BACTERIAL CONTAMINATION OF SINGLE- AND MULTIPLE-DOSE PARENTERAL INJECTION VIALS AFTER OPENING AND ANTIBIOTIC SUSCEPTIBILITY OF ISOLATES AT JIMMA MEDICAL CENTER, JIMMA, SOUTHWEST ETHIOPIA

BY:

ABAY TABOR

A THESIS SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, JIMMA UNIVERSITY INSTITUTE OF HEALTH SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE (MSc) IN MEDICAL MICROBIOLIGY.

MAY, 2022

JIMMA, ETHIOPIA

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEATH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

BACTERIAL CONTAMINATION OF SINGLE- AND MULTIPLE-DOSE PARENTERAL INJECTION VIALS AFTER OPENING AND ANTIBIOTIC SUSCEPTIBILITY OF ISOLATES AT JIMMA MEDICAL CENTER, JIMMA, AND SOUTH WEST ETHIOPIA

BY:

ABAY TABOR

ADVISORS:

Mr. Zewudineh, S/Mariam (MSc, Assistant Professor): School of Medical Laboratory Science, Jimma University

Mr. Yared, Alemu (BSc, MSc): School of Medical Laboratory Science, Jimma University

MAY, 2022

JIMMA, ETHIOPIA

Abstract

Background: *Injectable liquid medications are provided in either single or multiple dose vials for parenteral administration under the appropriate aseptic conditions. These vials are prone to bacterial contamination after opening and could be potential reservoirs of microorganisms that could be transmitted to the patient through the parenteral route. There have been reports of outbreaks of infectious diseases, especially in low and middle-income countries emanating from improper handling and use of parental medications. The present study aims at assessing the magnitude of the problem and associated factors at Jimma Medical Center, Jimma Southwest Ethiopia.*

Methods: *An institutional based cross-sectional study design was conducted at Jimma Medical Center from July 2021 to October 2021. One hundred microliters of parental medications were withdrawn with a sterile needle and syringe from a total of 384 parental medications (61.5% multiple and 38.5% single-dose vials) that were administered in 11 wards and 3 intensive care units. Besides, self-administered structured questionnaire was used to collect data about risk factors for vial contamination from nurses. Samples were processed onto appropriate culture media and bacteria were isolated and identified using gram staining and a series of biochemical tests. An antimicrobial susceptibility test was performed based on Kirby Bauer Disk diffusion technique. Data were entered by Epi data and analyzed by SPSS version 23 the P-value of less than 0.05 was considered as statistically significant.*

Result: *The overall prevalence of vial contamination due to aerobic bacteria was 21(5.5%) among multiple-dose and none of the single-dose vials. The highest contamination 8(38.1%) was found in the pediatric ward. P. aeruginosa 6(28.5 %) and K. pneumoniae 5(23.8%) were the most common agents of vial contamination. Multidrug resistance rate of isolates was found among 95.2% of the isolates with all gram-negative isolates showed multidrug resistance rate against tested antibiotics. In multivariate logistic regression analysis, vial contamination was strongly associated with reuse of syringe and/or needle, medication drawing environment, and storage conditions.*

Conclusions: *In this study, the prevalence of vial contamination was high. The bacterial isolates from vials were also highly resistant to commonly prescribed antimicrobial drugs. Healthcare professionals must strictly adhere to basic infection control practices as per standard guidelines.*

Key words: *Vial contamination, multiple dose Vial, Single dose Vial, Antimicrobial susceptibility*

Acknowledgement

First and for most, I would like to express my heartfelt thanks to my Almighty God, for all the grace and encouragement that enabled me to accomplish my study properly despite all the ups and downs of life.

Secondly, my appreciation goes to my advisors Mr. Zewudineh S/Mariam (Ass. prof) and Mr. Yared Alemu (MSc) for their precious guidance, continuous support and never-ending patience throughout the preparation of my thesis, without you this study would never reach its present stage.

Moreover, I would like to acknowledge Mr. Kasahun Gorems bacteriology lab expert for his technical, material, and constructive ideas support. I also would like to acknowledge Mrs. Rahel Tamrat (MSc) for providing her effective guidelines for the research proposal ahead of the main research. I would like to extend my acknowledgement to patients/parents who participated in conducting the study, without them the study would have not been materialized.

My special thanks go to the School of Graduate Studies of Jimma University, the school of Medical Laboratory Sciences, and Mizan Tepi University for giving me this opportunity and for their technical and financial support for the study. Finally, my special thanks go to those who have supported me in every possible way, practically and psychologically, throughout the completion of this research.

Table of contents

Pages

List of tables **pages**

List of figures **pages**

List of Abbreviations and Acronyms

µL	Micro Liter
AMR	Antimicrobial resistance
ASA	American Society of Anesthesiology
AST	Antimicrobial Susceptibility Testing
BSI	Blood Stream Infections
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standard Institute
CoNS	Coagulase-Negative *staphylococci*
FDA	Food and Drug Administration
HAI	Hospital-Acquired Infection
HCAI	Health Care- Associated Infection
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCWs	Health Care Workers
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
JMC	Jimma Medical Center
MCH	Mueller Hinton Agar
MDR	Multi Drug Resistance
MDV	Multiple Dose Vial
MRSA	Methicillin-Resistant *Staphylococcus aureus*
SASA	South African Society of Anaesthesiologists
SDV	Single Dose Vial
SPSS	Statistical Package for Social Science
TSIA	Triple Sugar Iron Agar

CHAPTER ONE: INTRODUCTION

1.1. Background

A nosocomial infection, also known as hospital-acquired infection (HAI) or healthcare-associated infection (HCAI) is an infection that is acquired in a hospital or other health care facility (1). This infection can be acquired in the hospital, nursing homes, rehabilitation facilities, outpatient clinics, diagnostic laboratories, or other clinical settings which were absent at the time of admission (2). There is the possibility of nosocomial infection transmission when parenteral medications are not accessed in an aseptic manner (1,2). Globally in health care settings, unsafe injection procedures have been recorded (3).

Parenteral medications are usually given out in multiple and single-dose vials (MDVs and SDVs) (4). MDVs are a liquid medication vial intended for parenteral administration (injection or infusion) that contains more than one dose of medication (5). Antimicrobial preservatives such as benzyl alcohol, benzethonium chloride, methylparaben, propylparaben, and metabisulphite are commonly used in these vials, which are labeled as such by the manufacturer. On the other hand, the preservatives can prevent bacterial contaminations if health workers are adherent to safe injection practices (6).

MDVs can offer certain potential advantages of convenience and presumed cost reduction over SDVs (7). However, these vials are susceptible to bacterial contamination and their use has been reported to be a possible source of infections. Various factors affect the standard and sterility of medication present in these vials. The common factors are sterility of the techniques employed by healthcare personnel, injection of environmental air into the vial during extraction, storage conditions like temperature, and exposure to the sun (5). In addition to those most of the recorded outbreaks associated with MDVs have been linked directly to poor aseptic techniques such as lack of hand hygiene, administering of the same solution to more than one patient, entering a vial with a used syringe or needle, and leaving the needle in the stopper (6,7).

SDVs are also liquid medications intended for parental administration or injection these vials are used for a single patient in a single case or procedure (8). SDVs or single-use vials are labeled as such by the manufacturer and typically lack an antimicrobial preservative. Preservative-free and lipid-containing drugs are more susceptible to contamination (9). Multiple outbreaks have occurred as a result of healthcare workers are using single-dose or single-use vials multiple times (8).

Bacterial contaminations of MDVs and SDVs are a potential cause of different infections (4). Gram-negative and positive bacteria cause hospital-acquired infections arising from extrinsic contamination (4). MDVs contamination is hypothesized to occur after a single syringe is used to administer medication to an infected patient, and the same syringe is then used to remove additional medication from the vials. If another patient is given contaminated MDVs, an iatrogenic infection can spread (10).

Recommendations from the Centers for Disease Control and Prevention (CDC) state that, "MDVs should be dedicated to a single patient whenever possible. If MDVs must be used for more than one patient, they should be only kept and accessed in a dedicated medication preparation area away from immediate patient treatment areas (5). This is to prevent inadvertent contamination of the vial through direct or indirect contact with potentially contaminated surfaces or equipment that could lead to infections in subsequent patients. If a MDVs enters an immediate patient treatment area, it should be dedicated for single-patient use only." (5). The name of the patient should be written on the vial as well as the date and time of first use (5).

The national department of health in South Africa provides guidance on the use of MDVs. However, this aligned with the CDC recommendations but it pertains largely to vaccines (11). The South African Society of Anaesthesiologists (SASA) guidelines for infection control in anesthesia give ambiguous advice on the safe use of MDVs. This document fails to give a precise definition of multiple-vials, exact disinfection procedure, the importance of adherence to manufacturer's instructions, criteria for use on multiple patients, disposal of the vial, or storage limitations once opened (12). A significant number of medications used in anesthesia are intended for multiple dosing (12,13).

There is a report that explains the administration of contaminated MDV and SDVs by *P. aeruginosa*, *S. aureus*, *S. marcescens*, *K. pneumoniae*, group A *Streptococcus*, and *E. cloacae* resulted in several cases of bloodstream infection, bacterial meningitides, wound infection, and death in the receiving patients (9,14). Several disease outbreaks and individual cases were reported in human medicine due to the intake of contaminated medicines (15).

According to a study conducted in the anesthesiology unit in South Africa 6.36%, of MDVs microbial contamination was identified (13). On the other hand, based on the research in a major teaching hospital in Shiraz Iran 5.6% of bacterial contamination was identified with no difference in contamination rate among different wards or the medication type. The most commonly identified organisms were part of the normal flora (16). Similarly, 5.36% of microbial contamination was identified in SDVs and MDV in a pulmonary teaching hospital in Tehran, Iran (4). There is no investigation on bacterial contamination of single- and multiple-dose parenteral injection vials in the Ethiopia context. Therefore, the aim of this study was to assess the prevalence of bacterial contamination of single- and multiple-dose parenteral injection vials after opening and antibiotic susceptibility of isolates at Jimma Medical Center, Jimma, Southwest.

1.2. Statement of the problem

Injectable drugs are widely used to prevent, diagnose, and treat various diseases in healthcare settings (17). This includes chemotherapy, intravenous antibiotics, vaccines, and medications used for anesthesia. Medical injections are used in conjunction with surgery, endoscopy, pain control, and cosmetic or complementary and alternative medicine procedures (18). Sterile medication must start with every injection, safe manufacturing and pharmacy practices are important (19). The required medication must be prepared safely and administered in a manner that maintains sterility and minimizes the risk of infection. Safe administration relies on compliance with the protocols mentioned in the quality precautions guideline of CDC (19,20).

According to the CDC, injection protection has been recognized predominantly in low and middle-income countries as a public health concern (21). Approximately 20 million new hepatitis B virus (HBV) infections, 2 million new hepatitis C virus (HCV) infections, and 250,000 new human immunodeficiency virus (HIV) infections were included in the estimated global burden of illness associated with unsafe injections since 2000 (22). In recent years, the U.S. experience with outbreaks due to unsafe injection procedures has increased substantially (17).

In addition to the above, at least 49 outbreaks have occurred since 2001 because of the mishandling of injectable medical items. HBV or HCV transmission was involved in 21 of these outbreaks; the other 28 were outbreaks of bacterial infections, mostly invasive bloodstream infections (23). Although many of these outbreaks occurred in hospital settings, a high percentage occurred in pain management clinics, where injections are often given into the spine and other sterile areas using preservative-free medicines, and in cancer clinics that typically provide chemotherapy or other infusion services to patients who may be immune-impaired (17,23). Moreover, during this period, more than 150,000 patients were required notice to undergo blood-borne pathogen testing after following their potential exposure to unsafe injections (17,23).

The CDC is aware that the misuse of SDVs has been associated with at least 19 outbreaks of blood-borne and bacterial infections since 2007. Seven were blood-borne pathogen infections, and twelve were bacterial infections (23). Most of these outbreaks occurred in the outpatient setting, while eight occurred in the pain remediation clinics (17,23). These examples probably underestimate the harm resulting from the misuse of SDVs, according to CDC. Due to the difficulties of tracing the misuse of vials to pathogens, the adverse effect of misusing a vial is usually not seen immediately (24). Adverse effects related to unsafe injection procedures and lapses in infection management practices are underreported, and it remains a challenge to quantify the true incidence of such occurrences (23).

As far as the current researcher's knowledge, there has been limited research conducted specifically on bacterial contamination of single- and multiple-dose parenteral injection vials after opening and antibiotic susceptibility of the isolates in the study area. Hence, the frequency of bacterial contamination of single- and multiple-dose parenteral injection vials after opening and antibiotic susceptibility of isolates is the first topic to be conducted in the Ethiopian context. Therefore the desire and motivation for the study emerged in part from recognizing those problems in the study setting.

1.3 Significance of the study

Patient safety is a global healthcare issue affecting countries at all levels of development. Bacterial infection is the most predominant type of HAI and is a major public health problem particularly in developing countries. Unsafe injection practices are common in developing nations. Such practices through infected vials, syringes, and needles place injection recipients' at large risk of infection. It is essential to investigate whether safe injection practices were adhered to when intravenous medications were prepared and administered.

Jimma medical center is one of the hospitals in Oromia regional state providing health services for its catchment communities. However, currently, no adequate information has so far been made available on the prevalence of contamination of vials in the study area. Therefore, determining the prevalence of bacterial contamination of single- and multiple-dose parenteral injection vials after opening and antibiotic resistance profiles of the isolates is helpful to preserve local knowledge and contribute to the nation to have multi-centered data. Besides, updated information on the burden of hospital-acquired bacterial infections and drug resistance profiles are required to take corrective measures. Finally, the findings of this study would be used as a baseline for health care workers to improve their clinical services by properly adhering to disease prevention and control practices and also used as baseline for other researchers who want to conduct further research on the area.

CHAPTER TWO: LITERATURE REVIEW

2.1. Microorganisms cultured from contaminated vials

Several parenteral medications and pharmaceutical drugs are dispensed in MDVs that may be used over a period of time for one or more patients in hospitals and other healthcare facilities (25). Many of these medications contain a preservative to inhibit microbial growth however, microbial contamination introduced during use could theoretically cause infection in patients receiving the medications (25,26). Although several reports suggest that this has occurred, several studies have shown that some medications or pharmaceuticals available in MDVs allow the persistence or proliferation of microorganisms after being contaminated (27,28).

2.2. Bacteria

Bacteria are a common pathogen involved in the contamination of MDVs (13). Commonly identified gram-positive bacteria in different reports including coagulase-negative *staphylococci*, *Corynebacterium* species, *Propionibacterium* species, as well as *Staphylococcus aureus*, *Bacillus* species, and *Micrococcus* species (28,29). Potentially pathogenic gram-negative organisms were also significantly isolated from all medication sites than gram-positive organisms. Commonly identified gram-negative bacteria are *Serratia marcescens, Pseudomonas, Proteus,* and *Enterobacter* species (28,30). In 1976, the three main *Enterobacter* species: *E. cloacae, E. aerogenes (Klebsiella aerogenes), and E. agglomerans*, became well-known as pathogens following a national outbreak of septicemia in 378 patients at 25 hospitals caused by infected intravenous solutions (31). This is due to the bacteria easily replicating in glucose-containing parental fluids and they continue to cause sporadic outbreaks (32).

According to a Study conducted at an outpatient pain clinic in West Virginia eight cases of severe methicillin-susceptible *S. aureus* infection were identified among 110 patients who received epidural injections (33). The facility's infection management evaluation reported the reason was non-adherence to healthy injection practices (33). Another retrospective cohort study reported four laboratory-confirmed case-patients three with *K. pneumoniae* and one with *Enterobacter aerogenes* were identified after invasive pain management procedures at an outpatient facility (34). The three cases of *K. pneumoniae* and *E. aerogenes* positive blood

cultures were indistinguishable by pulse-field gel electrophoresis, to the culture from an open vial. The infection control flaws have been discovered the cause as the reuse of single-use vials for several patients (34). In July 2001, in Germany two patients were died because of meningitis caused by *Pseudomonas aeruginosa* in a hospital, the reason for their infections was a contaminated iomeprol used as MDV over 8 days (35).

There is a report that explains the administration of contaminated MDVs and SDVs with *Pseudomonas aeruginosa*, *Enterobacter cloacae,* and *Serratia marcescens* were resulted in several cases of bloodstream infection, bacterial meningitides, wound infection, and death in the receiving patients (9,14,36). According to a study conducted in Tehran, Iran 5.36% of vials microbial contamination was identified (4). The most frequent contaminated medication was insulin. Gram-positive bacteria were more significantly involved than gram-negative ones with the highest frequency for *Staphylococcus epidermidis* (4).

Based on a cross-sectional study conducted in Shiraz, southwestern Iran 5.6% prevalence of MDVs contamination by aerobic bacteria was identified. The most commonly identified organisms were part of the normal commensally flora (16). Gram-positive bacteria were more significant and accounted for 88.9% than gram-negative ones 11.1%, with the highest frequency for *Staphylococcus epidermidis* 44.4%, and the lowest for *Actinomyces viscosus* 2.8% (16).

2.3. Antimicrobial drug resistance

Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century that threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi no longer susceptible to the common medicines used to treat them (37). AMR) is increasing rapidly worldwide, causing an estimated 700,000 deaths annually over the past decade, en route to becoming the leading global threat to public health by 2050 with an estimated 10 million deaths per year (more than heart disease, cancer, and stroke), while reducing global wealth by US$100 trillion (38).

The problem of AMR is especially urgent regarding antibiotic resistance in bacteria. Over several decades, to varying degrees, bacteria causing common or severe infections have developed resistance to each new antibiotic coming to market. Faced with this reality, the need

for action to avert a developing global crisis in health care is imperative (37,38). The threat of AMR is growing at an alarming rate and the situation is perhaps aggravated in developing countries due to gross abuse in the use of anti-microbial (39). Misuse of antimicrobials is facilitated in developing countries by their availability over the counter, without prescription and through unregulated supply chains (39,40).

AMR is spreading at an alarming rate, and the issue is likely to worsen in developing countries due to widespread antimicrobial misuse (39). The availability of antimicrobials over the counter, without a prescription, and through unregulated supply chains facilitate their misuse in developing nations (39,40). On the other hand, even among the developed nation some patients miss doses either by mistake or deliberate, especially in cases where signs and symptoms begin to subside after an initial favorable therapeutic response (41).

2.4 Standard precautions related to the use of vials

MDVs are labeled by the manufacturer and usually contain an antimicrobial preservative that inhibits bacterial growth (42). The preservative has no effect on viruses and does not protect against contamination when healthcare personnel fails to follow safe injection practices (42). CDC suggests that MDVs should be devoted to a single patient wherever possible, the vial should not be stored or accessed in the immediate patient care area if MDVs have to be used with more than one patient, and both the needle and the syringe used to access the MDVs must be sterile (23). The introduction of standard precautions is the primary technique for preventing the spread of infectious agents between patients and healthcare workers in health care facilities (19).

2.4.1 Hand washing

Hand hygiene is a general term that applies to hand washing, antiseptic hand-wash, antiseptic hand rub, or surgical hand antisepsis (43). It is the best and easiest way of preventing the spreading of microorganisms. However, about 50% of infections associated with health care occur due to poor hand hygiene of health care providers (44). Hand hygiene should be performed either with soap and running water or with alcohol rubs. It is extremely important to establish an aseptic technique when handling MDVs (45).

Nosocomial infections are the major cause of morbidity, mortality, and health care costs among hospitalized patients worldwide due to poor hand hygiene. The high prevalence of these infections 19%, in developing countries, poses a challenge to health care providers (46). The hands of health care providers are the most common vehicle for the transmission of infections associated with health care (47). A study conducted in health institutions of Bahir Dar city administration showed that 82.5% of health professionals had hand hygiene practice after completing the procedure they perform and about 50.8% wash their hands before the procedure. The overall hand hygiene practice score was 69.0% (48).

2.4.2 Use of glove (Wearing Glove)

Medical gloves are disposable personal protective equipment used during medical examinations and procedures that help prevent cross-contamination between HCWs and patients (49). Gloves should be worn for invasive procedures, any contact with sterile sites, non-intact skin, mucous membranes, and exposure to blood, all other body fluids, and contaminated instruments. Several of prospective controlled trials provide evidence that wearing gloves can help to reduce the transmission of pathogens in healthcare settings (49).

Gloves are worn to protect the hands from organic matter and microorganism contamination and also to reduce the chance of HCW transmitting microorganisms to patients and vice versa (50,51). Nevertheless, inappropriate use of gloves is observed regularly worldwide. The observational studies found that healthcare workers did not always remove gloves after previous care and gloves were not always changed between each patient contact (52,53). A study done in Shenen Gibe Hospital, South West Ethiopia revealed that 49% of health care providers did not comply with the recommended procedures of glove utilization (54).

2.4.3 Disinfecting of the vial (top) septum

At concentrations of 60-70% or greater isopropyl alcohol has germicidal activity against both gram-negative and gram-positive bacteria, such as *E. coli* or *MRSA*, respectively (55). The 2010 American Society of Anesthesiology (ASA) guidelines support swabbing vial tops. Use aseptic technique, including use of an alcohol swab or appropriate disinfectant, to cleanse the vial's rubber septum before entering is recommended. After cleaning the neck of glass ampules with an

alcohol swab it should be dry before opening (56). In 2010, the American Journal of Infection Control also recently supported disinfecting vial stoppers while using friction with sterile 70% isopropyl alcohol (24).

2.4.4 Avoid reuse of needles or syringes

CDC stated that needle and/or syringe reuse and the improper use of medication vials are preventable healthcare errors that should never occur (57). Reuse of needles and syringes between patients allows direct transmission of potentially infected blood and body fluids from one patient to the next (58). Once a syringe is empty, the inside barrel is open to the air and can become exposed to contaminants that could infect a patient. Another potential cause of transmission stems from the multiple uses of SDVs (58). These items do not contain additives that inhibit the growth of microorganisms, if they are accessed multiple times become contaminated and potentially cause an infection (58).

In a survey conducted on syringe and needle safety among student registered nurse anesthetists in the United States showed that 14 (4%) have administered medications from the same syringe to multiple patients, 59 (18%) have reused a needle on the same patient, 71 (22%) have reused a syringe or needle to withdraw medication from a MDV, and 160 (49%) have reentered a single-use medication vial to prepare doses for multiple patients (59). Similarly according to research conducted in the Northwest Province of Cameroon injection equipment reuse is practiced by 44% of health workers at public hospitals (60).

2.4.5 Storage temperature of medication

The medication storage room is often the “heart” in the ward since nearly all patients at hospitals receive drugs of different types (61). Environmental controls are essential to maintaining drug safety, quality, and efficacy (62). The drug must be transported, handle and store in a way that reduces the risk of exposure to temperatures outside the labeled storage conditions (63). Temperature is one of the most important parameters to control. It should be controlled and monitored using calibrated monitoring devices and records of temperature and alarms should be maintained (61,64).

Written procedures should be available describing the actions to be taken in the event of temperature is outside the labeled storage conditions (62). Provide thermostatic temperature-controlled systems for all temperature controlled rooms, cold rooms, freezer rooms, refrigerators, and freezers, used to store time and temperature-sensitive pharmaceutical products (63).

2.4.6. Medication drawing environment

Medications should be drawn up in a proper and clean medication preparation area that must be free from potential sources of contamination (61). Furthermore, any equipment that might have contact with body fluids or blood, such as contaminated items used in a procedure, should not be in the medication preparation area. The medication preparation environment should be disinfected and cleaned on a regular basis and at any time if there is evidence of soiling (64).

In addition, in the medicine preparation area, there should be easy access to vital supplies such as needles and syringes in their sterile packaging, alcohol-based hand rub, and alcohol wipes to ensure that employees adhere to the aseptic technique (61,64).

2.5 Labeling

The correct labeling of medication practice is a key element to safe medication administration (65). Food and drug administration (FDA) recommends that the appropriate package type term appears on all components of the labeling of injectable medical products for human use so the user will be able to easily identify the package type. Standardized labeling is one of the processes that contribute to the safe administration of injectable medicines (55). The labeling standard sets out the requirements for the user of containers for injectable medicines and fluids (bags, bottles, and syringes) in which the medicine can no longer be identified by its original packaging (66).

Labeling recommendations include patient name, patient identifier, medication added to the container, amount of medication added (including units), the total volume of diluent in a container (ml), concentration (units/ml), date and time of preparation, prepared by (signature), and route of administration (65). Labels on fluid bags and bottles should be placed on the front and the name of the fluid, batch number and expiry date should remain visible (66).

In a study conducted in Germany, Hannover hospital identified 113 (50%) of MDVs were undated from 227 total vials. On two MDVs, no medication type was indicated, and on seven vials concentration was not written (35). The other investigation in university hospital Innsbruck, Austria reported that in twenty-seven samples of MDV the date of the first use was not noted (67). Incomplete or inaccurate labeling of injectable medicines and fluids is a recognized risk to the safe administration of medicines (68).

Neglecting information or not using a label at all, can result in the wrong medication being administered or medicines being administered to the wrong patient (68). A study done in the United States investigated the medication errors un- labeled bag containing magnesium sulfate was accidentally administered to a patient who already had a bag of magnesium sulfate in progress (69). The unlabeled bag was prepared for another patient. The patient suffered a respiratory arrest and anoxic encephalopathy as a result of the overdose (69).

CHAPTER THREE: OBJECTIVES

3.1 General Objective

The general objective of this study was to assess bacterial contamination of single- and multiple-dose parenteral injection vials after opening, antibiotic susceptibility of isolates and associated risk factor at Jimma Medical Center, Jimma Southwest Ethiopia.

3.2 Specific Objectives

The specific objectives of the study were:

- To determine the prevalence of bacterial contamination of single- and multiple-dose injection vials.
- To determine antimicrobial susceptibility test for bacterial isolates from injection vials.
- To assess the associated risk factor for vial bacterial contamination.

CHAPTER FOUR: MATERIALS AND METHODS

4.1 Study setting

The study was conducted at Jimma Medical Center (JMC). JMC is one of the oldest public hospitals found in Jimma town Oromia regional state. The town is located 350 Km to the South West of Addis Ababa with geographical coordinates of approximately 7^{o}40'N latitude and 36^{o} 50'E longitude and an altitude of 1750-2000m above sea level; temperature range of 20-30^{o}C and average annual rainfall of 800-2500mm^{3}. The medical center served annually for 20,000 Inpatients, 205,000 Outpatient visits, and 11,000 emergency cases. JMC is categorized into different departments (units) which is suitable for service provision like Medical, Surgical, Pediatrics, Ophthalmology, Neonatology, Maternity, Gynecology, Orthopedics, Oncology, Psychiatry, Maxillofacial wards. The Unit and clinic include Burn, ICU, Endoscopy, Dialysis, Dressing and Suturing, Dental and ART as well as different outpatient departments are included in JMC.

4.2 Study design & periods

An institutional-based cross-sectional study design was conducted at JMC, from July 2021 to October 2021.

4.3 Source of population

The source populations were parenteral administered solutions for medication purposes at JMC.

4.4 Study population

Single- and Multiple-dose parenteral injection vials that were administered for patients from all wards and three ICUs of JMC during the study period

4.5. Inclusion and exclusion criteria

4.5.1 Inclusion criteria

Parenteral administered solution for therapeutic purposes during the data collection period

4.5.2 Exclusion criteria

Parenteral administered solutions for immunization and contraceptive purpose were excluded

4.6 Sample size determination

The sample size was calculated based on single population proportion

formula$= \frac{(\frac{Z\alpha}{2})^2 x\ p(1-p)}{(d)^2} = \frac{(1.96)^2 \times 0.5(1-0.5)}{(0.05)^2} = 384$

Whereas, n = sample size required

Zα/2= Critical value at 95% confidence level (1.96)

P = proportion of population (50%)

d = margin of sampling error tolerated (5%)

4.7. Sampling technique

A consecutive sampling technique was applied. Until the desired sample size reached all injectable medications that fulfilled the inclusion criteria and questionnaire were collected from Medical, Surgical, Pediatrics, Ophthalmology, Neonatology, Maternity, Gynecology, Orthopedics, Oncology, Psychiatry, Maxillofacial wards, and three ICU which are Medical, Adult, and Pediatric ICU. To avoid sampling bias particularly those samples that have some common similarities were coded to avoid repeating the same vial.

4.8 Study variables

4.8.1 Dependent variable

- ✓ Bacterial contamination of vials
- ✓ Antimicrobial susceptibility pattern

4.8.2 Independent variables

- Hand washing
- Wearing glove
- Reuse of syringe and/or needle
- Expiration date
- Medication drawing environment
- Disinfecting the top of vial
- Labeling opening day
- Storage condition

4.9 Data collection instrument

4.9.1 Questionnaire

A pre-tested self-administer questionnaire was used to collect factors that cause vial contamination by attending nurses. Data were collected from a total of 384 nurses who were working in the department that the sample were collected (ANNEX-III).

4.9.2 Specimen collection

During data collection each sample was labeled with a specific serial number; the detailed labeling of this information was recorded in a separate sheet for each sample. Details of each sample include: date and time of sample collection, name of the medication, ward, type of dose, preservative status, date and time of opening or preparation, storage condition, and expiration date of the vials were recorded.

All SDVs and MDV injectable drugs that opened and currently in use were well mixed before sampling and then rubber stoppers were swabbed with 70% alcohol. Using sterile techniques the vials were inverted and 100 µl of the medication was withdrawn with a sterile 1 ml insulin syringe then the sample was immediately transported to the microbiology laboratory.

4.10. Bacterial isolation and identification

4.10.1 Bacterial isolation

The sample was inoculated into Blood and MacConkey agar (OXOID, UK) by streak plate technique then incubated at 37 °C for 24 hr. All the isolates were subjected to colony morphology; gram staining, and standard confirmatory identification tests (70). **(**ANNEX-I).

4.10.2 Bacterial identification

4.10.2.1 Gram-positive bacteria

All bacteria growth was identified according to the protocol of clinical and laboratory standards institute (CLSI) by using gram staining reaction, colony morphology, and type of hemolysis on blood agar. Moreover, conventional biochemical tests like catalase and coagulase test were used for the identification of gram-positive bacteria (70).

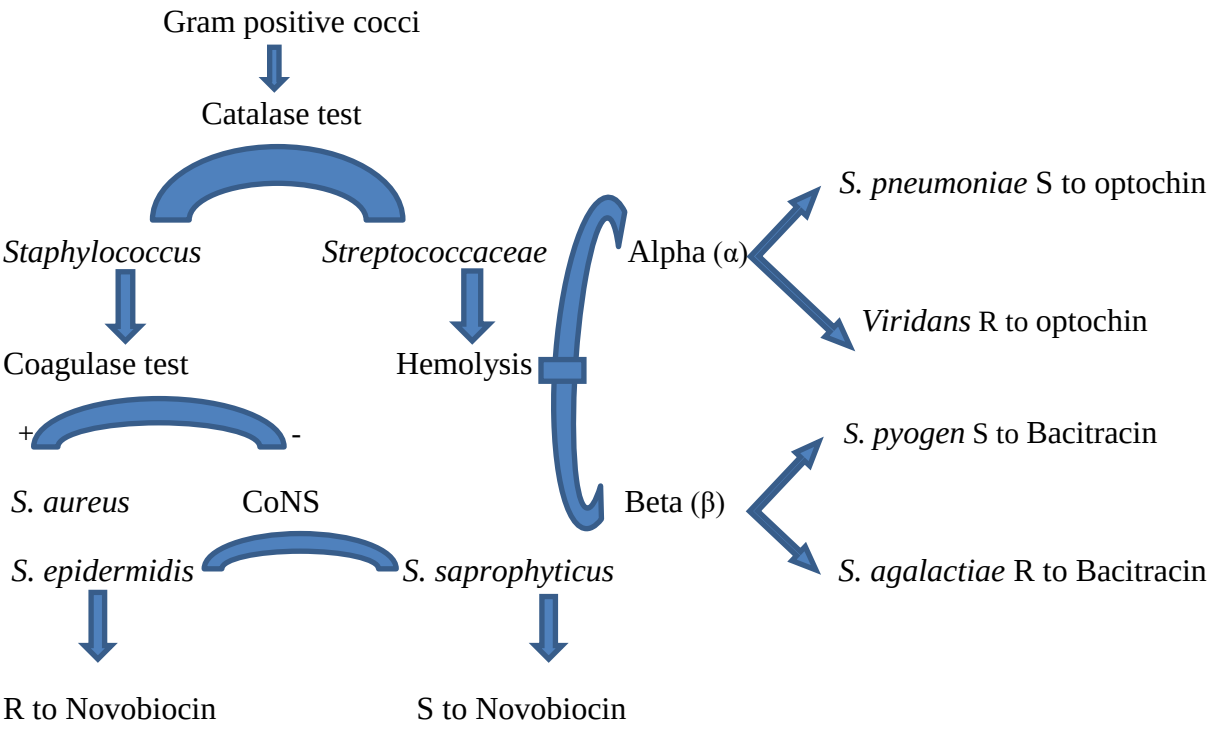


Figure1: Identification Flowchart of Gram Positive Cocci

4.10.2.2 Gram negative bacteria

A Gram-negative bacterium was identified by colony characteristics on Mackonkey agar, gram reaction, and carbohydrate fermentation. A single isolated colony was inoculated into biochemical media (OXOID, UK) Triple sugar iron agar, Urea, Citrate, lysine iron agar, and SIM (Sulfur, Indole, and Motility) then incubated aerobically for 24 hours. After overnight incubation, the expected bacteria were identified by a series of biochemical tests indole production, hydrolysis of urea, gas production, citrate utilization, sugar fermentation, oxidase test, hydrogen sulfide production, and lysine decarboxylase and motility characteristics (70).

4.11. Antimicrobial susceptibility test on bacterial isolates

An antimicrobial susceptibility test was carried out using Kirby-Bauer`s disc diffusion technique on Muller-Hinton agar (MHA) according to CLSI (71). The suspension of the bacterium was prepared by picking a pure colony with a sterile loop, suspended in sterile normal saline. The turbidity of this suspension was adjusted by comparing with 0.5 McFarland standards. After adjusting the turbidity the sterile cotton swab was dipped into the suspension of the isolate in normal saline, squeezed free from excess fluid against the surface of the tube. The swab was then being evenly distributed to the entire surface of MHA. The plate is left at room temperature to dry for 15-20 minutes. The discs were aseptically impregnated with proper spacing on the surface of the inoculated agar plates then pressed firmly onto the agar with sterile forceps, and incubated at 37°C for 24 hours.

The drugs tested for bacteria were include Gentamicin (CN-10 μg), Ciprofloxacin (CIP-5 μg), Trimethoprim/ sulphamethoxazole (SXT-25 μg), Ceftazidime (CAZ-30 μg), Meropenem (MEM-10 μg), Imipenem (IMI-10 μg), Ampicillin (AMP-30 μg), Chloramphenicol (CLR -30 μg), Tetracycline (TET-30 μg), Amikacin (AMI-30 μg), Ceftriaxone (CTR-30 μg), Cefoxtin (CFO-30μg), Clindamycin (CLI -2 μg), Penicillin (P-10 U) and Erythromycin (ERY-15 μg) were used (71). The diameter of inhibition around the discs was measured to the nearest millimeter and interpreted as sensitive (S), intermediate (I), or resistant (R) according to the defined breakpoints of CLSI (71). Reference strain of *E. coli* (ATCC® 25922) and *S. aureus* (ATCC® 25923) were used for quality control for antimicrobial susceptibility test (72).

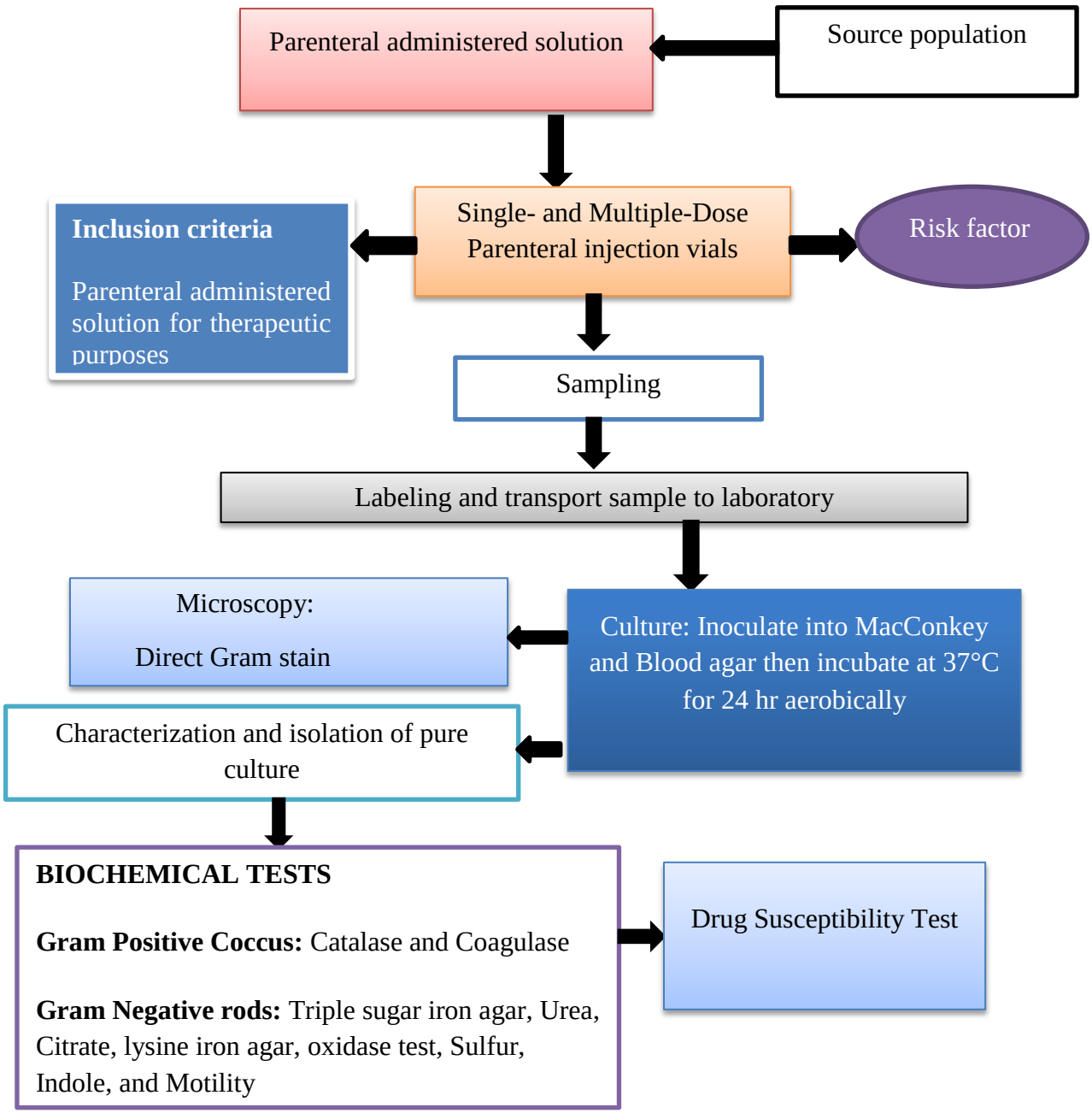


Figure 2: Flow Chart for the Bacteria Identification and Susceptibility Test from Contaminated Vials

4.12 Statistical analysis

Data entry was done by epi data version 3.1 then double-checked and exported to SPSS version 23 for further analysis. Frequency and percentage were calculated to summarize the results and presented in tables and figures. Dependent and independent variables association and strength of associated factors were determined using bivariate logistic regression and a variable showing statistically association were further analyzed for multivariate logistic regression and a value of less than 0.05 was considered as statistically significant.

4.13. Data quality control

The reliability of the study findings was guaranteed by implementing recommended quality control measures throughout the whole process of the laboratory works. All materials, equipment, and procedures were adequately controlled. Aseptic techniques were used in all the steps of specimen collection, transportation, and inoculation onto culture media to minimize contamination. All culture plates were prepared according to the directions of the manufacturers. From the prepared media 5% were incubated at 37 degree Celsius for overnight if there is any contamination or not, Control bacteria strains: *Escherichia coli* (ATCC 25922) and *S.aureus* (ATCC 25923) were used to ensure quality control of culture plates and antimicrobial susceptibility testing discs (72).

4.14 Ethical considerations

Ethical clearance was obtained from Jimma University Ethical Review Board; a support letter from the school of medical laboratory science was also obtained and submitted to Jimma medical center's administrative body. After adequately explaining the objective and purpose of the study, permission was granted from the JMC administrative body and from the patient or patient family to withdraw samples from vials. Vials with a positive result were communicated about their result to their respective health professionals.

4.15 Information dissemination

The finding of the study is submitted in the form of a thesis for partial fulfillment of a Master Degree to Jimma University institute of health science, School of Medical Laboratory Science. In addition, the result is disseminated to the JMC administrators and other responsible stakeholders. Besides, an attempt will be done that the findings get presented in different scientific meetings and workshops. Finally, a manuscript will be prepared and would be submitted to a national or international journal for publication.

4.16 Operational definitions

Injectable drug is all parenterally administered solution for therapeutic purpose

Single-dose vials are considered as an injectable drug that intended to be used only once after opening

Multi-dose vial: In this study, a multi-dose vial is considered as any kind of injectable container that is used at least once after opening and kept in the proper manner for subsequent use.

Safe injection practices are injection procedures that aim to maintain basic levels of patient safety and avoid vial contamination

Bacterial contamination is the presence of bacteria in the parenterally administered solution as evidenced by isolation in culture.

Multiple drug resistance (**MDR)** is bacteria that are resistant to one antibiotic in three or more classes of drugs

A risk factor is any attribute or exposures that increases or decrease the likelihood of vial contamination

Re-use of needle or syringe is the reuse of syringe and/or needle to enter a medication vial for the same patient or more than one patient

Medication drawing environment is a clean medication preparation room that is free from potential sources of contamination

Medication storage condition is a maintained condition for storing medication according to a manufacturer like temperature, air, light, and humidity

CHAPTER FIVE: RESULT

5.1. Socio demographic characteristic

A total of 384 nurse participants were included during the study period to collect data associated with vial contamination. Their age ranged from 24 to 64 with a mean age of 32 years and a standard deviation of 6.054 years. The mean work experiences of participants were 6 years and the majority of them had less than six years 231(60.2%) of work experience. Data were collected from eleven wards and three ICUs accordingly (Table1).

Table: 1 Socio Demographic Characteristic of Nurses' Staff at JMC, Jimma, Southwest, Ethiopia, from July 2021 to October 2021.

Variables		Frequency	Percent (%)
Ward	Medical	56	14.6
	Pediatrics	47	12.2
	Surgical	45	11.7
	Ophthalmology	32	8.3
	Neonatology	31	8.1
	Maternity	26	6.8
	Gynecology	24	6.3
	Oncology	22	5.7
	Orthopedics	20	5.2
	Maxillofacial	15	3.9
	Psychiatry	12	3.1
	Adult ICU	24	6.3
	Pediatric ICU	18	4.7
	Medical ICU	12	3.1
Work	≤6	231	60.2
Experience	>6	153	39.8

5.2. Overall solution and their features

From a total of 384 samples, 236 (61.5%) vials were MDVs and the remaining 148 (38.5%) were SDVs collected from eleven ward and three ICU units, with 36 medication types. The highest numbers of parenteral medications were collected from three major wards: Pediatrics 55(14.3%), Medical 53(13.8%), and Surgical 48 (12.5%) respectively. The most sampled medications were ceftriaxone 107(27.9%), metronidazole 51(13.3%), and normal saline 46(12%) (Table2). Of the total MDVs, 157(66.5%) of the medications were preservative-free whereas the rest 79(33.3%) contain preservatives. However, all SDVs 148(38.5%) were preservative-free. Almost three fourth (77.1%) of the medications had been stored at room temperature while the rest 88(22.9%) were stored in the refrigerator. Of collected parenteral medication, 73(19%) were stored out of manufacturer recommendation. None of MDVs medications were dated with opening day. Almost all vials were being used within their expiration period, but 4 insulin vials' expiration date label was not legible upon checking.

Table 2: Name of Medication, Ward and Respective Number of Sampled Vials at JMC, Jimma, Southwest, Ethiopia, from July 2021 to October 2021.

Medication	**Ward/unit**	**Respective number of sampled vials**	**Frequency**	**Percent (%)**
Ceftriaxone	Pediatric, Medical, Surgical, Orthopedic, Maternity, Gynecology, Neonatology, Maxillofacial, Ophthalmology, Pedi ICU, Oncology and Adult ICU	16,4,13, 17,11,7, 8,13,3,6, 7,2	107	27.9
Ceftazidime	Pediatric, Neonatology, Ophthalmology, Oncology, Pedi ICU	6, 1,7,1,2	17	4.4
Vancomycin	Pediatric, Medical, Surgical, Neonatology, Ophthalmology, Pedi ICU, Oncology and Adult ICU	6,2,2,3,9,7,3,1	33	8.6
R insulin	Pediatric, Medical, Medical ICU	6,11,2	19	4.9
NPH insulin	Medical, Neonatology, Medical ICU	10, 2, 2	14	3.6
NaCl (normal saline)	Pediatric, Medical, Surgical, Maternity, Neonatology, Gynecology, Pedi ICU, Medical ICU, Oncology	12,1,9, 3,4,2, 4,3,8	46	12
Dextrose 5%	Pediatric, Surgical, Maternity, Neonatology, Pedi ICU, Oncology	2,4,1,1,3,6	17	4.4

Sodium lactate	Pediatric	1	1	0.3
Furosamide	Medical	3	3	0.8
Metronidazole	Pediatric, Medical, Surgical, Orthopedic, Maternity, Gynecology, Neonatology, Maxillofacial, Pedi ICU, Oncology	2,8,8,9,5,9,3,4, 2,1	51	13.3
Propofol	Surgical, Operation room and anesthesiology	1,2	3	0.8
Ketamine	Surgical, Adult ICU, Operation room	1,2,3	6	1.6
Thiopentone	Operation room and anesthesiology	1	1	0.3
Suxamethopin um	Surgical, Operation room and anesthesiology	2,1	3	0.8
Vecuronium	Operation room and anesthesiology	1	1	0.3
Morphine	Pedi ICU, Pedi oncology and Adult ICU	1,1,1	3	0.8
Pethioline	Operation room and anesthesiology	1	1	0.3
Metoclopromide	Operation room and anesthesiology	1	1	0.3
Afropine	Operation room and anesthesiology	1	1	0.3
Ciprofloxacin	Surgical, Pedi oncology	2, 1	3	0.8
Gentamycin	Pedi oncology	1	1	0.3
Heparin	Medical, Surgical, Orthopedic, Medical ICU	13,2,2,1	18	4.7
Potassium chloride	Surgical	1	1	0.3
Dexamethasone	Neonatology, Pedi ICU	1,2	3	0.8
Hydro cortisone	Pedi ICU	2	2	0.5
Calcium gluconate	Neonatology	1	1	0.3
Ampicilin	Pediatric, Pedi ICU	1,3	4	1.0
Lidocaine	Surgical, Pedi ICU	3,1	4	1.0
Magnesium sulfate	Maternity	6	6	1.6
Omeprazole	Pediatric, Adult ICU	1,3	4	1.0
Ondansetron	Pedi oncology	2	2	0.5
Bupivacaine	Surgical	1	1	0.3
Sodium pentothal	Surgical	1	1	0.3
Tramadole	Medical	1	1	0.3
Modicate	Psychiatry	2	2	0.5
Distilled water	Pediatric	2	2	0.5
Total			384	100

5.3. Prevalence of contamination in different department

Contaminations were detected among five wards and two intensive care units. The highest 8(38.1%) and the lowest 1(4.8%) prevalence of contamination were observed between pediatric and surgical wards respectively (Fig 3). However, contaminations were not detected in the following wards ophthalmology, maternity, gynecology, orthopedics, psychiatry, maxillofacial wards, and medical ICU.

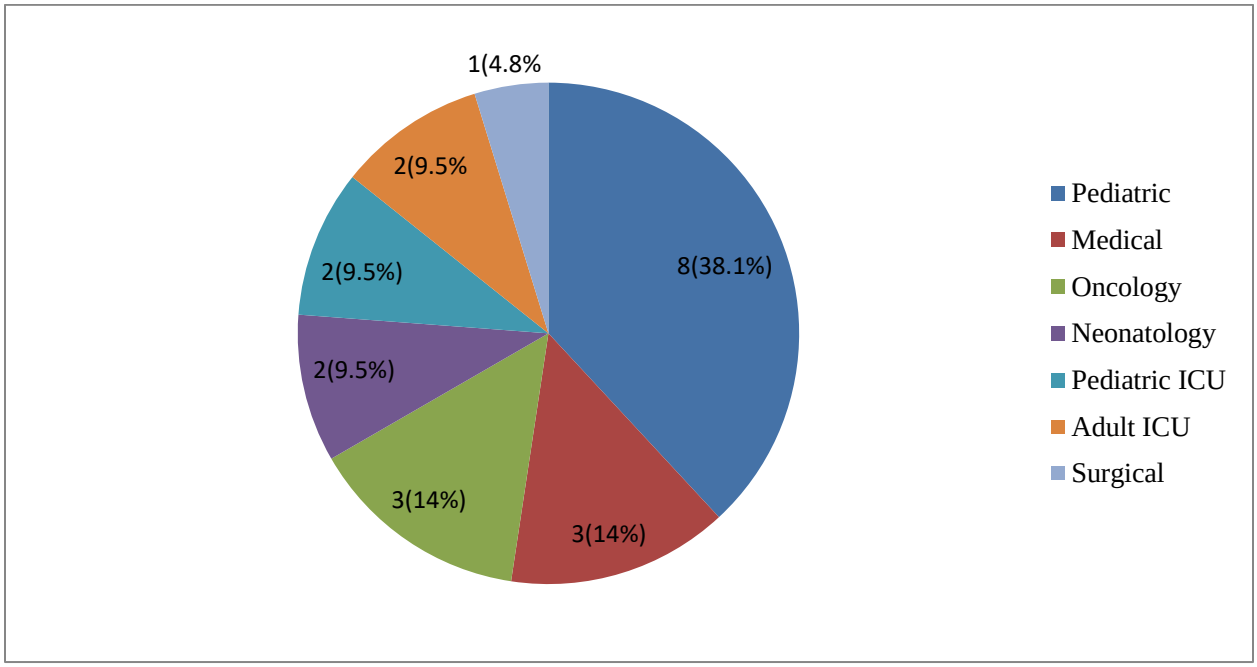


Figure: 3 Distributions of Contaminations among Ward and Intensive Care Unit at JMC, Jimma, Southwest, Ethiopia, from July 2021 to October 2021

5.4. Prevalence of contamination according to type of medication

From a total of 36 types of medication, contaminations were identified among 9 medications (Table 3). The most frequent contaminated parenteral solutions were listed in the following descending order normal saline 10(47.6%), dextrose 3(14.3%), and omeprazole 2(9.5%). Contaminations were not detected in any of the antibiotics vials. No mixed contamination was detected in any of the MDVs. Of contaminated parenteral medications, 4(44.4%) were with preservatives. Though the remaining medication 5(55.6 %) were preservative-free. All contaminated medications were stored at room temperature.

Table 3: Distribution and Frequency of Isolated Bacteria, Source of Medication, Storage Condition, Preservative Status and Wards of Vial at JMC, Jimma, Southwest, Ethiopia, from July 2021 to October 2021

Ward /unit	Isolated bacteria	Source of medication	Storage condition	preservative status	Frequency	Percentage
Medical	CoNS	NPH insulin	RT	Present	1	0.3
	Pseudomonas aeruginosa	R insulin	RT	Present	1	0.3
	Acinetobacter spp	Heparin	RT	Present	1	0.3
Pediatric	CoNS	NS	RT	Absent	1	0.3
	K. pneumoniae	Dextrose NS	RT	Absent	2	0.5
	K. aerogenes	Potassium chloride	RT	Absent	1	0.3
	Pseudomonas aeruginosa	NS	RT	Absent	2	0.5
	Acinetobacter spp	NS	RT	Absent	2	0.5
Surgical	*K. pneumoniae*	Propofol	RT	Present	1	0.3
Neonatology	CoNS	NS	RT	Absent	1	0.3
	Pseudomonas aeruginosa	NS	RT	Absent	1	0.3
Oncology	*K. pneumoniae*	NS	RT	Absent	1	0.3
	Pseudomonas aeruginosa	NS	RT	Absent	1	0.3
	Citrobacter koseri	Dextrose	RT	Absent	1	0.3
Pedi ICU	*K. pneumoniae*	Dextrose	RT	Absent	1	0.3
	K. aerogenes	Sodium lactate	RT	Absent	1	0.3
Adult ICU	*Pseudomonas aeruginosa*	Omeprazole	RT	Absent	1	0.3
	K. oxytoca	Omeprazole	RT	Absent	1	0.3
Total					21	5.5%

CoNS = Coagulase negative *staphylococci*, RT= Room temperature, NS= Normal saline, R insulin = Regular insulin

5.5. Prevalence of isolated bacteria

From the total 384 parenteral administered solutions enrolled in the study, 21 bacterial were isolated. The overall prevalence of contamination in this study was 5.5%. Out of total bacteria isolates, five different pathogenic bacteria species were identified. Gram-negative bacteria were more dominant than gram-positive. Gram-negative and gram-positive bacteria were involved in 18 (85.7%) and 3 (14.3%) of contaminations respectively. *Pseudomonas aeruginosa* is the most common gram-negative bacteria followed by *K. pneumoniae* constituting 6 (28.5%) and 5 (23.8%) respectively. Coagulase-negative *staphylococcus* species was the single isolate from gram-positive bacteria (Figure 4).

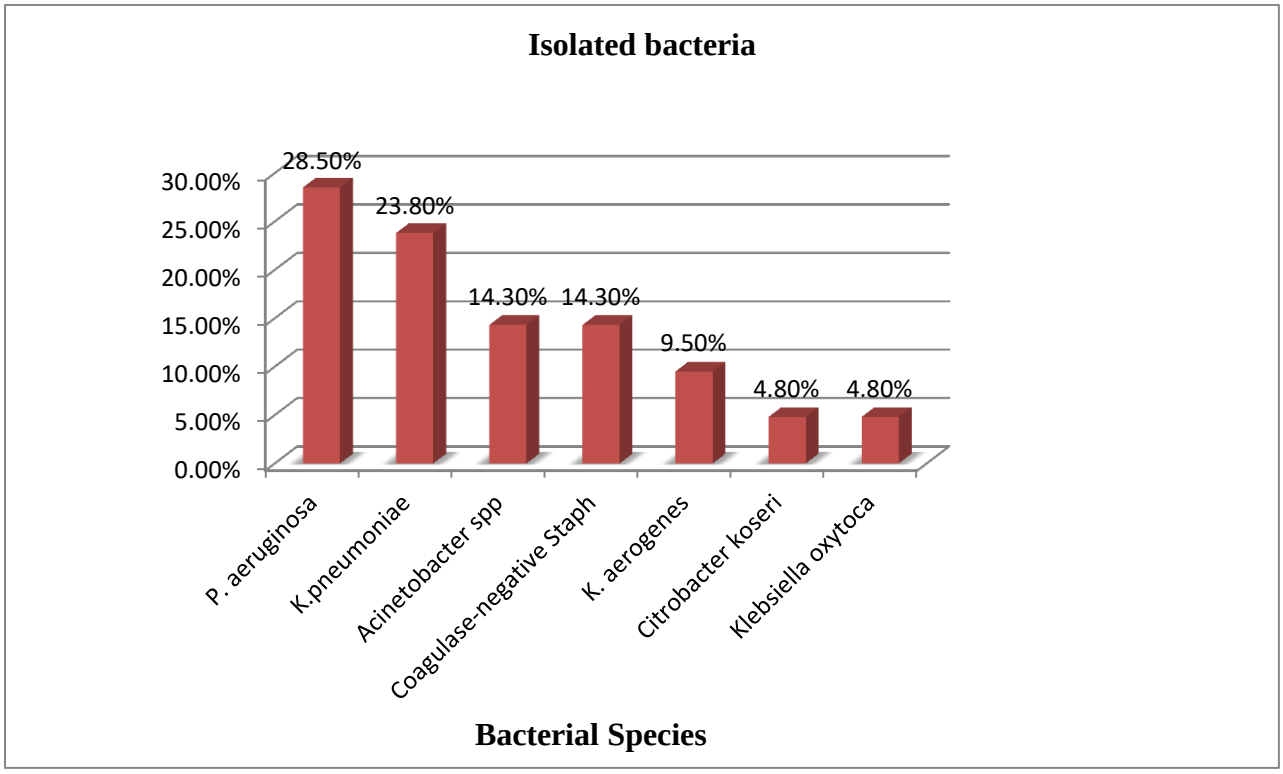


Figure 4: Prevalence and Types of Isolated Bacteria among Contaminated Vial at JMC, Jimma, Southwest, Ethiopia, from July 2021 to October 2021.

5.6. Antimicrobial susceptibility patterns

In this study, a total of 15 different types of antimicrobial agents were used to test the antimicrobial pattern of the pathogenic bacteria isolated from contaminated vials. Both gram-negative and gram-positive isolates revealed a different level of resistance pattern to the antimicrobials tested. Of the total, 18(85.7%) gram-negative isolates were sensitive to imipenem 14(77.8%), amikacin 11(61%), chloramphenicol 10(55.6%), and gentamicin 9(50%). Antimicrobial drug resistance profiles of the gram-negative bacterial isolate was revealed a relatively high resistance rate against ceftriaxone 16 (88.9 %) and ceftazidime 14(77.8%).

Pseudomanas aeruginosa is the most frequently isolated bacterium that showed 6(100%) resistance rate to each of the following antibiotics: gentamicin, tetracycline, ceftriaxone, sulfamethoxazole-trimethoprim and ceftazidime. However, it showed sensitivity to amikacin, imipenem, and chloramphenicol 3(50%), 2(33.3%), and 1(17%) respectively. *K. pneumoniae* is the second common isolate that showed 3(60%) resistance rates to tetracycline, ceftriaxone, sulfamethoxazole-trimethoprim, and meropenem; however, it was sensitive 5(100%) for imipenem and chloramphenicol and as well as 5(80%) for amikacin. Among *Acinetobacter* spp isolate 3(100%) resistance rates were seen to ceftriaxone and ceftazidime. conversely, they were 2(66.7%) sensitive to chloramphenicol, gentamicin, and meropenem. The drug resistance profile of gram-negative bacteria is presented in table 4.

Among gram-positive bacteria, 3(100%) level of resistance were found to penicillin and followed by 2(66.7%) to ampicillin, tetracycline, ciprofloxacin, and erythromycin. However, gram-positive bacteria also showed 1(33.3%) of sensitivity to gentamicin, sulfamethoxazole-trimethoprim, and clindamycin. Out of total CoNS isolate methicillin-resistant were accounted for 2(66.7%) whereas the remaining 1(33.3%) was methicillin-sensitive. The drug resistance profile of gram-positive bacteria is presented in table 4.

Table 4: Antimicrobial Resistance Profiles of Bacterial Isolates (n = 21) at JMC, Jimma, Southwest, Ethiopia, from July 2021 to October 2021.

Bacterial Isolates	Total Isolate	**Resistance pattern of antimicrobial agents (%)**														
		CLR	CN	AMI	TET	CTR	CIP	SXT	CAZ	MEM	IMI	AMP	CFO	P	CLI	ERY
K. pneumoniae	5	0	2(40)	1(20)	3(60)	3(60)	2(40)	3(60)	2(40)	3(60)	0	NA	NA	NA	NA	NA
P. aeruginosa	6	5(83)	6(100)	3(50)	6(100)	6(100)	5(83)	6(100)	6(100)	5(83)	4(66.7)	NA	NA	NA	NA	NA
Acinetobacter spp	3	1(33.3)	1(33.3)	2(66.7)	2(66.7)	3(100)	2(66.7)	2(66.7)	3(100)	1(33.3)	0	NA	NA	NA	NA	NA
K. aerogenes	2	1(50)	0	0	0	2(100)	1(50)	2(100)	2(100)	1(50)	0	NA	NA	NA	NA	NA
Citrobacter koseri	1	0	0	0	0	1(100)	1(100)	1(100)	1(100)	0	0	NA	NA	NA	NA	NA
K. oxytoca	1	1(100)	0	1(100)	1(100)	1(100)	1(100)	1(100)	0	0	0	NA	NA	NA	NA	NA
Total	18	8 (44.4)	9(50)	7(39)	12(66.7)	16(88.9)	12(66.7)	15(83)	14(77.8)	10(55.5)	4(22.2)	NA	NA	NA	NA	NA
CoNS	3	1(33.3)	1(33.3)	NA	2(66.7)	NA	2(66.7)	1(33.3)	NA	NA	NA	2(66.7)	2(66.7)	3(100)	1(33.3)	2(66.7)

Note: CoNS Coagulase-negative *staphylococci*, NA not applicable, CLR chloramphenicol, CN gentamicin, AMI amikacin, TET tetracycline, CTR ceftriaxone, CIP ciprofloxacin, SXT sulfamethoxazole-trimethoprim, CAZ ceftazidime, MEM meropenem, IMI imipenem, AMP ampicillin, CFO cefoxitin, ERY erythromycin, P Penicillin, CLI clindamycin

Antibiogram showed that almost all isolates 20(95.2%) of bacteria were resistant to three or more classes of commonly used antimicrobial agents. Observed multiple drug resistance (MDR) for three and four antimicrobial agents was 1(4.8%), 4(19%) respectively. The frequency of MDR was found in all gram-negative bacteria 18 (100%), whereas 2(66.7%) out of the total three gram-positive bacteria showed MDR. None of the isolates showed sensitivity to all used antibiotics.

Table 5: Multiple Drug Resistance Patterns (Antibiogram) of Isolated Bacteria at JMC, Jimma, Southwest, Ethiopia, from July 2021 to October 2021.

Types of Spp.	ANTIBIOGRAM PATTERN						
	R0	R1	R2	R3	R4	≥ R5	Overall MDR (%)
Klebsiella spp (N=8)	0	0	0	0	2(25%)	6(75%)	8(100%)
Acinetobacter spp (N=3)	0	0	0	1(33.3%)	0	2(66.7%)	3(100%)
Citrobacter koseri (N=1)	0	0	0	0	1(100%)	0	1(100%)
P. aeruginosa (N=6)	0	0	0	0	0	6(100%)	6(100%)
CoNS (N=3)	0		1(33.3%)	0	1(33.3%)	1(33.3%)	2(66.7%)
Total (N=21)	0	0	1(4.8%)	1(4.8%)	4(19%)	15(71.4%)	20(95.2%)

R0= Sensitive to all drugs, R1= Resistance to one drug, R2= Resistance to two drugs, R3= Resistance to three drugs, R4= Resistance to four drugs and R5= Resistance to five and above drugs.

5.7. Factors associated with vial contamination

In bivariate logistic regression analysis, vial contamination was strongly associated with re-use of syringe and/or needle, medication drawing environment, and storage conditions of the vial (P<0.05). As shown in table 6 statistical significance difference was observed with re-use of syringe and/or needle, medication drawing environment, and storage conditions with crude odds ratio (COR)(95%CI) 2.5[1.011-6.183], 0.377[0.153-0.934] and 0.042[0.006-0.314] respectively.

The results of this study indicate that the chance of vial contamination by re-use of syringe and/or needle was increased by 2.5 times (COR; 2.5[95 % CI, 1.011-6.183] than the use of sterile needle and syringe. Similarly, the odds of vial contamination increased by 0.377 times when drawing medication in a contaminated environment 0.377[0.153-0.934]. The chance of vial contamination was increased by 0.042 fold when the vial are stored out of the manufacturer's order than storing vials according to the manufacturer's order 0.042 [0.006-0.314].

In bivariate logistic regression analysis, re-use of syringe and/or needle with significance value (p=0.047), medication drawing environment (p=0.035) and storage conditions (p=0.002) was the candidate variable for multivariate logistic regression analysis. In multivariate analysis, vial contamination was significantly associated with all candidate variables, reuse of needle and/or syringe, medication drawing environment and storage conditions showed statistically significant association with vial contamination (p=0.032), [AOR (95%CI) =2.830 (1.095-7.319)], (p=0.036), [AOR (95%CI) =2.768 (1.071-7.153)] and (p=0.001), [AOR (95%CI) = 28.65 (3.765-218.068)] respectively.

Table 6: Bivariate and Multivariate Logistic Analysis of Risk Factor for Vial Contamination at JMC, Jimma, Southwest, Ethiopia, from July 2021 to October 2021

Variables		Frequency	Percentage	COR (95%CI)	p-value	AOR (95%CI)	p-value
Sex	Male	156	40.6	1.654 [0.685-3.994]	0.263		
	Female	228	59.4	1*			
Age	≤32	228	59.4	1.393[0.549-3.533]	0.486		
	>32	156	40.6	1*			
Department	Ward	330	85.9	1.588[0.359-7.022]	0.542		
	ICU	54	14.1	1*			
Experience	≤6	231	60.2	1.081[0.437-2.673]	0.866		
	>6	153	39.8	1*			
Single vial for a single patient	YES	359	95.8	1.160 [0.146-9.228]	0.888		
	NO	16	4.2	1*			
Re-use needle or syringe	YES	156	40.6	2.5[1.011-6.183]	0.047	2.830 [1.095-7.319]	0.032
	NO	228	59.4	1*			
Properly hand washing	YES	205	53.4	2. 400 [0.946-6.08]	0.065		
	NO	179	46.6	1*			
Disinfected top of the vial	YES	45	11.7	0.541 [0.174-1.686]	0.290		
	NO	339	88.3	1*			
Drawn medication in clean area	YES	233	60.7	1*			
	NO	151	39.3	0. 377 [0.153-0.934]	0.035	2.768 [1.071-7.153]	0.036
Checked expiration date before use	YES	371	96.6	0.294 [0.061-1.435]	0.131		
	NO	13	3.4	1*			
Use new glove before injection	YES	331	81	4.948 [0.653-37.485]	0.122		
	NO	73	19	1*			
Check opening date	YES	148	38.5	0.623 [0. 236-1.642]	0.338		
	NO	236	61.5	1*			
Store the vials accordingly	YES	199	51.8	1*			
	NO	185	48.2	0.042 [0.006-0.314]	0.002	28.65[3.765-218.068]	0.001
Vials can be contaminated	YES	246	64.1	1.129 [0.445-2.869]	0.798		
	NO	138	35.9	1*			

Key: OR= Odd ratio, CI = Confidence Interval, COR= Crude Odds ratio, AOR= Adjusted Odds ratio, 1* reference category

CHAPTER SIX: DISCUSSIONS

In the present study, bacterial contamination was detected in vials containing preservatives as well as preservative-free. This finding emphasizes the importance of safe medication injection practices, regardless of the preservative content of vials. Outbreaks of sepsis infection related to unsafe injection practices indicate that some vials do not adhere to basic principles of infection control. Therefore, the presence of preservatives itself cannot be guaranteed to contamination-free medication practices unless the aseptic technique used among the nurses working in different wards and exposure of the contents of the vials to environmental factors is controlled.

In the current study, the overall prevalence of vial contamination rate was 5.5%. This prevalence was consistent with two other previous studies from Iran that showed a 5.6% and 5.36% contamination rate respectively (4,16). However, the result from this study was higher than other similar studies conducted in Germany 0.9% and Austria 4 % (35,67). The possible reason for this difference might be due to the sample size, study period, type of collected sample and reuse of needle and/or syringe, medication drawing environment and storage condition might be a possible reason for the higher contamination rate of vials. In the current study, the length of the study period was longer than the previous study in Germany which collected a sample on a single day, and all used 227 MDVs were collected (35). Similarly, the study conducted in Austria incorporate only a total of 96 Vials from different wards except for intensive care units (67).

The prevalence of vial contamination in the current study was lower than the studies conducted in India 25% and South Africa 6.4% (6,13). This variation might be due to the sample size, kinds of wards include and aseptic technique. The previous study in India was a pilot study and only 40 MDVs were collected from different ICUs (6). Likewise, the research conducted in South Africa was limited to only 110 self-prepare multi-dose phenylephrine solutions and the samples were included from two obstetric theatres (13). Furthermore direct or indirect contact with potentially contaminated surfaces and poor aseptic techniques employed during successive uses might be a reason.

In the present study, the highest contamination of vial was found in pediatric ward 8(38.1%) and this finding is also supported by other study done in Shiraz, Southwest of Iran (16). However, these contradict with the report from another part of the world (4), which reported the highest

rate of vials contamination (14.28%) in interventional bronchoscopy unit. The reason for the highest contamination in the pediatric ward might be due to drugs administered for pediatric cases being based on the child's kg and stored for a long time at normal temperature.

In the current study, the most frequent contaminated solutions were normal saline 10(47.6%), this is inconsistent with similar studies in India and Iran where the most frequent contaminated solutions were insulin and heparin (4,6). The possible reason for inconsistency might be the type of medication that was frequently collected in the present study was normal saline and since normal saline is preservative-free it is more susceptible to contamination in addition that the previous study mainly conducted in India includes only insulin and heparin (6).

In this study, none of the MDVs medications were dated with opening day, this finding is also supported by findings from another study in the UK (73). But this result is inconsistence with similar studies in Austria and Germany the date of first use was given on 27 (28.1%), and 114 (50%) vials respectively (35,67). The difference might be due to the extraordinary degree of nurses not performing a simple act that contributes to quality control. From a total of MDVs, 66.5% of samples were repeatedly used even though they were preservative-free. This result is higher than the previous reports in Austria 28% (67). This study confirmed microorganisms can survive in the presence of a preservative, as a number of the contaminated vials were containing a preservative. This finding is consistent with previous studies conducted at different places of the world (4,6,67).

In the present study, from the total bacteria isolates, gram-negative bacteria 18(85.7%) were more dominant than gram-positive bacteria 3(14.3%). The leading isolated bacterium was *Pseudomonas aeruginosa* 6(28.5 %) and followed by *K. pneumoniae* 5(23.8%). Our finding is in concordance with a similar investigation conducted in the USA (14). However, this result contradicts two other studies done in Shiraz, Southwest of Iran, and Tehran, northern Iran that reported 88.9% and 81.82% prevalence of gram-positive bacteria respectively (4,16). The reason might be due to the highest percentage of hospital-acquired infections being related to gram-negative bacteria due to mechanical transfer of bacteria on hands, clothing of health care workers, and cross-contamination among admitted patients. In current study of gram-positive

bacteria, CoNS has confirmed 3(14.3%) prevalence. This reflection were supported by other investigations in Iran, India, and Germany (4,6,35).

Antimicrobial resistance represents a global health crisis and one of the most serious threats humans face today (37). In this study also the antimicrobial resistance of bacteria became an existing problem. This study found that among gram-positive bacteria, CoNS had the highest resistance to commonly prescribed antibiotics. It had 3(100%) resistance rate to penicillin and 2(66.7%) to methicillin. The drug resistance pattern of gram-negative bacteria also showed the highest rate of resistance to ceftriaxone 16(88.9%) and ceftazidime 14(77.8%). Overall, 20(95.2%) of the bacterial isolates from this study were characterized as MDR pathogenic bacteria.

The possible reason might be most hospital-acquired bacteria are more resistant and can be spread from patient to patient in healthcare facilities, often via the contaminated hands of healthcare personnel, contaminated medical or surgical equipment, or the inanimate hospital environment. These organisms are highly efficient at up-regulating or acquiring genes that code for mechanisms of antibiotic drug resistance.

In this study, 40.6% of participants responded that they re-use syringes and/or needles. This finding is in agreement with a study in Cameroon (44%) (60). However, this result is higher than the previous survey reports in the USA where 22% of anesthetists nurses have reused a syringe or needle to withdraw medication from a multidose vial (59). In the current study, unsafe practices concerning not washing hands (46.6%) and not wearing or changing gloves were (19%). This result was lower than the study conducted in India which showed that 95.4% and 61.6% of not washing hands and wearing/changing gloves respectively (74). This much difference may be due to the fact that non -adherence of health care professionals to the most important aseptic technique.

Multivariate analysis on reuse of syringe and/or needle, medication drawing environment, and storage conditions were analyzed concerning vial contamination in this study. The rate of vial contamination with respect to three variables has shown some level of importance and the weight was significant. In this analysis, reuse syringe and/or needle, medication drawing environment

and storage conditions showed statistically significant association with vial contamination (p=0.032), [AOR (95%CI) =2.830 (1.095-7.319)], (p=0. 036), [AOR (95%CI) = 2.768 (1.071-7.153)] and (p=0.001), [AOR (95%CI) = 28.65 (3.765 -218.068)] respectively. Reuse of needle and/or syringe, medication drawing environment, and storage conditions of vials were more likely associated with vial contamination. However, other factors like proper hand washing, disinfected on top of the vial, expiration date, use of new glove before injection, and other variables tested as a possible risk factors for vial contamination were not seen as significant predictors.

6.1. Limitation of the Study

- This study did not include anaerobic bacteria which can also be the causative agent for vial contamination
- Similar studies were absent in Ethiopia to compare the result

.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. Conclusion

The present study confirmed microbial contamination of the parenteral administered solutions and this may indicate an infection threat. The overall prevalence of bacteria was high and most of the isolates were gram-negative bacteria. The majority of the bacterial isolates had multiple drug-resistant features. The use of MDVs is a convenient and economical option in developing countries like Ethiopia. Conversely, they are also associated with the risk of contamination and nosocomial outbreaks of life-threatening bloodstream infections. In current study reuse of needle and/or syringe, medication drawing environment, and storage conditions of vials were more likely associated with vial contamination. The present study also revealed that there is a gap among healthcare professionals with regard to basic infection control practices as per standard guidelines to minimize the incidence of hospital-acquired infections.

7. 2. Recommendation

- A periodic training program should be installed for health care workers regarding aseptic techniques.
- Use single-dose vials instead of MDVs to reduce the risk of contamination
- A clean environment should be provided for the preparation and administration of drugs
- Drugs should be stored according to the manufacturer recommendations
- Avoid the use of preservative-free solutions as MDV
- Reuse of needle and/or syringe must be avoided
- Further study is warranted on the assessment of the efficacy of preservatives present in MDV solutions

References

1. Rosenthal VD, Maki DG, Mehta Y, Leblebicioglu H, Memish ZA, Al-Mousa HH, et al. International Nosocomial Infection Control Consortium (INICC) report, data summary of 43 countries for 2007-2012. Device-associated module. Am J Infect Control. 2014 42(9):942–56.
2. WHO. Report on the Burden of Endemic Health Care-Associated Infection Worldwide Clean Care is Safer Care. World Health Organization. 2011;
3. Simonsen L, Kane A, Lloyd J, Zaffran M, Kane M. Unsafe injections in the developing world and transmission of bloodborne pathogens : a review / L. Simonsen ... [et al.]. Bull World Heal Organ Int J Public Heal 1999 ; 77(10) 789-800.
4. Baniasadi S, Dorudinia A, Mobarhan M, Karimi Gamishan M, Fahimi F. Microbial contamination of single- and multiple-dose vials after opening in a pulmonary teaching hospital. Brazilian J Infect Dis. 2013;17(1):69–73.
5. Centers for Disease Control and Prevention. Questions about multi-dose vials. www.cdc.gov/injectionsafety/providers/provider_faqs_multivials.html [accessed 12.06.21].
6. Bhatia M, Mishra B, Loomba PS, Dogra V. A pilot study for evaluation of knowledge and common practises of nursing staff regarding use of multidose injection vials and their microbial contamination rate in a super-specialty hospital. J Educ Health Promot. 2018.14;7:120.
7. Longfield R, Longfield J, Smith LP, Hyams KC, Strohmer ME. Multidose medication vial sterility: An in-use study and a review of the literature. Infect Control. 1984;5(4):165–9.
8. Centers for Disease Control and Prevention. Questions about single-dose/single-use vials. www.cdc.gov/injectionsafety/providers/provider_faqs_singlevials.html [accessed 12.06.21].
9. Khalili H, Sheikhbabayi M, Samadi N, Jamalifar H, Dalili D, Samadi N. Bacterial contamination of single-and multiple-dose vials after multiple use and intravenous admixtures in three different hospitals in Iran. Iran J Pharm Res. 2013;12(1):205–9.

10. Plott RT, Wagner RFJ, Tyring SK. Iatrogenic contamination of multidose vials in simulated use. A reassessment of current patient injection technique. Arch Dermatol. 1990;126(11):1441–4.

11. Ashok A, Brison M, LeTallec Y. Guidelines for Handling Temperature Sensitive Vaccines and Pharmaceuticals 2017:19; 35(17):2217-23.

12. Samuel R, Nel D, Joubert I, De Bruin J, Dwyer S, Fourie A, et al. SASA Guidelines for Infection Control in Anaesthesia in South Africa 2014 SASA Guidelines for Infection Control in. SAJAA. 2014;20(3):S1–39.

13. Van Den Heever A, Scribante J, Perrie H, Lowman W. Microbial contamination and labelling of self-prepared, multi-dose phenylephrine solutions used at a teaching hospital. South African J Anaesth Analg. 2016;22(6):18–22.

14. Archibald LK, Ramos M, Arduino MJ, Aguero SM, Deseda C, Banerjee S, et al. Enterobacter cloacae and Pseudomonas aeruginosa polymicrobial bloodstream infections traced to extrinsic contamination of a dextrose multidose vial. J Pediatr. 1998;133(5):640-4.

15. Kirschke DL, Jones TF, Stratton CW, Barnett JA, Schaffner W. Outbreak of joint and soft-tissue infections associated with injections from a multiple-dose medication vial. Clin Infect Dis. 2003;36(11):1369–73.

16. Motamedifar M, Askarian M. The prevalence of multidose vial contamination by aerobic bacteria in a major teaching hospital, Shiraz, Iran, 2006. Am J Infect Control [Internet]. 2009;37(9):773–7. Available from: http://dx.doi.org/10.1016/j.ajic.2008.12.009

17. Hutin YJ, Hauri AM, Armstrong GL. Use of injections in healthcare settings worldwide, 2000: literature review and regional estimates. 2003 6; 327(7423):1075.

18. Birkhead G, Hamilton TE, Kossover R, Perz J, Gangadharan D, Iskander J. CDC grand rounds: preventing unsafe injection practices in the US health-care system. MMWR Morb Mortal Wkly Rep. 2013 May;62(21):423–5.

19. Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee. 2007 Guideline for Isolation Precautions: Preventing

Transmission of Infectious Agents in Healthcare Settings (updated July 2019). Centers Diasease Control Prev. 2019;1–232.

20. Siegel JD, Rhinehart E, Jackson M, Chiarello L. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. Am J Infect Control. 2007;35(10 SUPPL. 2).

21. Kermode M. Unsafe injections in low-income country health settings: need for injection safety promotion to prevent the spread of blood-borne viruses. Health Promot Int. 2004;19(1):95–103.

22. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJL. Selected major risk factors and global and regional burden of disease. Lancet (London, England). 2002;360(9343):1347–60.

23. Commission TJ. Sentinel Event Alert Issue 52: Preventing infection from the misuse of vials. 2014;(52).

24. Dolan SA, Felizardo G, Barnes S, Cox TR, Patrick M, Ward KS, et al. APIC position paper: Safe injection, infusion, and medication vial practices in health care. Am J Infect Control. 2010;38(3):167–72.

25. Highsmith AK, Greenhood GP, Allen JR. Growth of nosocomial pathogens in multiple-dose parenteral medication vials. J Clin Microbiol. 1982;15(6):1024–8.

26. Bothe J. Study shows contamination in multiple-dose vials. AORN J. 1973;17(5).

27. Rosenzweig Al. Potential Health Hazards In The Multiple-Dose Vial. Hospitals (Lond). 1964 Jun;38:71–4.

28. Tamrat L, Gelaw Y, Beyene G, Gize A. Microbial Contamination and Antimicrobial Resistance in Use of Ophthalmic Solutions at the Department of Ophthalmology, Jimma University Specialized Hospital, Southwest Ethiopia. Can J Infect Dis Med Microbiol. 2019;2019:6–8.

29. Tsegaw A, Tsegaw A, Abula T, Assefa Y. Bacterial Contamination of Multi-dose Eye Drops at Ophthalmology Department, University of Gondar, Northwest Ethiopia. Middle East Afr J Ophthalmol. 2017;24(2):81–6.

30. Rahman MQ, Tejwani D, Wilson JA, Butcher I, Ramaesh K. Microbial contamination of preservative free eye drops in multiple application containers. Br J Ophthalmol. 2006;90(2):139–41.

31. Mackel DC, Maki DG, Anderson RL, Rhame FS, Bennett J V. Nationwide epidemic of septicemia caused by contaminated intravenous products: mechanisms of intrinsic contamination. J Clin Microbiol. 1975;2(6):486–97.

32. Maki DG, Anderson RL, Shulman JA. In-use contamination of intravenous infusion fluid. Appl Microbiol. 1974;28(5):778–84.

33. Radcliffe R, Meites E, Briscoe J, Gupta R, Fosheim G, McAllister SK, et al. Severe methicillin-susceptible Staphylococcus aureus infections associated with epidural injections at an outpatient pain clinic. Am J Infect Control. 2012;40(2):144–9.

34. Wong MR, Del Rosso P, Heine L, Volpe V, Lee L, Kornblum J, et al. An outbreak of Klebsiella pneumoniae and Enterobacter aerogenes bacteremia after interventional pain management procedures, New York City, 2008. Reg Anesth Pain Med. 2010;35(6):496–9.

35. Mattner F, Gastmeier P. Bacterial contamination of multiple-dose vials: a prevalence study. Am J Infect Control. 2004;32(1):12–6.

36. Henry B, Plante-Jenkins C, Ostrowska K. An outbreak of Serratia marcescens associated with the anesthetic agent propofol. Am J Infect Control. 2001;29(5):312–5.

37. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015/09/07. 2015;109(7):309–18.

38. Cegielski JP, Tudor C, Volchenkov G V., Jensen PA. Antimicrobial drug resistance and infection prevention/control: lessons from tuberculosis. Int J Infect Control. 2021;1:1–12.

39. Byarugaba DK. A view on antimicrobial resistance in developing countries and responsible risk factors. Int J Antimicrob Agents. 2004;24(2):105–10.

40. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, et al. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infect Dis. 2005 Aug;5(8):481–93.

41. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in

developing countries: Causes and control strategies. Antimicrob Resist Infect Control. 2017;6(1):1–8.

42. Miller DC, Smith C. The Safe Use of Multi-Dose and Single-Dose Vials. Pain Medicine. 2019.

43. Centers for Disease Control and Prevention. Hand washing: Clean hands save lives.:2021.

44. World Heath Organization. Global Patient Safety Challenge 2005/2006; Clean Care is Safer; WHO Guidelines for Hand Hygiene in Health Care. 2005.

45. Nishimura Y, Hagiya H, Keitoku K, Koyama T, Otsuka F. Impact of the world hand hygiene and global hand washing days on public awareness between 2016 and 2020: Google trends analysis. Am J Infect Control. 2022: 1;50 (2):141-7.

46. Jemal S. Knowledge and Practices of Hand Washing among Health Professionals in Dubti Referral Hospital , Dubti , Afar , Northeast Ethiopia. 2018;2018:1–8.

47. Anderson JL, Warren CA, Perez E, Louis RI, Phillips S, Wheeler J, et al. Gender and ethnic differences in hand hygiene practices among college students. Am J Infect Control. 2008;36(5):361–8.

48. Gulilat K. Assessment of Knowledge, Attitude And Practice of Health Care Workers on Infection Prevention in Health Institution Bahir Dar City Administration. Sci J Public Heal. 2014;2(5):384.

49. Flores A. Appropriate glove use in the prevention of cross-infection. Nurs Stand. 2007;21(35):45–8.

50. Casewell M, Phillips I. Hands as route of transmission for Klebsiella species. Br Med J. 1977;2(6098):1315–7.

51. Saloojee H, Steenhoff A. The health professional's role in preventing nosocomial infections. Postgrad Med J. 2001;77(903):16–9.

52. Ehrenkranz NJ, Alfonso BC. Failure of bland soap handwash to prevent hand transfer of patient bacteria to urethral catheters. Infect Control Hosp Epidemiol. 1991;12(11):654–62.

53. Thompson BL, Dwyer DM, Ussery XT, Denman S, Vacek P, Schwartz B. Handwashing and glove use in a long-term-care facility. Infect Control Hosp Epidemiol. 1997;18(2):97–103.

54. Alemu B. Knowledge and Practices of Hand Washing and Glove Utiliz ation Among the Health Care Providers of Shenen Gibe Hospital, South West Ethiopia. Sci J Public Heal. 2015;3:391.

55. Patel J, Smith C, Spine Intervention Society's Patient Safety Committee. Swabbing Vial Tops. Pain Medicine. 2019 Aug 1;20(8):1633-4.

56. American Association of Nurse Anesthetists. Infection Prevention and Control Guidelines for Anesthesia Care. 2015;1–35.

57. Wilson WO. Infection control issue: Understanding and addressing the prevalence of unsafe injection practices in healthcare. AANA J. 2008;76(4):251–3.

58. Kossover-Smith RA, Coutts K, Hatfield KM, Cochran R, Akselrod H, Schaefer MK, et al. A survey of physician and nurse knowledge, attitudes, and practices around injection safety. Am J Infect Control. 2017;45(9):1018–23.

59. Ford K. Survey of Syringe and Needle Safety Amoung Student Registered Nurse Anesthetists: 2013;81(1):37–42.

60. Okwen MP, Ngem BY, Alomba FA, Capo M V, Reid SR, Ewang EC. Uncovering high rates of unsafe injection equipment reuse in rural Cameroon: validation of a survey instrument that probes for specific misconceptions. Harm Reduct J. 2011;8:4.

61. Andersen B. Medicine Storage and Preparation Room: Practice and Theory. In 2019. p. 871–80.

62. WHO. Model requirements for the storage and transport of time and temperature sensitive pharmaceutical products. 2010;:1–42.

63. Ziehr H. Annex 9: Model guidance for the storage and transport of time and temperature–sensitive pharmaceutical products. WHO Tech Rep Ser No961, 2011. 2011;(961):324–72.

64. Grissinger M. Physical Environments That Promote Safe Medication Use. 2012;37(7):377–8.

65. Merry AF, Shipp DH, Lowinger JS. The contribution of labelling to safe medication administration in anaesthetic practice. Best Pract Res Clin Anaesthesiol. 2011 Jun;25(2):145–59.

66. Food and Drug Administration. Selection of the Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use Guidance for Industry. Md. 2018;20993:301–796.

67. Nogler-Semenitz E, Lass-Flörl C, Nogler M, Speer G, Dierich MP. Bacterial contamination of solutions for parenteral administration for single- and multiple-dose vials after multiple use in the hospital. Wiener Medizinische Wochenschrift. 2007;157(15–16):398–401.

68. Australian Commission on Safety and Quality in Health Care. National Standard for User-applied Labelling of Injectable Medicines, Fluids and Lines. 2015.

69. Grissinger M. Preventing Magnesium Toxicity in Obstetrics. Vol. 34, Pharmacy and Therapeutics. 2009. p. 403.

70. Cheesbrough M. District Laboratory Practice in Tropical Countries [Internet]. 2nd ed. Cambridge: Cambridge University Press; 2005.

71. CLSI. Performance Standards for Antimicrobial Susceptibility Testing.31th ed. CLSI supplement M100S, PA. Clinical and Laboratory Standards Institute. 2021

72. Dis CI, Bhat V, Vira H. Quality Control Issues in Antibiotic Susceptibility Testing by Disc Diffusion Technique. Clin Infect Dis. 2018;2(1):1–2.

73. Melnyk PS, Shevchuk YM, Conly JM, Richardson CJ. Contamination study of multiple-dose vials. Ann Pharmacother. 1993;27(3):274–8.

74. Rehan HS, Chopra D, Sah RK, Chawla T, Agarwal A, Sharma GK. Injection practices of healthcare professionals in a Tertiary Care Hospital. J Infect Public Health. 2012 Apr;5(2):177–81.

ANNEX: I: Standard laboratory procedure

Culture media preparation

1. Read the label on a bottle of dehydrated agar media. It specifies the amount of dehydrated powder required to make 1 liter (1,000 ml) of medium. Carefully follow manufacturer instructions. The amount needed for 1/2 liter was calculated and weigh out by analytical balance.
2. 500 ml of distilled water was placed in an Erlenmeyer flask. The weighed, dehydrated agar powder was added and placing stirrer in to it.
3. The flask was set on tripod over hot plate by avoiding excessive heating
4. After the agar mixture is completely dissolved, the flask removed from the hot plate, the stir bar also removed prior to sterilization. It closed with the cap, and sterilized in the autoclave.
5. Sterilized agar media was cooled to 45 to 50^{o}C or recommended temperature before addition of heat labile supplements/additives (e.g., sheep blood).
6. After removing the plug or cap with the little finger of right hand and correct volume of media was dispense into Petri dishes with in Biosafety cabinet
   - 18-20 ml for 15x100 mm plates
   - 60-70 ml for 15x150 mm plates
7. After pouring media, leave lids ajar for 20 minutes to avoid excess moisture on surface of the agar. After approximately 20 minutes, place the lids back on.
8. Slant tubed media that used in biochemical test was prepared to provide a deep butt (2 to 3 cm) and a short slant.
9. The prepared media was labeled and placed at 35 -37°C incubators for 24 hours to ensure they are sterile (free of contaminating bacteria) before use.

B. Collection and processing of sample

The investigator collected sample of injection solution with special care to avoid contamination of the specimen. By using sterile needle and syringe the sample of medication vials was collected from different wards and intensive care units of Jimma medical center.

1. The basic sample information was labeled on prepared check list
2. The sample was inoculated into MacConkey and blood agar then incubated at 37 °C for 24 hr
3. Examination and report the culture; by looking for colony characteristics, hemolytic characteristics and perform gram reaction & biochemical test.
4. Drug susceptibility pattern of the isolated organism was determined

Gram stain procedures

1. Prepare a thin smear of the culture or specimen was observed.
2. Allow to air-dry and fix the smear.
3. Cover the fixed smear with crystal violet for 1 min.
4. Rinse with clean water and tip off all the water.
5. Cover the smear with Lugol's iodine for 1 min.
6. Wash off the iodine with clean water.
7. Add acetone-alcohol for 30 sec.
8. Wash the smear immediately with clean water.
9. Cover the smear with safranin for 1-2 minutes.
10. Rinse with clean water.
11. Wipe the back of the slide and place in a draining rack for the smear to air-dry.
12. Examine microscopically, first with the 40x objective and then with the oil immersion objective for white cells, bacteria and other structures.

13. Result interpretation

- Gram- positive bacteria ----------Dark purple

- Gram- negative bacteria ----------Pale to dark red.

D. Biochemical testing procedures

Identification of Gram-positive bacteria: Gram-positive cocci were identified based on their gram reaction, catalase and coagulase test results.

Catalase test: This test used to differentiate *staphylococci* (+ve) from *streptococci (*-ve)

Procedure

1. Pour 2-3 ml of 3% hydrogen peroxide to a slide
2. Using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution
3. Look for immediate bubbling
4. Interpretation: Active bubbling--positive test
   No release of bubbles-negative test

Coagulase test: This test is used to differentiate *S. aureus* from other *Staphylococcus* spp

Procedure

1. 2 ml of physiological saline was added on test tubes
2. Emulsify the test organism & add one drop of plasma to suspensions and mix gently.
3. Incubate at 35- 37 0c for 4 hours & look for clumping of the organism
4. Interpretation : Clumping ----------*S.aureus*
   No clumping ----------other staphylococcus species

Identification of Gram-negative bacteria: was based on their test result with a series of biochemical tests

Oxidase Test

A piece of oxidase strip was soaked with a few drops of oxidase reagent. A colony of the test organism was then smeared on the filter paper. When the organism is oxidase producing, the phenylenediamine in the reagent to a deep purple color

Indole test

Few colonies of culture was inoculated in to peptone water and incubated at 37°C for 24 hours. Few drops of kovac`s reagent was added and gently shake to mix well and then color change was observed. If the layer of indicator reagent turns to red within 1 minute, it is indole positive. If the layers of indicator reagent remain yellow it is indole negative.

Urease test

Urea agar was inoculated heavily over the entire surface of the slants in test tube. The cap was loosened and then incubated at 37°C for 3-12 hours. A urease positive culture was producing a pinkish red color in the medium. Urease negative organisms were not change the color of medium.

Triple Sugar Iron (TSI) Agar Slant

Using s sterile inoculating needle, stab the butt of the TSI slant twice then streak back and forth along the surface of the agar with the organism then incubate at 37°C for 24hr. If acid slant acid butt (yellow-yellow): glucose and/or lactose fermented. If alkaline slant-alkaline butt (red-red): glucose not fermented. The presence of black precipitate (butt) indicates hydrogen sulfide production, and the presence of splits or cracks with air bubble indicate gas production.

Citrate utilization test using simmon`s citrate agar

Simmon`s citrate slope was prepared in test tube as recommended by manufacturer. The slope was then stabbed and incubated at 37°C aerobically for 24 hr. Blue color indicates a positive reaction and if simmon`s citrate agar slope remained as green in color indicate negative reaction.

Motility test

Motility agar were prepared and inoculated with a straight inoculating needle making a single stab about 1-2 cm down into the medium. The motility was examined after it incubated at 37°C for 24 hr. Motility positive if there is diffuse growth away from the line of inoculation.

Lysine decarboxylase

Decarboxylation of lysine can be detected by culturing bacteria in a medium containing the desired amino acid, glucose and pH indicator bromcresol purple. The bacterium that was positive for decaboxylate lysine turn the medium purple.

Biochemical test for gram negative rods

Table 1: Identification flowchart of lactose positive rods

Group I	Lactose	Indole	Urea	Manitol	H_2S	Gas/glucose	Citrate	Motility	Lysine	Organisms	Additional
Lactose positive rods			+	+	+	+	+	+	-	Citrobacter	Urea(+)
				+			+	+	-	Enterobacter cloacae	
					-	+		-	+	Klebsiella pneumonae	Maonate(+)
							-/+	-	-/+	Kleb. Ozanae	
		-				-	-	-	-	Kleb.rhinosco[(1)] Shi.sonnei[(2)]	
					-		+	+	+	Serratia[(3)]	
			-			+	+	+	+	Ent.aerogens[(4)] or Hafnia[(5)]	
				+					-	Ent.cloacae[(6)] or citrobacter	
	+				+	+	+	+	-	Citrobactor	
									+	Arizona	Maonate(+)
			+	+	-	+	+	-	+	Klebs.oxytoca	
							+	+	-	Citrobacter diversus	
						+	-	+	+	E.coli	
		+						-	+	E.coli	
			-	+	-	-	-	-	-	E.coli(A-D)	
				-	-	-	-	-	-	Sh.dysentriae or E.coli(A-D)	

NB! [(1)] Ornithine (-), [(2)] Ornithine (+), [(3)] Gas variable week [(4)] Addenitol, inistol(+), [(5)] Addenitol,inistol(-) MR(-),VP(-) [(6)] MR(+),VP(-)

Table 2: Identification flowchart of lactose negative gram-negative rods

Group II	Lactose	Indole	Urea	Manitol	H_2S	Gas/glucose	Citrate	Motility	Lysine	Organism	Additional test
Lactose negative				+/-	-	+	+	+	-	Providencia rettreri	PAD(+)
					-	+	-	+	-	Morganella morganii	PAD(+)
			+	-	+	+	-/+	+	-	Proteus vulgaris	PAD(+)
					-	+	+	+	-	Providencia alcalifacients(A)	PAD(+)
		+				-	+	+	-	Provedencia stuartii	PAD(+)
				-			-	-	-	Shigella dysentriae	
					+	+	-	+	+	Edwardiesella	
			-				-	-	+	E.coli(A-D)	
	-			+	-	-			-	Shigella spp	
							+	+	-	Provedencia stuartii(B)	PAD(+)
				-	-	-	-	-	-	Shigella dysentriae	
			-			-	-	-	-	Shigella spp	
		-		+		+	-	+	-	Salmonella Group "A"	
						+	+	+	+	Salmonella or Arizona	
					+	-	-	+	+	Salmonella typhi	Vi(+)
			+	-	+	+	+/-	+	-	Proteus mirabilis	PAD(+)
Non-fermentative bacteria	-	-	**D**	+/-	-	-	+	+/-	+/-	Pseudomonas aerogenosa	Cat,Oxi(+)
	+/-	-	**-(s)**	-	-	+	+/-	-	-	Acinetobacter	Cat,Oxi(-)
	-	-	-	-	-	-	**d**	+		Alcaligenes spp	Oxi (+)
	d	-	**d**	+	-	+/**d**	+	+	+	Serratina mescosoes	Oxi (-)

NB! "+" 90% or above are positive, "-"90% or above are negative, "+/-" majority are positive, "-/+" majority are negative.

Antimicrobial sensitivity testing

Procedure

1. A suspension of the test organism was prepared by emulsifying several colonies of the organism in a small volume of normal saline.

2. The turbidity of suspension was matched with turbidity standard.

3. With a sterile swab sample was taken from the suspension (the swab squeezed against the side of the test tube to remove the excess fluid).

4. The inoculums were spread evenly over the Muller-Hinton agar plate with the swab.

5. Using a sterile forceps, the antimicrobial disc is place on the inoculated plate and incubated at 37°C for 24 hours.

7. The test was read after checking that the bacterial growth is neither heavy nor light. The radius of the inhibition zone is measure.

8. Interpretation of the test organism to each antibiotic interpreted as sensitive or resistance as per the standard.

Sensitive – zone of radius is wider or equal to the control

Resistance – no zone of inhibition.

Table 3: Zone diameter interpretive standards for both gram positive coccus and gram negative rods

Antimicrobial agent	**Disk content**	**Zone diameter nearest whole mm**		
		sensetive(s)	intermidiate (i)	resistant(r)
Amoxicillin clavulanic acid*(AMC)*	20/10µg	≥13	14-17	≤ 13
Ceftriaxone(*CRO)*	30 µg	≥19	20-22	≤19
Cefoxitin (FOX)	30 µg	≥25	-	≤ 21
Gentamicin*(CN)*	10 µg	≥15	13-14	≤12
Ciprofloxacin*(CIP)*	5 µg	≥21	16-20	≤15
Trimethoprim-sulphamethoxazole*(SXT)*	1.25/23.75 µg	≥16	11-15	≤10
Erythromycin*(E)*	15 µg	≥23	14-22	≤13
Clindamycin (CC)	2 µg	≥19	16-18	≤15
Vancomycin (VA)	30 µg	≥ 17	-	-
Ampicillin*(AML)*	10 µg	≥17	14-16	≤13
Penicillin G,(*PG)*	10 unit	≥29		≤ 28
Chloramphenicol (CAF)	30 µg	≥18	13-17	≤12
Tobramycin (TOB)	10 µg	≥15	13-14	≤12
Amikacin (AK)	30 µg	≥17	15-16	≤14
Ceftazidime(CAZ)	30 µg	≥21	18-20	≤ 17
Cefepime (CEP)	30 µg	≥25	19-24	≤18
Cefuroxime(CXM)	30 µg	≥18	15-17	≤14
Cefotaxime (CFT)	30 µg	≥ 26	23-25	≤22

ANNEX: II: Information sheet

Name of principal investigator: Abay Tabor Bejiga

Name of the organization: Jimma University

Introduction

The information sheet is prepared by the principal investigator from Jimma University, school of medical laboratory science whose aim is to assess Bacterial contamination of single- and multiple-dose parenteral injection vials after opening and antibiotic susceptibility of isolates at Jimma Medical Center, Jimma, and South West Ethiopia.

Purpose: The purpose of this research is to assess bacterial contamination of single- and multiple-dose parenteral injection vials after opening and antibiotic susceptibility of isolates at Jimma Medical Center, Jimma, and South West Ethiopia.

Procedure: after they are willing to let the vials 100 µl of the medication was withdrawn with a sterile 1 ml insulin syringe then the sample was processed in medical microbiology laboratory of Jimma University.

Risk and discomfort: In this study no potential harm or injury expected. Since I follow standard operational procedures during sample obtaining, there is no any risk for vials

Benefits: The finding is give information about bacteria profile and drug resistance pattern with associated factors. It also important to improve the prevention and control practice of during injection procedure in jimma medical center.

Incentives: you would not be provided any incentive taking part in this research

Confidentiality: the information that I collect for this research project was be kept confidential. I give them a secret code for your answer and nobody would be given the results in a way that identify you. And also, the results kept private according to the law. The information I get will be written in published studies without your personal

Right to refuse or withdraw

You have full right to refuse from participating in this research if you do not wish to participate

Whom to contact:

If you have any questions contact to the principal investigator and you may ask at any time you want.

1. Abay Tabor Bejiga (BSC)-Jimma University, institute of Health Science, School of Medical Laboratory Sciences, Jimma, Ethiopia +251913128277 /abibiostaff@gmail.com
2. Name of main advisors: Mr. Zewudineh S/Mariam *(*MSc, Assistant Professor) Address: Jimma University +251913173050/ zedsweat@gmail.com
3. Name of Co-advisor: Mr. Yared Alemu (Msc) Address: Jimma University +251912303918/yared.alemu6@gmail.com

የተመራማሪ ስም- አባይ ታቦር ቤጅጋ
የተቋሙ ስም- ጅማ ዩኒቨርሲቲ

መግቢያ

የመረጃ ወረቀቱ የተዘጋጀው በጅማ ዩኒቨርሲቲ የሕክምና ቤተ ሙከራ ሳይንስ ትምህርት ክፍል ዋና ተመራማሪ ሲሆን ዓላማውም የባክቴሪያ ብክለትን እና የፀረ ተህዋሲያን ተጋላጭነት ሁኔታን ባለአንድ እና ባለብዙ መጠን ብልቃጦች ከተከፈተ በኋላ የባክቴሪያ ብክለትን መገምገም ነው። በጅማ የሕክምና ማዕከል በደቡብ ምዕራብ ኢትዮጵያ

ዓላማ

የዚህ ጥናት አላማ በደቡብ ምዕራብ ኢትዮጵያ በጅማ ህክምና ማዕከል የባክቴሪያ ብክለትን እና የፀረ ተህዋሲያን ተጋላጭነት ሁኔታን ባለአንድ እና ባለብዙ መጠን ብልቃጦች ከተከፈቱ በኋላ የባክቴሪያ ብክለትን መገምገም ነው።

ሂደቶች:-

ፍቃደኛ ከሆናችሁ ከመድሃኒቱ በጸዳ መርፌ እና ሲሪንጅ 100 µl ተወስዶ ከዚያም ናሙናው በጅማ ዩኒቨርሲቲ ሜዲካል ማይክሮባዮሎጂ ላብራቶሪ ውስጥ ይመረመራል ።

አደጋ እና የማይመቹ ነገሮች: በዚህ ጥናት ውስጥ ምንም ዓይነት ጉዳት አይጠበቅም። ናሙና በማግኘት ጊዜ መደበኛ አሠራር ህደቶችን ስለምከተል ፣ ለ ቀሪ መድሃኒቶች ምንም ሥጋት የለውም ።

የጥናቱ ጥቅም:

ግኝቱ ስለ ተህዋሲያን ባክቴሪያ መገለጫ እና የመድኃኒት መቋቋም ዘይቤን ከተጓዳኝ ምክንያቶች ጋር መረጃ ይሰጣል ። በተጨማሪም በጅማ የሕክምና ማዕከል ውስጥ በመርፌ የሚሰጡ መድሃኒቶችን አያያዝ ግንዛቤ ለማሻሻል ይጠቅማል።

ማበረታቻዎች- በዚህ ምርምር ውስጥ ለተሳትፎዎ ምንም ክፍያ የለም

ሚስጥራዊነት- ለዚህ ምርምር የሚሰበስበው መረጃ በሚስጥር ይቀመጣል ። የእርስዎ መልስ የምሥጢር ኮድ የሰጠዋል እናም ማንነታችሁን በሚለይበት መንገድ ውጤቱን ለማንም አይሰጥም ። ደግሞም ውጤቶቹ በሕጉ መሠረት የግል ሆነው ይቀመጣሉ።

ያለመሳተፍ መብት : መሳተፍ ካልፈለጉ በዚህ ምርምር ውስጥ ለመሳተፍ እምቢ ማለት ሙሉ መብት አለዎት ። ማንኛውም ጥያቄ ካለዎት ዋና ተመራማሪውን ያነጋግሩ እና በማንኛውም ጊዜ በፈለጉት ጊዜ መጠየቅ ይችላሉ።

ማንን ማነጋገር አለቦት: .

1) አባይ ታቦር ቤጅጋ (BSC) -ጅማ ዩኒቨርሲቲ የጤና ሳይንስ ተቋም ፣ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ቤት ፣ ጅማ+251913128277 /abibiostaff@gmail.com
2) የዋና አማካሪ ስም- አቶ ዘውዱነህ ስ / ማሪያም (MSc ፣ ረዳት ፕሮፌሰር) አድራሻ-ጅማ ዩኒቨርሲቲ +251913173050/ zedsweat@gmail.com
3) የተባባሪ አማካሪ ስም-አቶ ያሬድ አለሙ (MSc) አድራሻ-ጅማ ዩኒቨርሲቲ +251912303918/ yared.alemu6@gmail.com

Information sheet consent form (Afaan oromo)

Maqaa guutuu nama qorannocha : **Abbaay Taaboor Bajiiga**

Maqaa dhaabbaticha: **Yuuniiveersiitii Jimmaa**

Seensa

Waraqaa oddeeffannoo qorataan yuuniiveersiitii jimmaa kutaa barnoota saayiinsii laaboraatoorii mediikaala kaayyoon isaa " To assess Bacterial Contamination of Single- and Multiple-dose Parenteral Injection Vials After Opening and Antibiotic Susceptibility of Isolates at Jimma Medical Center, Jimma, South-West Ethiopia.

Kaayyoo

Kaayyoon qorannichaa faalamuu qorichoota lilmeen kennaman abba doozii tokkoo fi doozii baayee erga bilqaaxxiin isaanii banamee fi saaxilamummaa dhukkubaa sababee kanaan walqabatee dhaabbata fayyaa yuuniiveersiitii jimma keessatti uumamu qorachuudha.

Adeemsa duraaa fi duubee (prooseejerii)

Yoo bilqaaxxiiwaan kana irraa saamuuda akkan fudhadhuuf eeyyamamaa taatan, gaafii fi deebii akkasuumas saamuudawaan fudhachuufani.

Faayidaa qoronnichaarraa argamu

Firiin qorannoo kana irraa argamu ogeessota hojjii kana hojjetanuuf sadarkaa rakkoo kanaa akka hubataanuuf duubdeebii gaarii ta'uu danda'aa. Dhibamaaf immoo gara fuulduraatti yaalii faalama kana irraa mudachuu danda'urraaa hanbisuu danda'a.

Kaffallti

Qoranno kana irratti hirmaachuun hirmaataatiif kaffaltiin kaffalamu hinjiru

Iccitii

Iccitiin ykn odeeffannoon dhuunfaa sababa kanaan funaanam qorannoo kana keessatti iccitumaan isaa kan eegame waan ta'eef soda qaabachuu hinqabdu

Mirga hirmaataa

Hirmaataan hirmaachuuf hirmaachuu dhisuu, odeeffannoo fi saamuuda kennuu gidduuti dhaabuuf mirga guutu qaba. Qorannoo kana irrattii hirmaachuu dhiisuu isaatiin tajaajilli argachuu qabu kamillee sababa kanaa hafuu hin danda'u.

Dhimma kanaan woliqabatee odeeffannoo

Yoo odeeffannoo ykn gaafi dhimma kanaan waliqabatu qabaattan teessoowaan armaan gadii fayyadamuu dandeessuu

1. **Maqaa qorataa** : Aaddee Abbaay Taaboor Bajiga Bilibila qorataa +251913128277 /abibiostaff@gmail.com Yuuniversiitii Jimma
2. Yookin Obbo Zawudineh S/Maariyaam *(*MSc, Assistant Professor) teesso: Jimma University, +251913173050/ zedsweat@gmail.com
3. Obbo Yaared Alamuu (Msc) teesso: Jimma University +251912303918/ yared.alemu6@gmail.comANNEX: III: Questionnaire

Appendix III: Questionnaire

Dear respondents,

First of all, I would like to thank you for your cooperation to respond to this questionnaire. This questionnaire is designed to collect data for my M.Sc. thesis "Bacterial Contamination of Single- and Multiple-dose Parenteral Injection Vials after Opening and Antibiotic Susceptibility of Isolates at Jimma Medical Center, Jimma, and South-West Ethiopia". Thus, I kindly request you to give the required information. Your response is highly important for the success of this study. I would like to assure you that all the responses you give will be kept confidential and used only for the research purpose. As a further assurance, you don't need to write your name. Thank you in advance for your genuine cooperation.

Part I: Socio- Demographic characteristic of JMC nursing staff at Jimma, Southwest, Ethiopia.

1. Name of Wards you currently working

Medical ward	☐	Ophthalmology ward	☐
Surgical ward	☐	Maternity ward	☐
Pediatrics ward	☐	Gynecology ward	☐
Psychiatry ward	☐	Neonatology ward	☐
Oncology ward	☐	Maxillofacial ward	☐
Orthopedics wards	☐	Medical ICU	☐
Pediatric ICU	☐	Adult ICU	☐

2. How long have you worked in JMC

----------month

----------year

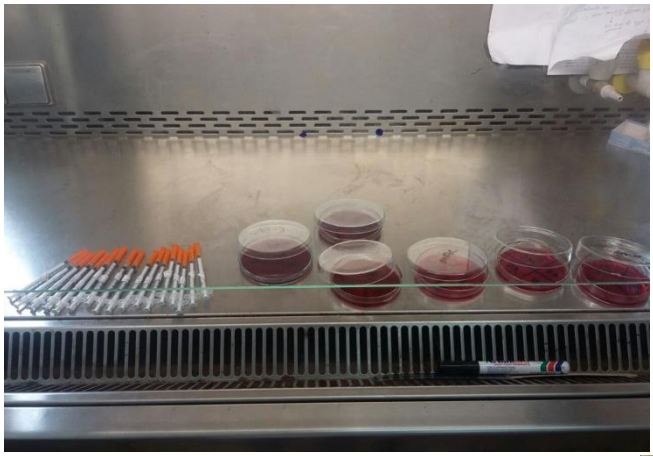
Part II: The associated risk factor for vial contamination in JMC at Jimma, Southwest, Ethiopia.

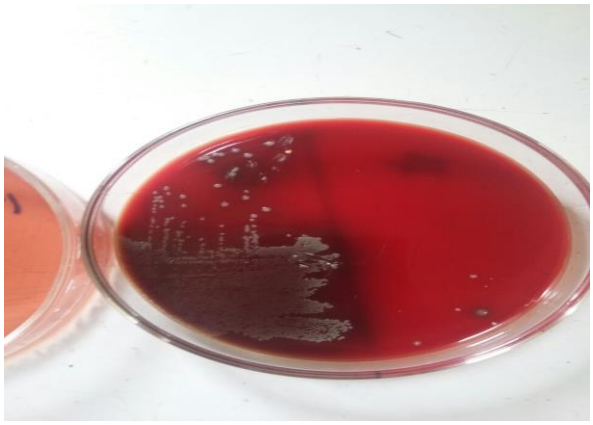
1. Do you use single vial for a single patient?
   YES ☐ NO ☐
2. Do you re-use syringe and/or needle for injection?
   YES ☐ NO ☐
3. Do you wash your hands properly before handling medication vials?
   YES ☐ NO ☐
4. Do you disinfect top of vial with alcohol before use?
   YES ☐ NO ☐
5. Do you draw a medication in a clear medication preparation area?
   YES ☐ NO ☐
6. Do you check expiry date before use?
   YES ☐ NO ☐
7. Do you use a new glove before every injection?
   YES ☐ NO ☐
8. Do you check opening date of vials if the vial was previously used**?**
   YES ☐ NO ☐
9. Do you store the vials according to the order of manufacturer?
   YES ☐ NO ☐
10. Do you think the vials and saline bag can be contaminated?
    YES ☐ NO ☐

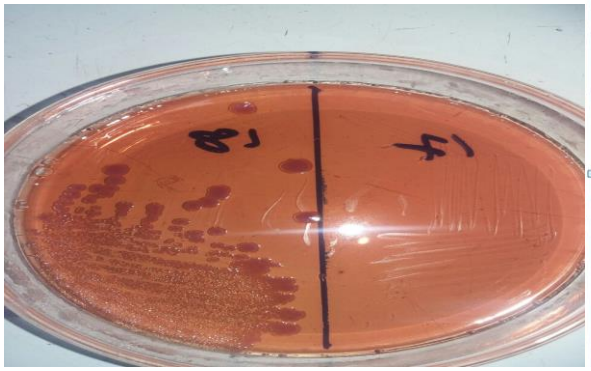
Appendix IV: Data collection Sheet

Sample number	
Date and time sample collected	
Name of medication	
Name of Wards Medical Ophthalmology Surgical Neonatology Pediatric Oncology Psychiatry Maxillofacial Orthopedics Maternity gynecology ward Medical ICU Adult ICU Pedi ICU	
Type of dose	
Preservative status	
Date and time of opening or preparation	
Opening/preparation date of vials	
Storage condition	
Expiration date	

ANNEX: V Photographs







The pictures which are listed above shows the main laboratory activities starting from ready samples for inoculation, overnight growth of bacteria on Blood and Mac media, Biochemical test reading and AST test result of two samples respectively.

ANNEX: VI: Declaration

I, the undersigned graduate student, hereby declare that this thesis is my original work, and it has not been presented for a degree in any other university for academic credit and that all sources of the materials used for this thesis have been duly acknowledged.

Name: Abay Tabor Signature _____ Date _____

Confirmation and Approval

The thesis on the title "**Bacterial contamination of single- and multiple-dose parenteral injection vials after opening and antibiotic susceptibility of isolates at Jimma Medical Center, Jimma, South-West Ethiopia",** Submitted to Jimma University for the award of the Degree of Master of science in medical microbiology is approved as the original work of Abay Tabor and all the sources were properly acknowledged.

Principal advisor: Zewudineh S/Mariam (MSc. Ass. Professor) Signature _____ Date_____

Co-advisor: Yared Alemu (BSc, MSc): Signature _____ Date_____

Thesis Evaluators

External Examiner _____Signature _____ Date_____

Internal Examiner _____Signature _____Date_____

Chairperson _____Signature _____ Date_____