P. aeruginosa* was such that *P. aeruginosa* isolation rate increased as the TBSA increased from 30% upwards. Patients with Burn area (TBSA) of ≥30% were 6.8 times more likely to have *P. aeruginosa* isolates whereas Patients with Burn area (TBSA) of 20-29 % were 3.1 times more likely to have *P. aeruginosa* isolates than those with TBS of <10% (reference).

Another highly associated significant level in the study was Hospital stay time, as the duration of hospital admission increased the isolation of *P. aeruginosa* had also increased. Patients with Hospital admission >1months were 4.6 times more likely to have *P. aeruginosa* isolates than those with 2-10 days hospital admission (reference) (Table 7).

Table 7: Bivariate and Multivariate analysis that shows the relationship between associated factors and Prevalence of *P. aeruginosa* among burn patients at Yekatit 12 Hospital Medical College burn center, Addis Ababa- Ethiopia, 2021

Variables	Category	COR (95% CI)	P-Value	AOR (95% CI)	P-Value
Age	0-15	(Ref.)			
	16-40	2.37(0.24-23.22)	0.46	1.47(0.1-33)	0.79
	41-60	4.20(0.22-79.32)	0.24	1.48(0.15-85)	0.32
	>60	4.8(0.1-7.5)	0.34	4.05(0.56-29)	0.17
Admission type	Burn OPD	(Ref.)			
	Burn IPD	9.2(2.6-31)	**0.001***	7.47(1.15-5.67)	**0.031***
Cause of burns	Scalds	(Ref.)			
	Open flame	1.5(0.15-11.09)	0.20	2.9(4.3-201.7)	0.33
	Chemical	1.3(0.32-73.5)	0.83	3.1(0.2-39)	0.74
	Electrical	1.8(0.7-48)	0.59	2.3(1.17-44.6)	0.42
	Others	0.3(0.03-42.4)	0.42	0.18(0.5-62)	0.34
Anatomical site	Trunk	(Ref.)			
	Extremities	1.4(0.81-14.32)	0.095	1.23(0.78-39)	0.74
	Whole body Parts	6.5(0.05-11.65)	0.84	9(1.10-45)	0.58
	Extremities and Perineum	1.02(0.18-5.95)	0.97	1.8(1.2-87)	0.44
	Head and Neck	2.12(0.35-12.95)	0.41	2.5(0.01-54)	0.78
	Head, Neck & Extremities	2.44(0.3-17.90)	0.38	4.2(0.14-73)	0.24
	Head, Neck, Extremities &Perineum	1.4(1.2-25)	0.56	3.3(1.7-45)	0.78
Level of burn	1st degree	(Ref.)			

	2nd degree	1.63(2.31-48)	**0.002***	1.02(0.81-12.90)	0.98
	3rd degree	3.56(1.6-69)	**0.014***	1.2(0.9-42.3)	0.29
	4th degree	10.2(5.2-20.68)	**0.018***	3.7(0.1-5.95)	0.82
Co-morbidity	HIV	(Ref.)			
	DM	0.3(0.03-3.40)	0.33	0.2(0.01-1.24)	0.63
	Epilepsy	0.36(0.02-7.3)	0.51	0.7(0.01-5.53)	0.21
	Others	0.14(0.4-20.7)	0.15	NA	
Total body surface area(TBSA)	<10%	(Ref.)			
	10−19%	3.9(1.6-37.6)	**0.001***	1.50(0.26-17.70)	**0.003***
	20−29%	7.73(0.2-26)	**0.002***	3.1(0.19-17.5)	**0.006***
	≥30%	8.3(0.58-16.47)	**0.000***	6.8(1.2-10.6)	**0.005***
Hospital stay Time	2-10days	(Ref.)			
	11-20days	1.40(0.21-10.80)	**0.012***	2.4(0.19-16)	**0.021***
	21-30days	5.9(0.6-6.04)	**0.017***	3.95(0.1-25.6)	**0.050***
	>1months	6.7(0.41-10.9)	**0.001***	4.6(2.9-21.5)	**0.011***

CHAPTER SIX: DISCUSSION

Prevalence of *P. aeruginosa* among burn patients

P. aeruginosa remains an important cause of opportunistic infection among burn wound patients and is known to have developed resistance to various antimicrobial agents in burn centers [**1**]. *P. aeruginosa* isolated from burn injuries have significant effect on the mortality and morbidity in hospitalized burn patients particularly in a developing country such as ours. This study assessed the current burden, antimicrobial resistance patterns and factors associated with the prevalence of *P. aeruginosa* among burn patients at Yekatit 12 Hospital Medical College from November 2020 to April, 2021.

This study enrolled 210 burn wound patients, the results showed that at least 27(12.86%) of the 210 participants were infected with *P. aeruginosa* which is in agreement with results of previous studies done in Germany (11.8%)[**39**], in India, Indonesia, and Turkey each with rate of 12% [**31**,**51**&**53**], 12.6% in Tanzania[**57**], 13.7% in Kenya [**56**], 14.5% in South Africa[**55**], and 14.6 % in Nepal[**54**]. However, the isolation rate from this study is higher than those reported from other previously done studies both in the country and elsewhere in the world :-(for examples, 4.8% in Yekatit 12 HMC [**60**], 6.25% in Nepal [**64**], and 9.3 % in China [**52**].) Contrary to this, considerably higher isolation rates were reported from various countries including 39.6% from Ethiopia[**61**], 22.4% from Malaysia [**16**], 22.6% from Beirut [**49**],44% from USA [**38**], 46.5% from Yemen [**48**], 50% from Palestine [**43**], 55% [**41**] and 32.1% [**67**] from India, 57% from Iran [**42**], 62.7% from Nigeria [**44**], 27% from Iraq [**45**], 24.9% from Pakistan [**46**], 30.2% from Ghana [**47**], 20.7% from Egypt [**17**], and 39.5% Kenya [**50**].

Such variations in *P. aeruginosa* isolation rates from burn wounds between studies could be due to one or a combination of the following reasons: due to(**1**) the type of inclusion criteria, (**2**) use of selective/differential media for culturing the target organism, (**3**) sampling protocols, and (**4**) differences in health care providing institutions wherein the patients were recruited from (e.g., dedicated burn center vs all-service provider; urban vs rural health care provider institution; availability vs unavailability of ideal health provider; differences in health care service : patient ratios or different patient loads; and differences in cleaning and hygiene practices).

Antibiotics resistance patterns of *P. aeruginosa* among burn patients

The antimicrobial resistance pattern of pathogens differs greatly amongst and within countries. Different pathogens and antimicrobial resistance patterns of bacteria keep changing with place and time, [**56**]. In this connection, resistance to antimicrobial agents is the main problem among *P. aeruginosa* strains isolated from wound infections in burn centers, [**75**].

The majority of antibiotics tested in this study, namely, -Imipenem, Meropenem, Amikacin, Piperacillin-tazobactam, Tobramycin and Cefepime were found to be the most active antimicrobial agents against *P. aeruginosa* isolates. On the contrary, Gentamycin was the most resisted antibiotic by the tested *P. aeruginosa* isolates (with 63% resistance and 30% sensitivity rate) followed by ceftazidime (with 26% resistance and 59% sensitivity rate) and ciprofloxacin (with 22% resistance and 67% sensitive rates), which might indicate that these drugs have been used frequently in the burn treatment center of the study site.

Comparable resistance rate by *P. aeruginosa* isolates was observed against Gentamycin in Tanzania [**76**], Kenya [**50**], and Pakistan [**77**]. Higher Gentamycin resistance by *P. aeruginosa* appear to also be a common phenomenon for some countries like India (84%) [**11**], Yemen (87%) [**48**], Iraq (88.5%) [**45**], and Malaysia (94.3%) [**16**]. However, some others reported lower resistance level e.g. 9.2% in South Africa [**55**], 13% in Ghana [**47**], 24.7% in Nigeria [**10**], 36% in Turkey [**53**] and 44.7% in Kenya [**56**].

From the finding of this study, it becomes clear that Imipenem was the most effective antibiotic with only 7.4% resistance, which is consistent with reports from studies done in South Africa [**55**], Nigeria [**10**], Tanzania [**76**], India [**31**], Germany [**39**], and Pakistan [**77**]. Unlike these observations, however, other studies have reported variable but considerably higher rates of Imipenem resistance: 17.5% in Nigeria[**10**], 31.7% in Kenya [**56**], 32% in Iraq [**45**], 46% in Turkey [**53**], 50% In Nepal [**54**], 61% in India [**11**], 66.7% in Pakistan [**46**], 73.9% in Malaysia [**16**], and 76% [**18**] and 94.7% [**24**] in Iran. Probably this drug is being used heavily in these countries.

The next most effective antibiotics were Amikacin, followed by Meropenem & Piperacillin-Tazobactam each with 11.11% resistance, which is comparable with resistance rate documented particularly for Amikacin from other studies including from Yemen [**48**], Ghana [**47**], Kenya [**56**], and South Africa [**55**]. However, there are much higher resistance rates reported for Amikacin: 21% in Turkey [**53**], 32% in Kenya [**50**], 32.1% in Nigeria [**10**], 48.1% in Iraq [**45**]**,** 50% in Malaysia [**16**], 54.3% in Tanzania [**76**], 73.2% in India [**11**], 75% in Pakistan [**46**], and 89.4% in Iran [**24**]. Overall, except for Gentamycin, sensitivity to all other tested antibiotics was over 50%. Compared with the susceptibility of *P. aeruginosa* to antimicrobials reported from the literature in several studies, low resistance level to systemic antibiotics was found in this study. This might be due to limited exposures of the pathogen to the new generation broad-spectrum antibiotics, or low development of cross-resistance among *P. aeruginosa* isolates from this settings, or due to the infrequent use of systemic antibiotics in the burn center under investigation.

The overall rate of MDR *P. aeruginosa* isolates was 33.33%, which goes in line with the result from studies done in Ethiopia, (36.5%) [**28**] and in Pakistan (29.24%) [**77**].However, the finding from this study is much higher than findings from Malaysia 5.74%[**14**],USA 10.2%[**25**],India 12%[**31**]and Egypt 12.3%[**17**].On the contrary, this finding is lower than the findings from other studies elsewhere which reported from as low as 40.7 % to as high as 100% [**10, 11, 24, 48, 52**]. The possible explanation for such disparity might be difference in study population, use of different antibiotic regimes, extensive use of antimicrobial drugs, presence of different persistent strains in hospitals, cross contamination of resistant isolates and the quality of hygiene in hospital environments.

Possible factors associated with *P. aeruginosa* infection among burn patients

Bivariate and multivariate regression analyses indicated that TBSA, Level of burn, Admission type and Length of Hospital stay had statistically significant (all with P value <0.05) association with *P. aeruginosa* infection. These findings are in line with reports from similar other studies. For example, the association of TBSA with burn wound infection by *P. aeruginosa* was also observed from other previous study in Ethiopia [**60**], Nigeria [**44**], Ghana [**78**], Canada [**38**], Netherlands [**9**], Iraq [**45**], Nepal [**54**], and Turkey [**23**]. Such associations may not be unexpected given that larger burn size means a greater area of unprotected body surface and a greater chance of colonization with the pathogen because of destruction of the surrounding structures which may facilitate colonization of microorganisms [**45**].

Similarly, the statistically significant associations of *P. aeruginosa* infection and Length of hospital stay and Admission type observed in this study were also reported previously from other studies elsewhere, including from Ghana [**78**], Nigeria [**44**], Iraq [**45**], Lebanon [**49**], Turkey [**23**] and USA [**25**]. The obvious explanation for this association of length of hospital stay and *P. aeruginosa* infection could be that the greater time of hospitalization of patients the most likely to contract and get colonized by the notoriously known most significant nosocomial opportunistic pathogen, *P. aeruginosa*. Moreover, because of time-related changes in succession of the predominant gram-positive burn wound colonizing flora during the early time to the late gram-negative bacteria (4-10 days after injury), it is expected to see significant positive associations between *P. aeruginosa* isolate rate and length of hospital stay, as most of the patients (21/27) from whom the pathogen was isolated comprised of those who stayed for 21-30 days.

6.2 Limitations

- It was done only in single burn center. It would have been better if it incorporated more health institutions for a better representation of study participants.
- Moreover, any other confirmatory test especially molecular test was not done.

CHAPTER SEVEN

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Overall, prevalence of *P. aeruginosa* among burn patients in the current study was almost 13%. The incidence of clinically significant *P. aeruginosa* in burn wound infection is low in this unit. However, *P. aeruginosa* is still the common cause of infection in our burn center as is the case elsewhere around the globe.

It was also observed that *P. aeruginosa* isolates were most sensitive to Imipenem, while they were most resistant to Gentamycin, indicating that the latter is no longer potent in the treatments of *P. aeruginosa* among burn wound infections in Yekatit 12 Hospital Medical College. Most of the *P. aeruginosa* isolates had a high level of sensitivity to examined antibiotics.

In addition, 33.3% of the isolates were Multi-drug resistant, which is not only of substantial concern in the treatment center but also enlightening as to the level of MDR *P. aeruginosa* one might expect in hospital wards handling burn patients. Infection with *P. aeruginosa* had significant association with Admission type (IPD), TBSA & Hospital Stay time, where *P. aeruginosa* isolation rate was high in IPD burn wards than OPD burn wards, high in TBSA≥ 30% and longer Hospital stay.

7.2 Recommendations

Based on these study findings, it is recommended that

- Although low prevalence of *P. aeruginosa* was observed among burn patients in this study, great attention is still needed during treatment because of the possibility of emergence of multidrug resistance strains.
- Gentamycin is no longer potent in treatments of *P. aeruginosa* among burn wound infections while Imipenem was the most potent antibiotics; so clinicians should be aware of these facts when considering treatment of burn wound due to *P. aeruginosa.*
- Regular antimicrobial sensitivity testing is need for *P. aeruginosa* among burn patients (and preferably also among other patients of *P. aeruginosa* infection) at Yekatit 12 Hospital Medical College to guide the choice of antibiotics.

- Multidrug-resistant isolates of *P. aeruginosa* reported should not be taken lightly, which needs special attention particularly in burn centers that requires prompt management of these cases.
- Infection control should be strictly followed in IPD burn wards and follow up their exposure to microorganism regularly.
- Great attention should be given for high TBSA and long Hospital stay time of burn patients.
- It would be valuable to conduct more in-depth similar studies with a large number of individual participants, more study sites and molecular characterization of the *P. aeruginosa* isolates.

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9. Annex

Annex 1-Specimen collection and Laboratory Procedures

A. Specimen Collection and Processing

Following removal of old, dressing of burn patients, the surface of the burns wound was cleaned with normal saline to prevent contamination. Each sample were collected by swabbing the wound with a sterile cotton- tip swab stick. Rotating the swab stick between the fingers, the swab stick was moved across the entire wound surface. The swab stick was quickly put in Stuart transport medium. The labeled sample was then be transported to the microbiology laboratory for culture and antimicrobial sensitivity test [**72**].

B. Laboratory procedures

Step 1: Media inoculation

The sample was inoculated on Blood agar and Mac Conkey agar aerobically at 37°C for 24 - 48 hours. *P. aeruginosa* were identified by its colony characteristics, pigment production, grape like odour, oxidase positivity, motility, gram staining (as gram-negative bacilli) [**72**].

Step 2: Morphology and Grams Staining

Inoculated media was removed from the incubator following overnight incubation and morphological characteristics of colonies noted and Gram staining done. Gram staining were done to identify pathogens based on their Gram staining reaction according to standard protocols [**72**].

Step 3: Biochemical tests

Depending on the bacteria pathogen isolated and the morphological characteristics, the Catalase, Motility, Citrate, Urease, Lysine iron agar, Indole test, Triple sugar iron and Oxidase test were performed for *P. aeruginosa* isolation.

Oxidase Test

The oxidase test was performed to aid in the identification of *P. aeruginosa*. Filter paper was soaked with few drops of oxidase reagent. Test organism was smeared on the filter paper. Oxidase producing *P.aeruginosa* oxidizes the phenylenediamine in the reagent showing a deep purple colour.

Catalase Test

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism was tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer, like *P. aeruginosa*.

Step 4: Antibiotic susceptibility tests

According to the standard operational procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar using Kirby Bauer disk diffusion method developed by Bauer to determine the susceptibility of the bacteria isolate to antibiotics according to standard protocols of CLSI 2019. *P. aeruginosa* ATCC 27853 strain was used for quality control in the study. The drugs, which were tested for *P. aeruginosa* bacteria was Ceftazidime (30µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Tobramycin (10µg), Cefepime (30µg), Meropenem (10µg), Imipenem (10µg), Amikacin (30 µg) and Piperacillin-tazobactam (30µg).The suspension of the test organism was prepared by picking parts of 4or 5 colonies of organisms with a sterile wire loop from pure culture and suspended in sterile broth. Turbidity of the broth culture was equilibrated to match 0.5 McFarland standards. The test organism was uniformly seeded over the Mueller-Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic impregnated paper disk into the agar medium. The medium was incubated at 35^0C for 18-24 hours. Thus, bacterial strains was classified into three groups: sensitive (S), intermediate (I) and resistant (R) as indicated in the manufacturers guide [**73**&**74**].

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY, AND PARASITOLOGY

Annex 2: Information sheet, Assent and Consent form

Annex I: Information Sheet for Adult Participant (English Versions)

Name of Organization:-Department of Microbiology. Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Principal Investigator: Fedasan Alemu

Title:-"Prevalence and antimicrobial-resistant patterns of *Pseudomonas aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia."

Introduction

You are invited to participate in a research to be conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams include principal investigator, advisors; from Addis Ababa University Microbiology, Immunology and Parasitology department and collaborators from Yekatit 12 Hospital Medical College. Please take as much time as you need to read or listen to the information provided here.

Purpose of the study

The purpose of the study is to determine prevalence, antimicrobial-resistant patterns and associated factors of *P. aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study. You can ask any question that is not clear to you and after that you can give your consent. You will be asked to give a small amount of burn wound specimen in the sample container that we will be provided to you.

Potential risks and discomforts

Burn wound swabs collection will have no effect but there might be a little pain while collecting specimens.

Confidentiality

In order to maintain the confidentiality of participant's personal information, your name will be kept confidential and samples will be coded. No personal information will be disclosed to third party or will not appear in any report from this study.

Potential benefits to participants and/or to the society

You will not receive any payment for your participation in this research study as compensation. Most importantly, this study will contribute to provide information or data for future and further nationwide study and to develop health programs for health policy makers.

Participation and withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time without consequences of any kind. You may also reject to give any sample. Your response to our request will not affect the service and care that you would normally get from the Hospital.

Contact Information:

In case of any questions, problem, unclear ideas and doubt relating to the study, please direct them to:

Fedasan Alemu (BSc, MSc Student), the principal Investigator of this study on:

Phone:-+251-912-744-508. Email: fedhanalebdi@gmail.com

Thank you for your Participation and Cooperation!

Information sheet for Adult participant Amharic version (ለአዋቂዎች ተሳታፊ የመረጃ ወረቀት)

የድርጅቱ ስም፡-የማይክሮባዮሎጂ ፣ ኢሙኖሎጂ እና ፓራሲቶሎጂ የሕክምና ትምህርት ክፍል ፣ የጤና ሳይንስ ኮሌጅ ፣ አዲስ አበባ ዩኒቨርሲቲ

ዋና ተመራማሪ፡-ፌደሳን አለሙ

የጥናቱ ርዕስ፡-“Prevalence and antimicrobial-resistant patterns of *Pseudomonas aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia.”

መግቢያ

ከአዲስ አበባ ዩኒቨርሲቲ በ MSC እጩ በሚካሄደው ጥናት እንዲሳተፉ ተጋብዘዋል ። የእርስዎ ተሳትፎ በርስዎ ፈቃደኝነት የተመሰራተ ነው ። የምርምር ቡድኖቹ ዋና ተመራማሪ ፣ አማካሪዎችን ከአዲስ አበባ ዩኒቨርሲቲ ማይክሮባዮሎጂ ፣ ኢሚኖሎጂ እና ፓራሲታሎጂ ዲፓርትመንት እና ከየካቲት 12 ሆስፒታል ሜዲካል ኮሌጅ ባልደረባዎች ጋር ነዉ። እበክዎ በመረጃ ወረቀቱ ውስጥ ያለዉን መረጃ ለማንበብ ወይም ለማዳመጥ ትንሽ ጊዜ ይውሰዱ ።

የጥናቱ ዓላማ

የጥናቱ ዓላማ በአሁኑ ጊዜ በአዲስ አበባ የካቲት 12 ሆስፒታል ሜዲካል ኮሌጅ በቀጣሎ ምክናት ተኝተዉ በሚገኙ ታማሚዎች የ*Pseudomonas aeruginosa መጠንና* ሚጠቀሙትን አብዛኞቹን አንቲባዮቲኮችን የሚቋቋሙ ኢነተሮባክቴሪያሲዎችን ለማወቅ ነው።

የጥናቱ ሂደቶች እና ተሳታፊ ለመሆን የሚጠበቅበዎት

ለመሳተፍ ፈቃደኛ ከሆኑ የጥናቱን ዓላማ መረዳት ያስፈልግዎታል ። ለእርስዎ ግልፅ ያልሆነ ማንኛውንም ጥያቄ መጠየቅ ይችላሉ እና ከዚያ በኋላ ፈቃድዎን መስጠት ይችላሉ ። እኛ በምናቀርብልዎ መያዣ ውስጥ ትንሽ መጠን ያለው ከተቀጠላ ሰዉናት ላይ ናሙና እንዲሰጡ ይጠየቃሉ ።

ሊጋጥሙ የሚችሉ አደጋዎች እና ችግሮች:ጊዜዎን ሳይጨምር ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም።

የጥናቱ ምስጢራዊነት

የተሳታፊዎችን የግል መረጃ ሚስጢራዊነት ለመጠበቅ ስም በሚስጥር የሚጠበቅ ሲሆን ናሙናዎች ኮድ ይደረጋሉ ። ምንም ዓይነት የግል መረጃ ለሶስተኛ ወገን አይገለጽም ወይም ከዚህ ጥናት በየትኛውም ሪፖርት አይደረግም ።

ጥናቱ ለተሳታፊዎች እና / ወይም ለህብረተሰቡ የሚስገኘዉ ጥቅሞች

በዚህ የምርምር ጥናት ውስጥ በመሳተፍዎ ምንም አይነት ክፍያ አይከፍሉም ። ከሁሉም በላይ ይህ ጥናት ለወደፊቱ እና ለበለጠ አገራዊ ጥናት መረጃን ወይም መረጃዎችን ለማቅረብ እና ለጤና ፖሊሲ አውጪዎች የጤና ፕሮግራሞችን ለማዘጋጀት አስተዋፅ ያደርጋል ።

የጥናቱ ተሳትፎ እና መውጣት ሁኔታ

ተሳትፎው በፈቃደኝነት ሲሆን እርስዎ በዚህ ጥናት ውስጥ ላለመሳተፍ መብት አልዎት ። ያለምንም መዘግየት በማንኛውም ሰዓት እና ቦታ መውጣት ይችላሉ ። እንዲሁም ማንኛውንም ናሙና ለመስጠት እምቢ ማለት ይችላሉ ። ለጥያቄያችን የሚሰጡት ምላሽ ከሆስፒታሉ በሚያገኙት አገልግሎት እና እንክብካቤ ላይ ምንም ተጽዕኖ የለውም ።

የግንኙነት መረጃ

ስለዚህ ጥናት ማንኛውንም ጥያቄ ካልዎት ለተጨማሪ መረጃ የሚከተሉትን ዋና ተመራማሪ አድራሻ በመጠቀም ያነጋግሩ።

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ኢሜል: fedhanalebdi@gmail.com

ስለ ተሳቲፎዉና ትብብሮት በጠም እናመሰግላን!

Annex II: Consent Form Adult Participant (English Version)

I……, after reading the information sheet and having been explained to by Fedasan Alemu (The Principal Investigator), I do voluntarily agree to take part in this research study on **"Prevalence And Antimicrobial-Resistance Patterns of *Pseudomonas aeruginosa* Among Burn Patients Attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia ."** I am aware that results of the study may not benefit me directly but it might benefit the future effective management of Burn patients. For this study burn wound sample will be required. The aim and possible risk of the study were explained to me well.

- I understand that participation in this study is voluntary; the information collected from me will remain confidential, and they will be reported with my approval and the information to be reported are only the results without my personal information.
- I know there will be no financial benefit to be provided for participating in this study.
- The interviewer explains for me, as there is no any risk or discomfort, and extra treatment.
- Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

Therefore, with full understanding of the information given above, I agreed to give the necessary information for laboratory analysis and participate in this study.

I _____ hereby give my consent for giving of the requested information and specimen for this study.

Participant code: _____ Signature: _____Date: ----------/--------/----------

Name of the interviewer _____ Signature: ---------- Date: ----------/--------/----------

Thank You for Your Participation and Cooperation!

Adult consent form Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

እኔ……የመረጃ ወረቀት ከአነበብኩና ከተገለጽልኝ ቦዓላ በፍቀዴ የዚህን የምርማረዉ ርህስ “Prevalence And Antimicrobial-Resistance Patterns of *Pseudomonas aeruginosa* Among Burn Patients Attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia” አንዱ ተሰተፊ ለመሆን ተስማሚቼለዉ። በዚህ የምርምር ጥናት ዉጤት በቀጣታ ለእኔ ምንም አይነት ክፍያ እነደሌለ ተረድቼ ነገር ግን ከዚህ ቦዓላ በቀጠሎ ምክናት ለተጎዱ ተከሚዎች ጥሩ የሆና የህኪምና አገልግሎትና እንክብከቤ ለመግንኛት እንደሚራደ አምናለዉ።ለጥናቱም የቀጠሎ ናሙና እንደሚያስፈልግ ተገልጾልኛል፤ናሙና ሲወሰዱም የከፈ ጉደት እነደሌለና በህክምናሄ ለይም ምንም አዓነት ችግር አለመኖሩን ተረዲቸለዉ።በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል ።በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል።የጥናቱንም አላማዎችም ተረድቻለሁ።

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ ።የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው የገንዘብ ክፍያ አለመኖሩን እና የጥናቱ ዉጤት ለፖሊሲ አዉጪዎች ፖሊሲዎቸዉን እንዲመርምሩ /እንዲያሻሽሉ እንደሚረዳቸዉ ተረድቻለሁ።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ ተረድቼ የሚያስፈለገዉ ናሙና እና በቂ መራጃ ለላቦራቶሪ ምርማረ ለመስጣት ተስማሚቼላዉ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

የተሰተፊዉ መለያ ቁጥር: _____ ፊርማ: _____ቀን: ----------/--------/----------

የተያቂዉ/ማርማሪዉ ስም _____ፊርማ: ---------- ቀን: ----------/--------/----------

ስለ ተሳቲፎዉና ትብብሮት በጠም እናመሰግላን!

Annex III: Information sheet for parents/guardians (English version)

Name of Organization:-Department of Microbiology. Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Principal Investigator: Fedasan Alemu

Title:-"Prevalence and antimicrobial-resistant patterns of *Pseudomonas aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia."

Introduction

We would like to conduct a research entitled "Prevalence and antimicrobial-resistant patterns of *Pseudomonas aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia."We are requesting you to allow your child to voluntarily participate in this study. We are going to inform you about the purpose, responsibility of investigators or data collectors to keep confidentiality and how we are going to use the data. Please take as much time as you need to read or listen to the information provided here.

Purpose of the study

The purpose of the study is to determine prevalence, antimicrobial-resistant patterns and associated factors of *P. aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College.

Procedures and the expected participation

If you agree your child to participate in this study and you sign the assent form the following will be done:

- ✓ We will ask a small amount of burn wound specimen from your child
- ✓ Review your child medical history
- ✓ You will have an interview with us some time about the child medical condition

Potential risks and discomforts: Burn wound swabs collection will have no effect but there might be a little pain while collecting specimens.

Confidentiality

In order to maintain the confidentiality of your child information, your child name will be kept confidential and samples will be coded. No personal information will be disclosed to third party or will not appear in any report from this study.

Potential benefits to participants and/or to the society

Your child will not receive any payment for the participation in this research study as compensation. Most importantly, this study will contribute to provide information or data for future and further nationwide study and to develop health programs for health policy makers.

Participation and withdrawal from the Study

Your child participation is voluntary and you have the right to refuse your child's participation in this study. You can stop your child to participate in the study at any time after giving your consent. You may also reject to give any sample from your child. Your response to our request will not affect the service and care that your child would normally get from the hospital.

Contact Information:

In case of any questions, problem, unclear ideas and doubt relating to the study, please direct them to:

Fedasan Alemu (BSc, MSc Student), the principal Investigator of this study on:

Phone:-+251-912-744-508. Email: fedhanalebdi@gmail.com

Thank you for your Participation and cooperation!

Information Sheet for Parents/Guardians (Amharic Version)

የድርጅቱ ስም፤ የማይክሮባዮሎጂ ፣ ኢሚኖሎጂ እና ፓራሲታቶሎጂ ሕክምና ትምህርት ክፍል ፣ የጤና ሳይንስ ኮሌጅ ፣ አዲስ አበባ ዩኒቨርሲቲ

የጥናቱ ርዕስ፡ "Prevalence and antimicrobial-resistant patterns of *Pseudomonas aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia."

የጥናቱ ምንነት ፡ *Pseudomonas aeruginosa* የሚባል ባከቴሪያ የተለያዩ በሽታዎችን የሚያምጡ እና በመድሃኒት ግትርነት እያሰቸገሩ ያሉ ባከቴሪያዎች ናቸዉ። በመሆኑም የችግሩን ተባባሽነት ለመቀነስ በየካቲት 12 ሆሰፒታል ሜዲካል ኮሌጅ ተኝተዉ በሚገኙ በተከሚዎች ጥናት ማካሄድ አስበናል።

የጥናቱ ሂደቶች እና ተሳታፊ ለመሆን የሚጠበቅበዎት

በዚህ ጥናት ውስጥ ልጅዎ/የሚያሳድጉት/ጓት ልጅ እንዲሳተፍ/እንድትሳተፍ ከተስማሙ የሚከተሉትን ነገሮች እናደርጋለን።ከልጆዎ አከል ትንሽ ናሙና እንወሰዳልን።

ለጥናቱ የሚያሰፈልጉ የልጆዎን የህክምና መረጃ እንያለን እና ከርስዎ አንደበት እንጠይቃለን።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ከልጆዎ ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥማትም/ዎም ይሁን እንጅ ትንሽ የእመም ስሜት ልኖር ይችለል።

የጥናቱ ሚስጥራዊነት

ስለ ልጆዎ የሰጡት ወይም የሰበሰብንዉ ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ናሙናዎች ኮድ ይደረጋሉ፤ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን ልጆዎ በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኘው/ኘዉ ጥቅም አይኖርም። ከሁሉም በላይ ይህ ጥናት ለወደፊቱ እና ለበለጠ አገራዊ ጥናት መረጃን

ወይም መረጃዎችን ለማቅረብ እና ለጤና ፖሊሲ አውጪዎች የጤና ፕሮግራሞችን ለማዘጋጀት አስተዋፅ ያደርጋል ።

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው?

በዚህ ጥናት ልጆዎ የሚሳተፈዉ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት በመሆኑ በማንኛውም ሰዓትና ቦታ ልጆዎን ከዚህ ጥናት የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ ልጆዎ ከጥናቱ በማግለልዎ ምክንያት የሚቀርብለት ምንም አይነት የሆስፒታል አገልግሎት አይቋረጥም ።ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት። እርስዎ ስለ ልጆዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን።

የግንኙነት መረጃ

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ልጆዎ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ።

ፌደሳን አለሙ (BSc, MSc Student), Principal Investigator

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ስለ ተሳቲፎዉና ትብብሮት በጠም እናማሰግላን!

Annex IV: Assent Form for Children Participant (English Version)

Name of study participant ----------

Name of the participant's family or Guardian ----------

I _____ understand that my parents /guardian have/has given permission for me to take part in a research entitled as "Prevalence and Antimicrobial-resistance Patterns of *Pseudomonas aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia ."

The study participant mentioned above who is not able to give informed consent himself because he/she is younger than 18 years not allowed to decide on him/herself.

• I agree samples to be collected from me.

• I understand that the information will be confidential.

• I understand that I can stop the study at any time.

• I understand it will not affect my current and future medical services.

• I agree that there is no payment as compensations for me.

It is therefore with full understanding; by taking a full responsibility, I gave my assent voluntarily to the researcher to use the information and specimen for this study.

Participant code: _____

Participant's signature/Finger print ---------- Date: ----------/--------/----------

Participant's family/Guardian signature ---------- Date: ----------/--------/----------

Name of the interviewer _____ Signature: ---------- Date: ----------/--------/----------

Thank you for your Participation and cooperation!

Assent form for Children Participant (Amharic Version)

የተሳታፊዉ ልጅ ስም----------

የተሳታፊዉ ልጅ ወለጅ ወይም አሳዳጊ ስም ----------

እኔ----------የተባልኩ ልጅ ወላጆቼ /አሳዳጊዎቼ "Prevalence, and antimicrobial resistance patterns of *Pseudomonas aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia" በሚል ርዕስ ለሚካሄደዉ ጥናት እንድሳተፍ መስማማታቸውን ተረድቻለሁ።

- እኔም ናሙና ለመስጠት ተስማምቻለሁ።
- ከእኔ የሚስበስበዉ መረጃዎች በሚስጥር እንደሚያዙ ተረድቻለሁ።
- በማንኛዉም ሰዓት ከጥናቱ ማቋረጥ እንደምችል ተረደቻለሁ።
- በጥናቱ ባለመሳተፌ/በማቋረጤ አሁን/ወደፊት የህክምና አግልጋሎቴ ላይ ችግር እንደማይፈጥርብኝ ተረደቻለሁ።
- እኔ በጥናቱ በመሳተፌ የማገኘዉ የገንዘብ ክፊያ እነደሌለ ተረደቻለሁ።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባ ተረድቼ የሚያስፈለገዉ ናሙና እና በቂ መራጃ ለላቦራቶሪ ምርማረ ለመስጣት ተስማሚቼላዉ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

የተሰተፊዉ መለያ ቁጥር: _____ ፊርማ: _____ቀን: ----------/--------/----------

የተሳታፊዉ ልጅ ወለጅ ወይም አሳዳጊ ፊርማ---------- ቀን: ----------/--------/----------

የተያቂዉ/ማርማሪዉ ስም _____ፊርማ: ---------- ቀን: ----------/--------/----------

ስለ ተሳቲፎዉና ትብብሮት በጠም እናመሰግላን!

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY, AND PARASITOLOGY

Annex 3:-Questionnaire

Questionnaire format for the assessment of the associated factors of *Pseudomonas aeruginosa*

Date of interview_____ Patient Code No_____ Ward----------

Part I. Study participant address

1. Zone/Subcity_____3. Woreda_____4. Kebele_____Phone No_____

Part II. Socio- demographic characteristics of the study participants

1. Age _____ (years)

2. Sex:-Male □ Female □

3. Marital Status: - Single□ Married □ Separate □ Widowed □ Divorced□

4. Educational Level:-Illiterate □ Elementary□ High School □ College and above □

5. Residence:-Urban □ Rural □

6. Family size: - <4□ 4 – 7□ >7 □

7. Occupation: - Gov. employed □ Self-employed □ Daily laborer □ Student □ Others----------

Part III: (A) Clinical information and Predisposition for *P. aeruginosa*

8. Etiology/cause of burns: Scalds [] Open flame [] Chemical [] Electrical [] Frictional burns [] Inhalational injury [] others []

9. Anatomical sites affected- Head &Neck [ ] Trunk [ ] Extremities [ ] Perineum [ ] Head, Neck &Extremities [ ] Extremities, Perineum & Trunk [ ] Head, Neck& Trunk [ ] Head, Neck Extremities &Trunk [ ] Whole body Parts [ ]

10. Level of the Burn wound: I. 1st Degree II.2nd Degree III.3rd Degree IV. 4th Degree

11. Is there any other comorbidity. Yes, □ If yes what type of diseases…….. No □

12. Previous history of Burn treatment: Yes □ No/New □

13. Total Body Surface Area/TBSA----------

14. Hospital Stay time----------

15. Date of occurrence……

16. At what time of day did the burns occur? Morning [] Afternoon [] Evening [] Night []

17. Time between the burns occurrence and reporting to the hospital.....

18. What was the cause of delay? Poverty (Money) [] Traditional medicine [] Distance -far away [] Referral [ ]

19. Antibiotics received…….

Contact person: - Name-_____Telephone of contact person:-……

Name and Signature of the Data Collector/Interviewer_____

(B). OUTCOME OF LABORATORY INVESTIGATION

20. Name of pathogen isolated……..

21. Antibiotics that are effective against the isolate…….

22. Antibiotics that are ineffective against the isolate……

Name and Signature of the Laboratory Technologist/Microbiologist_____

Thank You for Your Participation and Cooperation!

Declaration Sheet

I, Fedasan Alemu Abdi, declare that this thesis, Title "Prevalence and Antimicrobial-resistance Patterns of *Pseudomonas aeruginosa* among burn patients attending Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia "is my own original work, that it has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

Name of Principal Investigator: - Fedasan Alemu Abdi **Signature: ----------**

Place of Submission:-Addis Ababa University, College of Health Sciences, School of Medicine, Department of Microbiology, Immunology and Parasitology

Date of Submission: ---------/----------/----------

Approved by advisors:

Name	**Signature**	**Date**
1. Dr. Woldearegay Erku Abegaz	**----------**	**----------**
2. Dr. Alem Abrha Kalayu	**----------**	**----------**