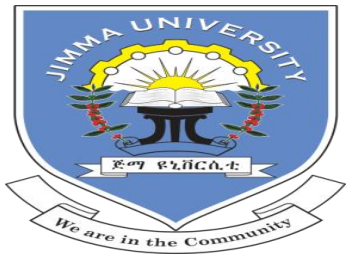

Jimma University

College of Natural Sciences

School of Post Graduate Studies

Department of Biology

Characterization of microbes associated with biomedical wastes of Jimma University Specialized Hospital, Southwest Ethiopia

By: Yasin Oljira

A Thesis Submitted to Department of Biology, College of Natural Sciences, Jimma University in partial fulfillment of the requirement for the Degree of Master of Science in Biology (Applied Microbiology)

February, 2020

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Principal advisor: Delelegn Woyessa (MSc, Assoc. Prof.)

Co-advisor: Shiferaw Demissie (MSc.)

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Acronyms

BMWM=Biomedical Waste Management

BMWs=Biomedical Wastes

CDC=Center for Disease Control and Prevention

EPA=Environmental Protection Agency

ICU= Intensive Care Unit

JUSH=Jimma University Specialized Hospital

MDR=Multiple Drug Resistance

NCCLS= National Committee for Clinical Laboratory Standards

Abstract

*For sustainable maintenance of hospital hygiene, health care regular management of biomedical waste is very crucial. The aim of this study was to isolate and characterize microbes from biomedical wastes discharged at Jimma University Specialized Hospital. The studies involved both cross-sectional and laboratory based experimental analysis of microbial load and safety. A total of 80 samples (20 each of bandage, glove, lancet and liquid wastes) were used for enumeration (aerobic mesophilic bacteria, Enterobacteriaceae, coliform, Staphylococci, yeast and molds) as well as isolation and characterization of microbes. Selected pathogens were also evaluated for their antibiotic susceptibility patterns. Regarding to the types of wastes discharged from JUSH, 37.5 % were solid wastes according to professional's response. The results of microbial analysis indicated that, the mean microbial counts (CFU/cm^2) were dominated by aerobic mesophilic bacteria (*6.54±0.28*), Enterobacteriaceae (*6.20±0.78*), coliforms (*6.16±0.22*), staphylococcus sp (*6.13±0.21*) and molds (*6.13±0.15*). Out of the total 520 isolates characterized, Entrococcus sp. were the most dominant (29.23%) followed by Staphylococci sp. (17.69%) and Escherichia 62 (11.92 %) but the least was Shigella (2.31%). A total of 8 sample positive for Salmonella sp, 7 Klebsiella sp, 6 Staphylococcus sp, 6 for Pseudomonas sp. Out of the six 6 Staphylococcus sp, majorities, 5 of them were resistant to clindamycin and tetracycline. However, 5 of Staphylococcus sp were susceptible to ciprofloxacin, Streptomycin and chloramphenicol. On the other hand, out of six (6) Pseudomonas sp all of them (6), were resistant to ampicillin, but 5 of them were resistant to tetracycline. Out of eight (8) Salmonella sp, all of them resistant to ampicillin, 6 resistant to naldixic acid and tetracycline. The hygienic status of JUSH is actually better and the managements, professionals and sanitarians should handle biomedical wastes in appropriate manner.*

Keywords*: Antibiotics, Biomedical, Microbes, Pathogens, Wastes,*

1. Introduction

Various scholars define hospital and medical waste in different ways. According to the Environmental Protection Agency and Center for Disease Control and Prevention (CDC, 2003), hospital waste refers to all wastes, biological or non-biological, that are discarded and not intended for further use. Medical waste refers to materials generated as a result of patient diagnosis, treatment, or immunization of human beings or animals. Health care waste consists of both organic and inorganic substances that enhance the growth of pathogenic microorganisms. Furthermore, wastes which are produced from health centers and health-care providers are considered as biomedical wastes (Windfeld and Brooks, 2015).

According to world health organization (WHO, 2012), wastes produced by the health-care providers are broadly categorized as general (non-hazardous) and hazardous waste. General waste constitutes about 85% of the total waste produced in the health care facilities and it is comparable to domestic waste (Hossain *et al*., 2011). This type of waste does not pose any risk to human being. The remaining 15% is, however, considered as hazardous which may pose a variety of environmental and health risks. Among this, about 10% is considered as infectious (waste generated from laboratory and washing, cleaning, house-keeping and disinfecting activities, blood and body fluids) with pathogens of humans (Chartier *et al*., 2014).

According to report of Moges *et al.* (2014) from Gondar University hospital, pathogens such as *Klebsiella spp.* 30 (26.5%) , *Pseudomonas spp*. 19 (16.8%), *Escherichia coli* (11.5%) and *Citrobacter spp* (11.5%), *Staphylococcus aureus* (8.2%), *Shigella dysentery* (2.18%) were isolated from hospital biomedical wastes. Similar study in Brazil reported that, the most common multi-resistant extended spectrum beta-lactamase producing isolates from hospital wastewater were *Klebsiella pneumoniae*, *Enterobacter cloacae* and *E. coli* (Chagas *et al*., 2011). Study conducted in Australia indicates that certain strain of *Staphylococcu*s *aureus* and *E. coli* can survive the path of treatment process until the inlet, including chlorination (USEPA, 2007).

The same study conducted in Belgium showed that, microbial contamination of biomedical waste (total ciliform, faecal coliform and *Escherichia coli*) exceeds WHO standard levels; *Shigella* spp and *Salmonella* spp were also isolated from biomedical waste samples (Chitnis *et al*.,2004). Hospital wastewater reveals bacterial counts that ranged between $1x10^2$ CFU to $1x10^8$

CFU/100 ml for coliforms; 1 to $4.8x10^5$ CFU/100 ml for *E. coli* and 4.4 to $1.5x10^6$ CFU/100 ml for Enterococci. The proportion of enteric group varied from 58% to 75% of the total bacteria (Keen and Patrick, 2013).

According to Moore *et al*.,(2010) it was found that $1.4x10^6$, $3.6x10^5$,$1.6x10^5$ and $5.5x10^4$ cfu/g (dry weight of sludge) for total coliforms, faecal coliforms, faecal streptococci and *Salmonella* spp, respectively. *Salmonella* species were detected in 37% of sludge from hospital wastewaters. Hospital wastewater enterococci count of 10^5 cfu per 100 ml was identified (Mulamattathil *et al*., 2000). People of developing countries often bear antibiotic resistant organisms (Calva *et al*., 1996). The majority of antibiotics used is only partially metabolized after administration, and are released via patient excreta into the municipal sewage system. Antibiotics used in hospitals and private households and released into effluent and municipal sewage indicates a selection pressure on bacteria (Khachatourians, 1998). Biomedical wastes and their microbial load are slightly neglected in our country. Even though there is specialized clinical settings, there is still hospital acquired infection in almost all Ethiopian hospitals.

Study conducted in India showed that, the presence of multiple drug resistance (MDR) bacteria in hospital samples ranged from 0.26% to 40%, which is alarmingly high to pose a serious problem to the communities. Simultaneous resistance for ampicillin, amoxicillin, piperacillin, second and third generation cephalosporin, cotrimoxazole, gentamycin, netilmycin and quinolones formed the common MDR pattern (Anitha and Jayraaj, 2012).

Long term exposure of microorganisms to low concentrations of antibiotics in wastewater and surface water has the potential for the development of antibiotic resistance (Smith *et al*., 1998). The pattern of resistance was almost the same for *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Pseudomonas* and strongly suggests prevalence of similar R-plasmids (Omar *et al*., 2014). Study conducted in Ethiopia, Gondar, also showed multiple drug resistance bacteria to the commonly used antibiotics are high in the hospital biomedical wastes (Goldstein *et al*., 2012).

If the hospital effluents are not treated, concentrated forms of infectious agents and antibiotic resistant microbes are shed into communities resulting in various infectious diseases (Sharma *et al*.,2010). The health sectors' role in the risk associated with communal disease epidemics, which are a direct result of infectious and hazardous waste from medical

facilities, is a pressing concern globally. The aim of the current study was to isolate and characterize microbes from biomedical wastes (BMW) in Jimma University Specialized Hospital (JUSH).

1.1. Statement of the Problem

Biomedical wastes have become an emerging problem worldwide and their management is still at infancy and gets attention near past due to increased awareness of nosocomial infection (Chakraborty *et al*., 2014). According to WHO (2005), 10-25% of BMWs produced by healthcare providers is hazardous with proportions varied from country to country ranging between 20% and 75% (Chartier *et al*.,2014).

BMWs generation rate in Ethiopia is unacceptably higher compared to some other countries and threshold set by WHO (Hayleeyesus and Cherinete, 2016). In Africa, like Libya BMWM is still in its immaturity stage and characterized by the lack of awareness on the impacts and could transmit more than 30 dangerous blood borne pathogens, with particular concern for infectious disease, for which there is strong evidence of transmission through biomedical wastes due to poor waste management (Sawalem *et al*., 2009). Moreover, BMWs are posing problems in most developing countries due to lack of awareness and trained clinical staffs in waste management framework and disposal of the hospital and other health care establishments that become an increasing issue of concern (Mathur *et al*., 2012).

Despite the risk it imposes in Ethiopia, it is a neglected activity by health service providers, due to lack of attention and deserves credible value by health institutions (Azage and Kumie, 2010). Like other hospitals JUSH generated a lot of solid and liquid wastes. There was no systematic research to assess biomedical waste management and microbes associated with it. Thus, the present study aimed to characterize microbes associated with biomedical wastes at JUSH.

1.2. Research Questions

- What are microbes associated with biomedical wastes in JUSH?
- Which pathogens dominated biomedical wastes in JUSH?

1.3. Objectives

1.3.1. General Objective

The general objective of this study was to characterize microbes associated with biomedical wastes in Jimma University Specialized Hospital, Southwest Ethiopia

1.3.2. Specific Objectives

The specific objectives were to:

- Evaluate practice towards BMWM among health care worker of JUSH
- Determine microbial load of biomedical wastes of JUSH
- Identify potential human pathogenic microbes
- Determine antibiotic susceptibility of pathogens isolated from BMWs of JUSH

1.4. Significance of the study

The result of this study showed the general characteristics of pathogenic microbes isolated from biomedical wastes. In addition to this it also indicates potential human pathogenic bacteria and fungi as well as antibiotic susceptibility of bacteria isolated from biomedical wastes. The findings from the study benefit clients, health care worker, health care facility managers, researchers, policy makers and other stakeholders by providing information for health care facilities to identify level of knowledge, attitude and practice among health care workers and factors contributing to noncompliance with waste management guidelines. So that the health care facilities could design targeted interventions to ensure safety of the patients it serves, its staffs and other clients. The study also provides information for policy makers and stakeholders about existing situations of biomedical waste management to plan measures to mitigate improper waste management. The study may identify gaps for researchers who would like to conduct detailed and comprehensive studies either in public or private health institutions.

2. Literature Review

2.1.1. Biomedical Wastes

Various scholars define hospital and medical waste in different ways. According to the Environmental Protection Agency (EPA) and Center for Disease Control and Prevention (CDC, 2003), hospital waste refers to all waste, biological or non- biological, that is discarded and not intended for further use. Medical waste refers to materials generated as a result of patient diagnosis, treatment, or immunization of human beings or animals. Biomedical waste management has recently emerged as an issue of major concern not only to hospitals, nursing home authorities but also to the environment. The bio-medical wastes generated from health care units depend upon a number of factors such as waste management methods, type of health care units, occupancy of healthcare units, specialization of healthcare units, ratio of reusable items in use, availability of infrastructure and resources (Mandal and Dutta, 2009).

The proper management of biomedical waste has become a worldwide humanitarian topic today. Although hazards of poor management of biomedical waste have aroused the concern world over, especially in the light of its far-reaching effects on human, health and the environment (Singh *et al*.,2007). Now it is a well-established fact that there are many adverse and harmful effects to the environment including human beings which are caused by the "Hospital waste" generated during the patient care. Hospital waste is a potential health hazard to the health care workers, public and flora and fauna of the area. The problems of the waste disposal in the hospitals and other health-care institutions have become issues with increasing concern (Chandra, 2009)

2.2. Categories of Biomedical waste

Biomedical wastes can be categorized based on their origin and physical, chemical or biological characteristics. According to Singh *et al*. (2014) review report, biomedical wastes may include wastes from human anatomical, animal, microbiological, biotechnological, sharps, discarded medicines, chemical, incineration ash, solid and liquid wastes. Each of these wastes has its own components, method of treatment and disposal (Table 1).

Table 1 Categories of biomedical wastes

Types of Wastes	Components	Method of treatment	References
Human	Human tissues, organs, body parts	Incineration or deep burial	Singh *et al*., 2014
Animal	All types of Animal tissues, organs, body parts and bleeding parts	Incineration or deep burial	Singh *et al*., 2014
Laboratory	Wastes from laboratory cultures, stocks or specimens of microorganisms	Local autoclaving or micro waving or incineration	Tudor *et al*.,2005
Sharps	Needles, syringes, scalpels and blades	Disinfections and chemical treatment	Saurabh and Ram, 2006
Discarded medicines	Outdated, contaminated and discarded medicines	Incineration or Destruction and disposal in landfills	Singh *et al*., 2014
Solid	Blood contaminated cotton, dressings, tubing's, catheters and intravenous sets	Incineration, autoclaving and chemical treatment	Hien *et al*.,2012
Liquid	Waste generated from laboratory and washing, cleaning, house-keeping and disinfecting activities	Disinfections by chemical treatment and discharge into drains	Saurabh and Ram, 2006
Incineration Ash	Ash from incineration of any biomedical waste	Disposal in municipal landfill	Ahmed *et al*.,2014
Chemical	Chemicals used in production of biological	Chemical treatment and discharges into drains	Hien *et al*.,2012

2.3. Microorganisms Associated with biomedical Wastes

The following groups of persons are at the risk of health care waste medical staff: doctors, nurses, and sanitary staff and hospital maintenance personnel; in and out-patients receiving treatment in healthcare facilities as well as their visitors. Workers in support services linked to healthcare facilities such as laundries, waste handling and transportation services; Workers in waste disposal facilities and the general public (Khan *et al*.,2017).

A number of (opportunistic) pathogenic bacteria, including *Pseudomonas spp., Lactobacillus spp., Staphylococcus spp., Micrococcus spp., Kocuria spp., Brevibacillus spp., Microbacterium oxydans,* and *Propionibacterium acnes*, were identified and reported from the various medical wastes. Commonly identified bacterial and viral pathogens such as *Pseudomonas spp., Corynebacterium diphtheriae, Escherichia coli, Staphylococcus spp.,* and respiratory synclinal virus have been reported to be part of the medical wastes. Medical waste should be carefully controlled and monitored to prevent nosocomial infection associated with the exposure to these wastes (Nascimento *et al*., 2009).

Nascimento *et al*.,(2009) reported that aliquots of leachate from health care waste in Brazil contained pathogenic strains of Staphylococcus spp., Gram-negative rods of the *Enterobacteriaceae* family and non fermenters. Bacterial resistance to all the antimicrobials tested was observed in all microbial groups, including resistance to more than one drug. This makes it possible to suggest that viable bacteria in health service waste represent risks to human and animal health.

Furthermore, occurrences of multi-resistant strains support the hypothesis that health service waste acts as a reservoir for resistance markers, with an environmental impact (Susan,1993). The lack of regional legislation concerning segregation, treatment and final disposal of waste may expose different populations to risks of transmission of infectious diseases associated with multi-resistant microorganisms (Kumar *et al*.,2015). The distribution of fungi in the hospitals wastes are coming from the clinical wastes specimens used for the diagnostic process. Healthcare wastes are general terms used to define the wastes generated from healthcare facilities. These wastes contain blood or human body fluids as well as heavily infectious loads (WHO, 2005).

The presence of fungi in the clinical wastes are related to the high contents of organic matter as well as pH which support the fungal growth. Among several fungal species isolated from the clinical wastes are *Fusarium sp., Mucor sp., Scopulariopsis sp., Paecilomyces sp., Aspergillus spp. Cladosporium spp., Penicillium spp., Basipetospora sp., Curvularia sp. Aureobasidium sp., Scytalidium sp. and Alternaria sp. Acremonium spp. and Alterneria spp* (Neely *et al*., 2001). *A. fumigatus, A. niger, T. harzianum* and *P. chrysosporium* were the most common (Noman *et al*., 2016). Generally microbes such as bacteria, virus, fungi and parasites the main pathogens isolated from BMWs (Table 2).

Table 2 Pathogens associated with BMWs

Microbial group	Type of disease caused	References
Bacterial	Tetanus, gas gangrene and other wound infection, anthrax, cholera, other diarrhea diseases, enteric fever, shigellosis and plague	Nyamogoba and Obala, 2002
Viral	Various Hepatitis, Poliomyelitis, HIV-infections, HBV, TB, STD and rabies	Ziebuhr *et al*., 2006
Parasitic	Amoebiasis, Giardiasis, Ascariasis, Ancylomastomiasis, Taeniasis, Echinococcosis, Malaria, Leishmaniasis and Filariasis	Hagen *et al*.,2001
Fungal	Various fungal infections like Candidiasis, Cryptococcoses and Coccidiodomycosis	Khan *et al*.,2017

2.4. Antibiotic Resistant Bacteria in Hospital Biomedical Wastes

Water is considered a vehicle for the propagation and dissemination of human associated bacteria. Safe drinking water is a fundamental human right and if contaminated with opportunistic pathogenic environmental bacteria, it may have health implications for consumers. Wastewater is referred to any water, whose quality has been adversely being abused by anthropogenic influence (Yadav *et al*., 2002).

This includes liquid waste discharged from domestic home, agricultural commercial sectors, pharmaceutical and hospital. Hospitals are an essential asset of any society, and waste production is inevitable outcome of service delivery. In hospitals water consumed by various parts such as hospitalization, surgery rooms, laboratories, administrative units, laundry, health services, kitchen and in the process its physical, chemical and biological quality decreased and converted to wastewater (Anitha, 2012).

Health care waste consists of solid, liquid and gaseous waste contaminated with organic and inorganic substance including pathogenic microorganisms, radiological chemicals, partially metabolized antibiotics which are usually generated from laboratory analysis of tissues and body fluids as well as excreted from patients (Nuñez & Moretton, 2007).The various sources of liquid waste in the hospital include outdoor and indoor departments, operation theatres, laboratories of microbiology, biochemistry, histopathology, blood bank, radiology and others. The major concern is the disposal of infectious wastes such as cultures and stocks of infectious agents, wastes from infected patients, wastes contaminated with blood and its derivatives, discarded diagnostic samples, contaminated materials (swabs, bandages) and equipment or disposable medical devices (Yadav *et al*.,2002).

The untreated hospital waste possess serious health hazards to the health care workers, public and air flora on the area source of pharmaceutical products in the environment are more than just consumers expelling unabsorbed medications through excretion into septic system and waste water treatment plants (Mesdaghinia *et al*.,2012). The basic principle of underlying wastewater management is the strict limit on the discharge of hazardous liquids into sewers without prior treatment so that living pathogenic organisms are not introduced into the environment (Manyele, 2004). Connection of hospital waste to the municipal sewage network may create problems such as public health risks and imbalance of the microbial community in the sewage systems, which in turn affect the biological treatment process and (Nemerow, 1978). It is very necessary to understand sources of waste that contribute pollutant to the individual waste streams and the shortcomings that will be encountered in an attempt to treat the waste. Low concentrations of antibiotics in the environment may select for resistant bacteria. These resistant bacteria from environments may be transmitted to humans, in whom they cause disease that cannot be treated by conventional antibiotics (Kummerer and Henninger,2003).

Waste effluent from hospitals and clinics contain high numbers of resistant bacterial strains and residual antibiotics at a concentration to which household waste quantitatively and qualitatively and found that general hospital waste contains bacteria with pathogenic potentials for humans compared to household waste (Schwartz *et al*.,2003). A variety of substances such as pharmaceuticals, radionuclide, antiseptics, disinfectants and solvents are used in hospitals for treatment, medical diagnostics, disinfection and research.

After application many non-metabolized drugs excreted from patients and residual chemicals enter into wastewater which finally interacts with micro flora of Hospital sewage (Sunday and Agbaji, 2012). These micro floras are composed by saprophytic bacteria from the atmosphere, soil, medical devices and water worked in the hospital practice; the pathogens are mainly released with the patient excreta (Pauwels and Verstraete, 2006). These bacteria that survive in Hospital wastewaters may be exposed to a wide range of biocides that could act as a selective pressure for the development of resistance. Due to heavy antibiotic use, hospital wastewater contains larger numbers of resistant organisms than domestic wastewater (Suma *et al*., 2014).

When the antimicrobial agents attack disease-causing bacteria, they also affect non-pathogenic bacteria in their course, thus they exterminate these bacteria and make room for more resistant bacterial growth (Nuñez & Moretton, 2007). It is clear that microorganisms can adapt to a variety of environmental, physical and chemical conditions, and it is therefore not surprising that resistance to extensively used antibiotic, antiseptics and disinfectants has been reported (Pathak *et al*.,1993). Many of these reports of resistance have often paralleled issues including inadequate cleaning, incorrect product use, or ineffective infection control practices, which cannot be underestimated (Yang *et al*., 2009).

Resistance can be either a natural property of an organism (intrinsic) or acquired by mutation or acquisition of plasmids or transposons (Linton *et al*., 1974). The nature of biofilm structure and the physiological attributes of biofilm organisms confer an inherent resistance to antimicrobial agents, whether these antimicrobial agents are antibiotics, disinfectants, or germicides (Rodney and Costerton, 2002). Intrinsic resistance is mostly demonstrated by gram-negative bacteria, bacterial spores and mycobacterium. Acquired, plasmid-mediated resistance commonly exists in both gram negative and positive bacteria and most widely associated with many antimicrobial agents which are conferred by R-factor (Olowe *et al*., 2004).

It has been speculated that low-level resistance may aid in the survival of microorganisms at residual levels of antibiotics, antiseptics and disinfectants; any possible clinical significance of this remains to be tested. With growing concerns about the development of biocide resistance and cross-resistance with antibiotics, it is clear that clinical isolates should be under continual surveillance and possible mechanisms should be investigated (Gerald and Russell, 1999). The public health impact of release of resistant bacteria to receiving environment can be explained by many ways.

First, if the resistant bacteria are carrying transmissible gene, they transfer resistant genes through conjugation or transduction so that infection caused by these bacteria are usually difficult to treat and also decrease antibiotic pool for treatment of bacterial infection. Second, this organism may act as vector or reservoir of resistant genes. Third, there will be increased nosocomial infection. Fourth, if infection occurs, it will increase cost of treatment and hospitalization (Nuñez & Moretton, 2007). Large quantities of disinfectants and antibiotics are used in hospitals for disinfection process and patient treatment respectively.

Most of the antibiotic taken by the patients is partially metabolized and excreted through feces and urine. After use, residual quantities of these products reach the wastewater, exposing the bacteria that survive in hospital wastewaters to a wide range of biocides that could act as a selective pressure for the development of resistance (Nuñez and Moretton, 2007). Increasing attention has been directed recently to the resistance of bacteria to antibiotics and disinfectants. The resistant bacteria isolated were diverse in nature. For example, study conducted in Buenos Aires City hospital, Brazil, the bacterial population resistant to disinfectants was mainly composed by *Enterobacteriaceae, Staphylococcus spp, and Bacillus spp,* which are highly associated to nosocomial infections (Nuñez and Moretton, 2007).

Study carried out in Nepal found out that healthcare liquid wastes were loaded with multiple drug resistance bacteria and seemed to pose a huge public health threat in the transfer of such resistance to the bacterial pathogens causing community acquired infections, thereby limiting our antibiotic pool (Sharma *et al.*, 2010). Also study conducted in Sweden demonstrated that high prevalence of Vancomycin Resistant Enterococci in Swedish sewage possibly due to antimicrobial drugs or chemicals released into the sewage system may sustain in the system (Aina *et al*., 2002).

Antimicrobial resistance may spread in aquatic environment (drinking and recreational water) and its role is not only as reservoir of clinical resistance genes, but also as a medium for spread and evolution of resistance genes and their vectors (Hilary, 1993). Therefore the potential for indigenous aquatic organisms to provide the source of new resistance genes and their associated genetic vectors and to function as hosts for the continued evolution of clinically important resistance genes deserves more intense and detailed investigation (Hilary, 1993).

However study conducted in U.S wastewater research division, municipal environmental research laboratory, U.S. Environmental Protection Agency observed the effect of UV light disinfection on antibiotic resistant coliforms in wastewater effluents and indicated UV irradiation effectively disinfected the wastewater effluent, the percentage of the total surviving coliform population resistant to tetracycline or chloramphenicol was significantly higher than the percentage of the total coliform population resistant to those antibiotics before UV irradiation and the finding was attributed to the mechanism of R-factor mediated resistance to tetracycline (Mark, 1982).

2.5. Nosocomial Infection related to Hospital biomedical wastes

Nosocomial infection also called "hospital acquired infection" can be defined as: An infection acquired in hospital by a patient who was admitted for a reason other than that infection (WHO, 2012). An infection occurring in a hospital or other health care facility was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility (Struelens, 1998). Despite progress in public health and hospital care infections continue to develop in hospitalized patients, and may also affect hospital staff.

Many factors promote infection among hospitalized patients: decreased immunity among patients; the increasing variety of medical procedures and invasive techniques creating potential routes of infection; and the transmission of drug-resistant bacteria among crowded hospital populations, where poor infection control practices may facilitate transmission.

Nosocomial infections occur worldwide and affect both developed and resource-poor countries.At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital (Shlaes, 1997). The most frequent nosocomial infections are infections of

surgical wounds, urinary tract infections and lower respiratory tract infections. The WHO studies, and others, have also shown that the highest prevalence of nosocomial infections occurs in intensive care units and in acute surgical and orthopaedic wards. Infection rates are higher among patients with increased susceptibility because of old age, underlying disease, or chemotherapy. There are many factors responsible for nosocomial infection such as microbial and environmental the most common agents.

Microbial agent

The patient is exposed to a variety of microorganisms during hospitalization (WHO, 2012). Contact between the patient and a microorganism does not by itself necessarily result in the development of clinical disease; other factors influence the nature and frequency of nosocomial infections. The likelihood of exposure leading to infection depends partly on the characteristics of the microorganisms, including resistance to antimicrobial agents, intrinsic virulence, and amount (inoculum) of infective material. Most infections acquired in hospital today are caused by microorganisms which are common in the general population, in whom they cause no or milder disease than among hospital patients (*Staphylococcus aureus, coagulase-negative staphylococci, enterococci, Enterobacteriaceae*) (Robert, 2011).

Many patients receive antimicrobial drugs. Through selection and exchange of genetic resistance elements, antibiotics promote the emergence of multi drug resistant strains of bacteria; microorganisms in the normal human flora sensitive to the given drug are suppressed, while resistant strains persist and may become endemic in the hospital. As an antimicrobial agent becomes widely used, bacteria resistant to this drug eventually emerge and may spread in the health care setting. Many strains of *pneumococci, staphylococci, enterococci,* and *tuberculosis* are currently resistant to most or all antimicrobials which were once effective (Arjana *et al*., 2012).

Environmental factors

Health care settings are an environment where both infected persons and persons at increased risk of infection congregate. Patients who become infected in the hospital are a further source of infection. Overcrowded population within the hospital, frequent transfers of patients from one unit to another, and concentration of patients highly susceptible to infection in one area. (E.g.

new born infants, burn patients, and intensive care) all contribute to the development of nosocomial infections. Microbial flora may contaminate objects, devices, and materials which subsequently contact susceptible body sites of patients. In addition new infections associated with water borne bacteria and parasites continue to be identified (Lee *et al*., 1998).

2.6. Principles of biomedical waste management

Biomedical waste is the waste which is generated during diagnosis, treatment or immunization of human beings or animals that may be contaminated with patients' body fluid which includes syringes, needles, ampoules, dressings, disposable plastics and microbiological wastes (Gautam *et al*., 2010). The main sources of BMWs are hospitals, clinics, other research facilities. BMWs should be considered as a reservoir of pathogenic microorganisms, which can cause contamination and infection. Proper BMWM include vital steps (segregation, collection, storage, transportation, treatment, and final disposal) of wastes generated in the healthcare establishment stages which require special attention (Fig.1).

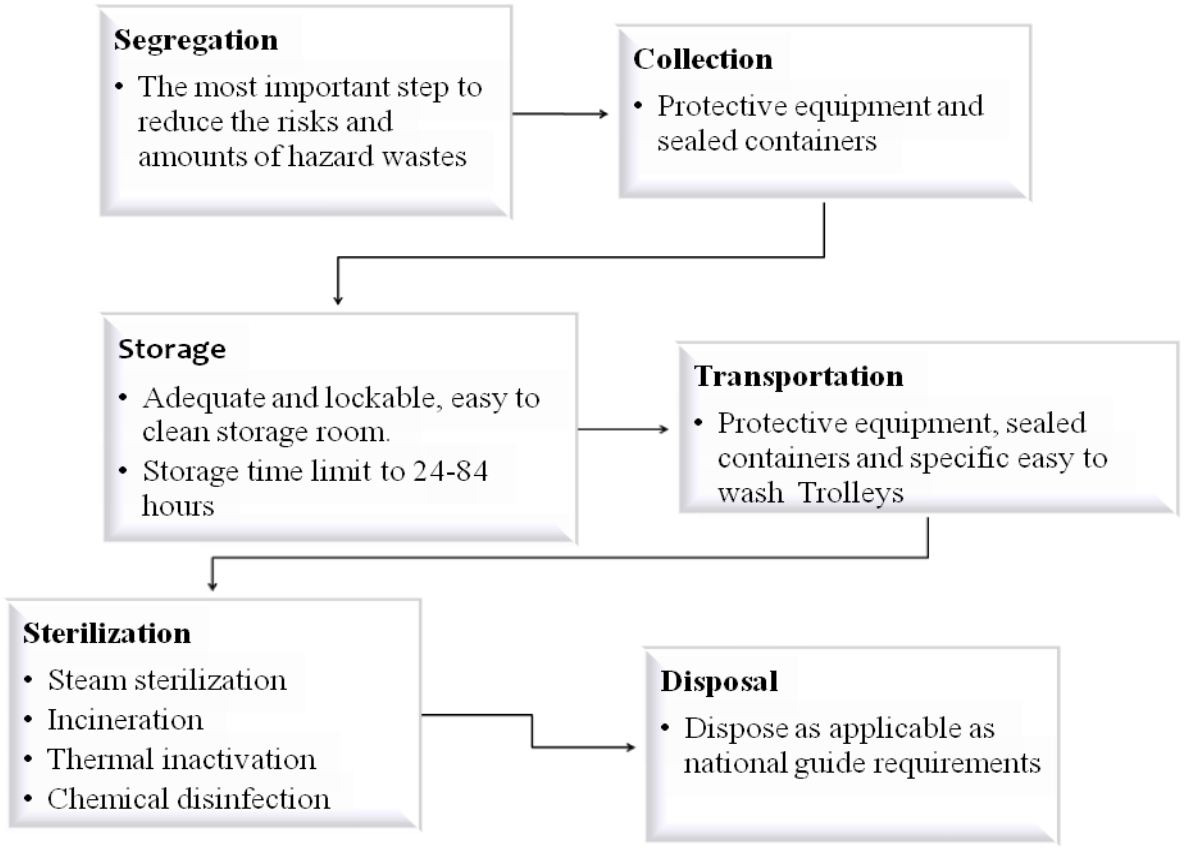

Fig. 1. Principles of biomedical waste management (Gautam *et al*., 2010)

3. Materials and Methods

3.1. Description of sampling site and study period

The study was conducted in Jimma University Specialized Hospital, which is found in Jimma town and located at 352 km southwest of Addis Ababa. Jimma town has geographical coordinates of $7^{o}41'$N latitude and $36^{o}50'$E longitude. The study area has an average altitude of 1, 780 m above sea level. It lies in between 1, 500 - 2,400 m above sea level which is considered ideal for agriculture as well as human settlement. The town is generally characterized by warm weather with a mean annual maximum temperature of 30°C and a mean annual minimum temperature of 14°C and annual rainfall ranges from 1138-1690 mm.

This hospital is one of the oldest public hospitals in Ethiopia and it is currently the only teaching and referral hospital in southwestern part of the country. It provides services for approximately 9000 inpatient and 80, 000 outpatient attendances a year from catchment population of about 15 million people. The clinical services given at the hospital are adult medical outpatient Department (OPD); surgical OPD; pediatric OPD; medical and surgical referral and follow-up; dental care and treatment; dermatological and venereal disease care and treatment; ophthalmology; psychiatry; physiotherapy; orthotic and prosthetic services; inpatient services for medical, surgical and trauma patients. Laboratory, pathology and radiology services are also given within the hospital. The Pharmacy services offered are inpatient and outpatient pharmaceutical drug information (JUSH, 2019).

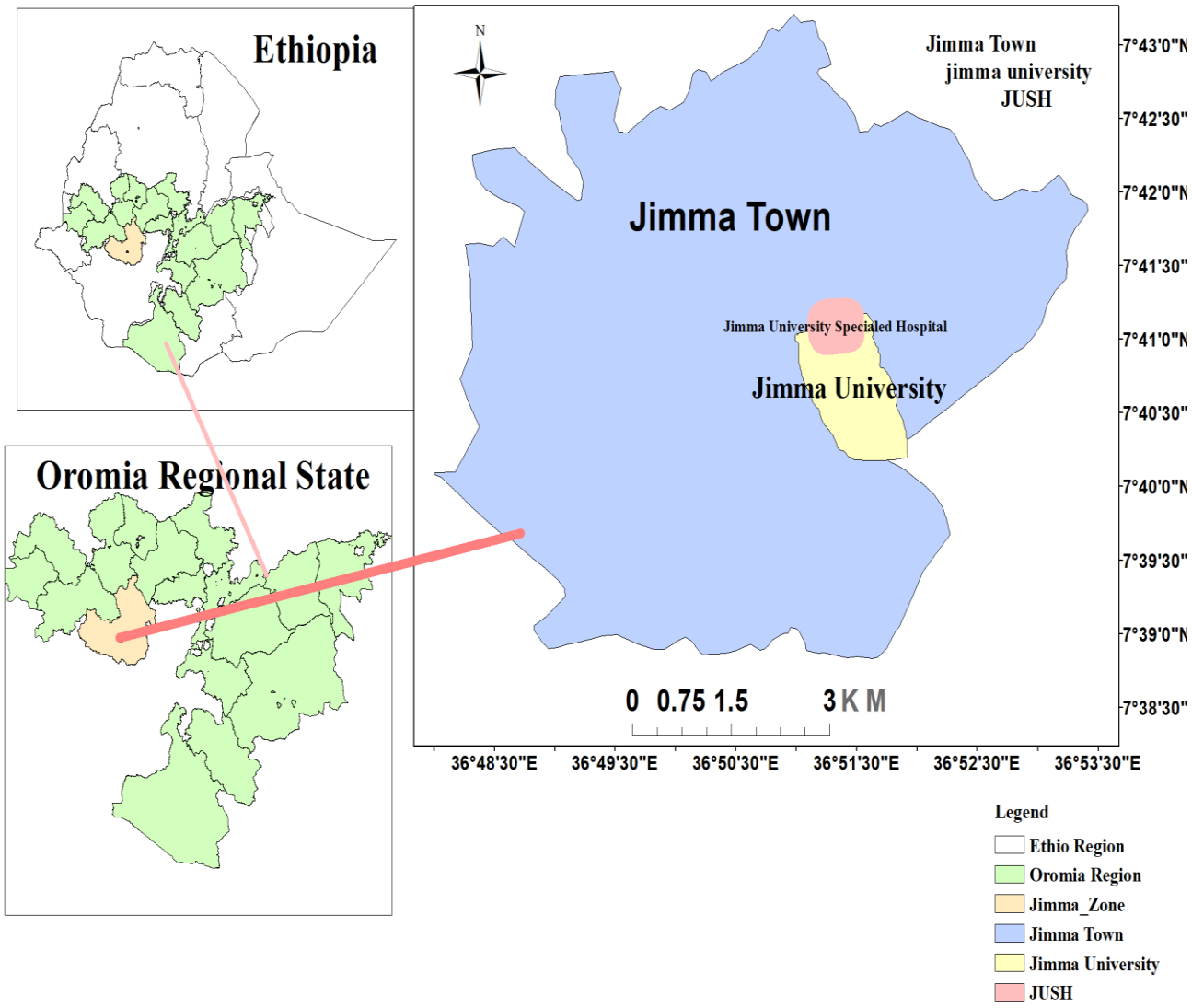

Fig. 2. Map of the study area (Jimma University Specialized Hospital)

3.2. Study Periods

The duration of this study was from November, 2018 to June, 2019.

3.3. Study design and population

Cross-sectional and experimental study designs were conducted for the study. The total workers of JUSH who engaged in the study were 1023 irrespective of their age and sex, with the proportion of 501 of professionals, 30 of Administrative staff and 492 of sanitarians. So, the sample size was calculated by using Cochran (1977) formula:

$$n = \frac{n_0}{1 + \frac{n_0}{N}} \qquad \text{Where} \qquad n_0 = \frac{Z^2_{\alpha/2} P(1-P)}{d^2}$$

n = total sample size
d= margin of error
N = total number of the population
p= proportion of population
α= level of significance
d = 0.05, P = 0.5 and α = 0.05, N= 1023

$$n_0 = \frac{(1.96)^2(0.5)(1-0.5)}{(0.05)^2} = 384$$

So, the sample size is determined by: $n = \frac{384}{1 + \frac{384}{1023}} = \mathbf{279}$

From the total sample 279, the sample sizes of respondents were 137 (Professionals), 8(Administrative staff) and 134 (sanitarians).

3.4. **Sampling technique**

A systematic random sampling technique was used to address representative workers of JUSH.

3.5. Data collection

Data about general biomedical waste management were collected using questionnaires from the workers of JUSH (Appendix 1) and data about the microbiology were conducted through experiment.

3.6. **Sample collection and preparation**

A total of 80 biomedical waste samples comprising of 20 samples each of (bandage, glove, lancet and liquid wastes) were collected from different disposing sites of Jimma University specialized hospital between the months of November 2018 to June 2019 at a time of waste disposal (between 8 AM to 9 AM).

Biomedical waste samples were collected from waste handlers using sanitarian's serving utensils and placed into sterile polyethylene bags. All biomedical waste samples were transported to Research and Postgraduate Laboratory, Department of Biology and the microbial analysis was

conducted within an hour after collection. These biomedical waste samples were kept in the refrigerator at 4^{o}C until microbial analysis was conducted.

Samples were prepared by swabbing an area of 1 cm^2 using cotton dipped by saline solution and mixing using vortex mixer. Then after, 1ml of each solid biomedical waste sample was transferred to 9 ml of saline solution. However for liquid wastes 1ml was directly transferred to 9 ml of saline solution. After homogenization, 1 ml of each biomedical waste was transferred aseptically into 9 ml of saline solution, and mixed thoroughly by using vortex. The homogenates were serially diluted from 10^{-1} to 10^{-6} and a volume of 0.1 ml aliquot of appropriate dilution was spread-plated on pre-solidified plates and incubated at appropriate temperature and time for enumeration of different microbial groups including aerobic mesophilic bacteria (AMB), Enterobacteriaceae, Coliform, Staphylococci and Molds. The colonies were counted from plate containing microbial colonies between 30 and 300. The counted colonies were expressed in colony forming units per cm^2 for microbes isolated from solid BMWs or colony forming units per ml for microbes isolated from liquid BMWs (CFU/cm^2 or CFU/ml) and later converted to log/cm^2 or log/ ml.

3.3. Microbial Enumeration and Isolation

3.3.1. Aerobic Mesophilic Bacterial Count

From appropriate dilutions, 0.1 ml of the aliquot was spread plated on Plate Count Agar (PCA) (Oxoid) and the plates were incubated at 37°C for 24 hrs.

3.3.2. Enterobacteriaceae Count

From appropriate dilutions, 0.1 ml of the aliquot was spread-plated on MacConkey agar (Oxoid) and incubated at 37^{o}C for 18 - 24 hrs. After which, pink to red purple colonies were counted as member of the family Enterobacteriaceae

3.3.3. Coliform count

From appropriate dilutions, 0.1 ml of aliquot was spread plated on pre-solidified surfaces of Violet Red Bile Agar (VRBA) (Oxoid) plates. Then the plates were incubated at 37^{o}C for 18 - 24

hrs. After this, purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms.

3.3.4. Staphylococci Count

From appropriate dilutions, 0.1 ml of the aliquot was spread plated onto Mannitol Salt Agar (MSA) (Oxoid) and incubated at 37°C for 36 hrs

3.6.1. Molds and yeast Counts

From appropriate dilutions, 0.1 ml aliquot was spread-plated on pre-solidified surfaces of Potato Dextrose Agar supplemented with 0.1 g chloramphenicol and incubated at 25^{o}C for 3-5 days (Katyayanee, 2017). Smooth (non-hairy) colonies without extension at periphery were counted as yeasts, whereas hairy colonies with extension at periphery were counted as moulds.

3.4. Microbial Analysis

After enumeration of AMB, 10 to 20 colonies with distinct morphological differences such as color, size and shape were randomly picked from countable plates and aseptically transferred into a tube containing 5 ml nutrient broth (Oxoid). The inoculated cultures were incubated at 37^{o}C for 24 hrs. Cultures were purified by repeated plating and preserved on slants at 4^{o}C. Finally, the obtained organisms were characterized to genus levels. The characterization of isolates was done based on manual of bacterial classification (John, 2012).

3.4.1. Cell Morphology

In order to assess the cell morphology of the pure culture, gram staining, motility test and endospore test were used. The morphological study includes cell shape, cell arrangement, presence or absence of endospore and motility.

Gram staining

A smear of pure isolates was prepared on a clean slide and allowed to air-dry and heat-fixed. The heat fixed smear was flooded with crystal violet dye for 1 minute and rinsed under tap water. Then, the slide was flooded with iodine solution for 1 minute and rinsed under tap water. After rinsing, the smear was decolorized with 96% of ethanol for 20 seconds and washed gently under tap water. Thereafter, the smear was counterstained by safranin and dried by absorbent paper.

Finally, the air-dried smear was observed under oil immersion objective. At the completion of the Gram Staining, gram-negative bacteria were stained pink/red and gram-positive bacteria were stained blue/purple (Gram, 1884).

Motility Test

A motility medium was prepared using a test tube. A purified broth culture was taken by a sterile needle and stabbed straight vertically into a test tube containing motility medium to the bottom of the tube and incubated at 37°C for 24 hours. A positive motility test was indicated by a red turbid area diffusing away from the line of inoculation and a negative test was indicated by red growth along the inoculation line only but no further (Shields and Cathcart, 2012).

Endospore Staining

Endospore test was done according to Schaeffer and Fulton (1933) method. A smear of isolates was prepared on a clean glass slide and allowed to air-dry. The air-dried smear was heat fixed. Heat fixed smear was flooded with 0.5% (w/v) malachite green solution and steamed using cotton dipped in 96% ethanol for 5 minutes. After cooling, the slide was washed with tap water and counterstained with safranin for 30 seconds. The slide was washed with tap water and air dried/blotted to be observed under the oil immersion lens (×1000) to check the presence of endo spore.

3.4.2. Biochemical Test

KOH-test (Test for Lipopolysaccharide)

Two drops of 3% KOH solution was placed on a clean microscopic slide. A colony was aseptically picked from the surface of nutrient agar using an inculcating loop and stirred in the KOH solution for 10 seconds to 2 minutes. The inoculating loop was raised slowly from the mass when the KOH solution became viscous, the thread of slime followed the loop for 0.5 to 2 cm or more in gram-negative bacteria. In case of no slime and the watery suspension did not follow the loop, the reaction was considered negative and the isolate was considered as gram positive bacteria (Gregerson, 1978).

Oxidation Fermentation (O/ F) Test

This test is used to assess the ability of the isolate to utilize glucose and to determine the metabolic way (i.e. fermentation or oxidation). Ingredients (g/l): Peptone, 2 g; yeast extract, 1 g; NaCl, 5 g; K_2HPO_4, 0.2 g; glucose,10 g; bromothymol blue, 0.08 g; agar, 2.5 g; distilled water, 1000 ml; pH, 7.10. Accordingly, test tubes containing 15 ml of freshly prepared medium for O/F test were autoclaved and immediately cooled under tap water to avoid dissolution of oxygen in the medium. Then, the broth cultures were inoculated into the medium by stabbing with a sterile straight wire to the bottom. An organism with oxidative metabolism displayed yellow in the upper half of the tube and green in the lower half. An organism with fermentative metabolism displayed yellow in both halves of the tube. Acid formation and growth regions were interpreted after 2 to 5 days of incubation at 37^{o}C (Hugh and Leifson, 1953).

Catalase Test

Catalase test was carried out after young colonies flooded with a 3% solution of H_2O_2.The formation of bubbles indicated the presence of catalase (MacFaddin, 1980).

Cytochrome Oxidase test

This test was conducted using the method outlined by Kovacs (1956). Accordingly, freshly prepared reagent A and B were mixed in the ratio of 2:3 immediately before use. Reagents: A, 1% α naphthol in absolute ethanol, B, 1% N, N – dimethyl –p- phenylenediammonium chloride in distilled water. The pure isolates from plate were rubbed on filter paper then three drops of the oxidase reagent were added onto the rubbed filter paper. Isolates were considered oxidase positive when the color changed to dark blue and negative when color was not changed within 30 seconds.

3.5. Isolation of human Pathogenic microbes from medical wastes

3.5.1. Isolation of *Staphylococcus aureus*

After counting staphylococci, golden yellow colonies on MSA plates were aseptically picked and transferred into 5 ml nutrient broth and incubated at 37^{o}C for 24 hrs for further purification. Then, a loopful of culture from the nutrient broth was streaked on nutrient agar supplemented with 0.75% NaCl and again incubated at 37^{o}C for 24 hrs. Finally, the distinct colonies were

characterized using the established microbiological methods. Gram-positive cocci with clustered arrangement under the microscope were subjected to preliminary biochemical tests (oxidase, catalase and coagulase tests) (Acco *et al*., 2003).

Coagulase test

Coagulase test was done using slide test and tube test procedures (Cheesbrough, 2006). In slide test, a colony of the purified isolates was emulsified in a drop of distilled water on two ends of clean glass slide to make thick suspensions. One was labeled as test and the other was as control. A loopful of human blood plasma was added to one of the suspensions and mixed gently. Clumping within 10 seconds was observed for coagulase positive organisms. On the other hand, Coagulase test was done using tube test. Accordingly, three test tubes were taken and labeled as test, negative control and positive control.

Each tube was filled with 0.5 ml of 1 in 10 diluted human's plasma. To the tube labeled test, 0.1 ml of overnight broth culture of test bacterium was added. To the tube labeled positive control, 0.1 ml of overnight broth culture of known *S. aureus* was added and to the tube labeled negative control, 0.1 ml of sterile broth was added. All the tubes were incubated at 37^{o}C and observed up to four hrs. Positive result was indicated by gelling of the plasma, which remains in place even after inverting the tube (Cheesbrough, 2006).

3.5.2. Isolation of *Bacillus cereus*

One ml of sample was added to 10 ml of saline solution and heated in a water bath kept 80 °C for 10 minutes and then cooled rapidly in tap water. From appropriate dilution, 0.1 ml aliquot was spread plated on pre-dried surface of *B. cereus* Agar medium which is a selective medium for *B. cereus* and incubated at 37^{o}C for 72 hrs. After incubation blue colonies with opaque halo on the medium was presumptive for *B. cereus.* The biochemical tests for *B. cereus* were subjected to endospore test, Gram staining, oxidase, catalase test, urease test and indole Production test.

Indole Production test

A loopful of 24-hours old pure culture of bacteria was transferred into 5 ml Tryptophan broth and incubated at 37^{o}C for 48 hours. In order to test for indole production, 5 drops of Kovac's

reagent was added directly into the tubes and the red colour indicate the positive for indole production (Smyth *et al*., 2005).

3.5.3. Isolation of *Salmonella* spp and *Shigella* spp.

For the detection of *Salmonella and Shigella spp.*1 cm^2 of swabbed solid BMWs were mixed with 9 ml of BPW and incubated at 37°C for 24 hrs. Then, 1 ml pre-enrichment broth culture was added to 10 ml of selenite cysteine broth (Oxoid) and again incubated at 37^oC for 24 hrs. Thereafter, a loopful of suspension from a tube was streaked onto Salmonella-Shigella Agar (Oxoid). The presumptive Salmonella and Shigella colonies were incubated at 37^oC for 24 hrs, then streaked onto Nutrient Agar (Oxoid) for purity, and incubated at 37°C for 24 hrs (Arvanitidou *et al*., 1998). Suspected Salmonella and Shigella colonies were picked and purified. Pure cultures were further tested for biochemical testes (Johnson and Case, 2007).

Triple Sugar Iron Agar (Oxoid)

The butt was stabbed and the slant was streaked and incubated at 37^oC for 24hrs to detect fermentation of glucose, sucrose and lactose as well as production of H_2S. The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H_2S was considered as presumptive for Salmonella spp. The Presence of alkaline (red) slant and acid (yellow) butt, without production of H_2S was considered as presumptive for Shigella.

Lysine Iron Agar (Oxoid)

The butt was stabbed and the slant was streaked and incubated at 37^oC for 24hrs. Then, the production of an alkaline reaction (purple color) throughout the medium was presumptive for *Salmonella spp*. The presence of alkaline slant and acid butt was considered presumptive for Shigella.

Urea Agar (Oxoid)

The slant was streaked and the tube was incubated at 37^oC for 24 hrs to assess the hydrolysis of urea. No color change was considered as negative and thus presumptive for *Shigella* and if not *Salmonella spp*. Urease producing organisms hydrolyze urea to form ammonia and the medium may change to purple red. Salmonella and Shigella did not produce the enzyme urease and the color of the urea slant was unchanged.

Simmons Citrate Agar (Oxoid)

The slant was streaked and the tube was incubated at 37^{o}C for 24hrs to determine citrate utilization as a sole source of carbon. The presence of growth and color change from green to blue was considered as presumptive for *Salmonella spp.,* and retained the original color for *Shigella spp.*

Sulfide Indole Motility (SIM) Medium (Oxoid)

The SIM medium was stabbed to the bottom and incubated at 37^{o}C for 24 hrs for the determination of H_2S production, indole production and motility. Production of indole was investigated by adding Kovac's reagent (HCl, 250 ml, amylalcohol, 750 ml and paradimethylamino-benzaldehyde 50g/l) to growth in this culture medium. The non-utilization of indole and absence of deep red color at the surface of agar was considered as presumptive for *Salmonella sp*., if not considered as presumptive for *Shigella sp.*

3.5.4. Isolation of *Klebsiella spp.*

The collected samples were swabbed on MacConkey agar for 24 hours at 37^{o}C. Then, mucoid colony on the medium is presumptive for *Klebsiella sp*. Further biochemical tests like motility, catalase, oxidase, Gram staining, indole production, citrate utilization, TSI and urea hydrolysis. Gram-negative, non-motile, lactose-fermenting, facultative anaerobic, rod-shaped bacterium, negative to indole production, positive to citrate utilization, TSI negative, positive for urea hydrolysis, catalase positive, negative for oxidase, non-endospore forming and lactose fermenters were taken as *K. pneumoniae*

3.5.5. Isolation of *Pseudomonas spp.*

For isolation of *Pseudomonas sp,* cotton dipped of saline solution and swab of 1cm^2(For solid wastes) and streaked on *Pseudomonas* Isolation agar base incubated at 37^{o}C for 48h. Then, after the presumptive colonies were subjected to preliminary tests like catalase, oxidase, TSI, and urease. The biochemical characters used for identification were, positive citrate utilization test, positive urease test, acid and abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests.

3.5.6. Isolation of *Escherichia spp.*

Solid BMWs were swabbed on MacConkey Agar. For liquid waste samples 1 ml was directly streaked on MacConkey Agar and lactose fermenting colonies were then sub cultured to Eosin Methylene Blue (EMB) and incubated aerobically at 37^{o}C for 24 hrs. Green metallic sheen colonies on EMB were considered as presumptive *E. coli* isolates.

Presumptive isolates were transferred in nutrient broth for further identification by biochemical tests. Then isolates were further characterized for their biochemical activity using the biochemical tests indole production and Gram staining to determine cell morphology and purity of the isolates. All the isolates that exhibited respective results were considered as *Escherichia sp*, isolates (Edwards and Ewing, 1972).

3.5.7. Isolation of pathogenic fungi

For isolation of pathogenic fungi, Swabs from solid BMWs samples and 1 ml liquid wastes were streaked on Sabouraud dextrose agar (SDA) incubated at 37^{o}C aerobically for 2-5 days. Then, pure colony was subjected to biochemical and further test. *C. albicans* identification test was done according to Forbes *et al* (2007), by diagnostic test germ tube formation The C. *albicans* was incubated in 0.5 ml of human serum at 37°C for 2-3 h to prevent other yeast species for germ tube formation. A drop of the suspension was transferred onto a microscopic slide for examination. A clean cover slip was placed over the drop and examined under low magnification for the presence of germ tubes. Other fungi like *Aspergillus sp.* and *Penicillium sp.* were identified by colony morphology and staining with Lacto phenol cotton blue and observe under microscope.

3.6. Antimicrobial Susceptibility Testing for Some Pathogens

Antimicrobial susceptibility testing for pathogens isolated from some biomedical wastes was performed using the disk diffusion method and the results were interpreted as per the criteria of the National Committee for Clinical Laboratory Standards (NCCLS, 2007). A suspected bacterial isolates was prepared and the turbidity of the inoculum was matched with the turbidity standard 0.5 McFarland (Bauer *et al*., 1966). McFarland is a Barium Sulphate standard against which the turbidity of the test and control inoculum was compared. This standard was prepared

by mixing two solutions; solution A and solution B. Solution A was 1% v/v solution of sulphuric acid (H_2SO_4) and solution B was 1% w/v solution of barium chloride ($BaCl_2$). To get 0.5 McFarland standard, concentration equivalents to cell density of about 10^7- 10^8 CFUg-1, an amount of 0.5ml $BaCl_2$ of 1% solution A was mixed with 99.5 ml H_2SO_4 of 1% solution B.

A small volume of the turbid solution was transferred to a screw-cap bottle of the same types as used for preparing test and control inoculums. Culture containing test tube with approximately equal concentration or density with 0.5 McFarland standards was used for inoculation of media. The standard was used after shaking immediately before use; and stored in a well-sealed container in a dark place at room temperature (20 - 28^{o}C) when not used. When matched with the standard, the inocula were confluent growth. Then, the standardized suspension was swabbed by cotton swab onto the Muller-Hinton Agar (Oxoid) and allowed to dry. Thereafter, the antibiotic discs were placed using forceps on the medium and incubated at 37°C for 18 hrs and the zones of inhibition were measured manually with a transparent ruler. The results of the antimicrobial susceptibility were interpreted based on the guidance of National Committee for Clinical Laboratory Standards (NCCLS, 2007). Finally, the isolates were classified as sensitive, intermediate, or resistant. Intermediates were considered as resistant for purpose of analysis.

The following standard drug discs (Oxoid) and their potency (µgml-1) were used depending up on the antibacterial spectrum, toxicity, effectiveness and availability (Vlkova *et al*., 2006). As a result, ampicillin (10), chloramphenicol (30), ciprofloxacin (5), gentamycin (10), kanamycin (30), naldixic Acid (30), streptomycin (10) and tetracycline (30) for Salmonella spp., *Pseudomonas spp*, *Klebsiella spp*., *Shigella spp*., and *Escherichia spp* whereas chloramphenicol (30), ciprofloxacin (5), clindamycin (2), erythromycin (15), gentamycin (10), kanamycin (30), penicillin G (10), streptomycin (10) and tetracycline (30) for Staphylococcus aureus and *Bacillus cereus*.

3.7. Data analysis

The percentage of coefficient of variation (% CV) was calculated to see if there is significant variation in counts within the food samples analyzed. The data obtained from the respondents were analyzed using SPSS software version 23. Mean values of biomedical waste samples from Jimma University specialized hospital were compared using one way ANOVA and the significance of differences were considered at 95% confidence interval ($P < 0.05$).

3.8. Ethical Consideration

Ethical clearance was obtained from Research Review and Ethical committee of College of Natural science, Jimma University. Respondents and concerned officials were informed about the purpose of the study. The consent was obtained from Jimma University specialized hospital human resource management bureau and hospital workers after a brief explanation of the objectives and benefits of the study.

4. Results

4.1. Socio-demographic status of JUSH workers

Socio-demographic characteristics of JUSH showed that, majorities 7 of 8 management groups and 50.36% of professions were males. However, the majority 99.25% of sanitarians were females. Educationally, 1of 8 the managements had diploma and 1 was each of sub-specialist, specialist, doctor, bachelor of science and nurse. From the groups of professionals, 34(24.82%) were doctors and 3 were sub specialists. Experience wise, 4 of the managements had 3-4 years' experience (Table 3).

Table 3 Socio-demographic status of Workers in JUSH, (Jimma, 2019)

Background information	Managements (N=8)		Professionals (N=137)		Sanitarians (N=134)	
	Frequency	%	Frequency	%	Frequency	%
Sex:						
Male	7	87.5	69	50.36	1	0.75
Female	1	12.5	68	49.64	133	99.25
Educational Status:						
Sub specialist	1	12.5	3	2.19	-	-
Specialist	1	12.5	7	5.11	-	-
Doctors	1	12.5	34	24.82	-	-
BSc.	1	12.5	62	45.3	-	-
Nurses	1	12.5	21	15.32	-	-
Diploma	2	25	10	7.3	1	0.75
Certificate	-	-	-	-	133	99.25
Intern	-	-	-	-	-	-
Department						
Emergency	1	12.5	18	13.13	-	-
Pediatrics	1	12.5	13	9.48	-	-
General Surgery	1	12.5	24	17.52	-	-
Anesthesia	1	12.5	14	10.22	-	-
OR	1	12.5	24	17.52	-	-
Medical Ward	1	12.5	31	22.63	-	-
ICU	1	12.5	14	10.22	-	-
TFG	1	12.5	-	-	134	100
Experience						
<6 months	-	-	15	10.95	59	44.02
6 months - 2 years	2	25	31	22.63	71	52.98
3- 4 Years	4	50	78	56.93	2	1.49
>5 years	2	25	13	9.48	2	1.49

4.2. **Transmission ways of nosocomial infection in JUSH**

From professionals, 62.77% of them stated that patients shake their hands at hospital with a large population and overcrowding of patients in the room. More than half of the professionals also revealed, there were a close contact between waste basket and patients in the JUSH (Table 4).

Table 4 Professionals Response on Transmission of nosocomial infection, (Jimma, 2019)

Ways of nosocomial infection transmission	Yes		No	
	Frequency	%	Frequency	%
No of a patients in the room	79	57.66	58	42.34
Contact between patients and waste baskets	69	50.36	68	49.64
Presence of re-infected patient	71	51.82	66	48.18
Hand shaking of patients	86	62.77	51	37.23
Using masks always	67	48.9	70	51.09
Specific tool to dispose cough droplets for each patient	60	43.8	77	56.2
Presence of flies, mosquitoes and ticks in patient room	123	89.78	14	10.22
BMW common for infectious disease in JUSH context	45	32.85	92	67.15

4.3. **Waste management system of JUSH**

To promote sustainable biomedical waste management system, only 3 of the 8 management bodies gave training or instructions for waste handlers, while 62.5% of the sanitarians had no training. Even though, 8 of the managements knew the universal precaution rule of BMWM, yet 77.61% of the sanitarians knew nothing about the guideline. On the other hand, half of the managements (4) and some of the sanitarians rated, JUSH hygienic status as better. Regarding to incinerators, 5 of the 8 managements responded as the number of incinerators was sufficient to burn solid wastes, still 98(73.13%) of sanitarians believed that, the numbers of incinerators were not enough. For transportation of biomedical wastes, a large number (57.46%) of the sanitarians transported by containers, whereas 36(26.87%) of them transported by carts. Moreover, solid wastes were generated more frequently than others (Table 5).

Table 5 Managements and sanitarians Response on BMWM handling JUSH, (Jimma, 2019)

BMWM management or handling	Managements (N=8)		Sanitarians (N=134)	
	Frequency	%	Frequency	%
Waste handlers got training on BMWM				
Yes	3	37.5	23	17.16
No	5	62.5	121	90.30
Awareness of universal precaution rule				
Yes	8	100	30	22.39
No	-	-	104	77.61
The hygienic condition of JUSH				
Best	2	25	34	25.37
Better	4	50	51	38.06
Fair	2	25	49	36.57
Means to transport the wastes mostly				
Wheeled Trolleys	2	25	21	15.67
Carts	3	37.5	36	26.87
Containers	1	12.5	77	57.46
Enough number of incinerators				
Yes	5	62.5	36	26.87
No	3	37.5	98	73.13
Frequency of solid waste incineration per a day				
Once	1	12.5	12	8.96
Twice	3	37.5	78	58.21
Three times	4	50	44	32.84
Procedure being applied for the waste management				
International	2	25	-	-
National	3	37.5	-	-
Institutional procedure	3	37.5	-	-
Hand washing facility near the waste depositing site				
Yes	6	75	64	47.76
No	2	25	70	52.24
Disposing infectious wastes in an open hole				
Yes	6	75	-	-
No	2	25	134	100
Types of BMWs mainly generated				
Infectious wastes (blood and body fluids)	2	25	19	19.71
Anatomical wastes (human tissues, body parts)	-	-	14	10.22
Sharp wastes	1	12.5	12	8.76
Chemical wastes reagents, solvents)	-	-	9	6.57
Pharmaceutical wastes (outdated meds)	-	-	11	8.03
Radioactive wastes and genotoxic wastes	1	12.5	27	13.87
Papers/food stuff	1	12.5	14	10.22
Solid wastes (bandages, gloves and plastics)	3	37.5	31	22.63

4.4. Microbial Counts

The average microbial count of both solid and liquid BMWs showed that AMB, Enterobacteriaceae, coliforms and staphylococci in all samples were above detectable level (3 Log CFU/cm^2). The mean counts (Log CFU/cm^2 or Log CFU/ml) of AMB (6.54 ± 0.28) and Enterobacteriaceae (6.20 ± 0.78) were higher in liquid wastes compared to solid ones, while staphylococci (6.13 ± 0.21), coliform (6.16 ± 0.22), and molds and yeasts (6.13 ± 0.15) were highest in bandage. Furthermore, the mean counts (Log CFU/cm^2) of all microbes were relatively small in lancet sample except staphylococci (Table 6).

The maximum counts (Log CFU/cm^2) of AMB (7.29, 6.91, 6.55 and 6.53) were in glove, liquid, lancet and bandage respectively. Whereas, the maximum mean counts of Enterobacteriaceae (6.69) was observed in glove. There was statistically significant difference ($P < 0.05$) among the mean counts of AMB, Staphylococci and Moulds in all biomedical waste samples between the groups. However, there was no significant difference ($P > 0.05$) of the mean counts in Enterococcus and coliforms among the four samples (Table 6).

Table 6 Microbial mean counts (log CFU/cm^2 ± SD or log CFU/ml ± SD) of BMWs

Types of BMW	No	Microbial mean counts (log CFU/cm^2 ± SD or log CFU/ml ± SD)									
		AMB	% CV	Entero.	% CV	Coliforms	% CV	Staph.	% CV	Molds and yeast	% CV
Bandage	20	6.25±0.27	4.32	6.20±0.24	3.87	6.16±0.22	3.57	6.13±0.21	3 .43	6.13±0.15	2.45
Lancet	20	5.92±0.83	12.15	5.83±0.75	12.86	5.31 ±0.82	15.44	5.28±0.81	15.34	5.48±0.78	14.23
Glove	20	6.23±0.73	11.71	6.06±0.74	12.21	5.94±0.75	12.63	5.73±0.94	16.40	5.52±1.05	19.02
Liquid	20	6.54±0.28	4.28	6.20±0.78	12.58	6.03 ±0.96	15.92	5.99±0.94	15.69	5.91±1.03	17.43
P-value	-	0.015	-	0.255	-	0.713	-	0.019	-	0.046	-

Where; AMB = Aerobic Mesophilic Bacteria, Entero = Enterobacteriaceae, Staph = Staphylococci and CV=Coefficient of variation

4.5. Microbial Analysis

Among 520 isolates, the most dominant genus was *Entrococcus* 152 (29.23%), followed by *Staphylococcus* 92 (17.69%), and the least was *Shigella* 12 (2.31%). Specifically, from (bandage, Glove, Lancet and liquid) sample, 144 (27.69%), 116 (22.31%), 130 (25%), 130 (25%) bacterial isolates were respectively isolated (Table 7). Detail morphological and biochemical characterization of the isolates were listed in appendix 4.

Table 7 Microbial analysis of isolates from BMWs of JUSH

Sample	**No**	**Bacterial genera**								
		Staphylococci	*Enterococci*	*Escherichia*	*Salmonella*	*Klebsiella*	*Shigella*	*Bacillus*	*Pseudomonas*	*Streptococci*
Bandage	144	29(20.14%)	49(34.03%)	15(10.45)	8(5.56%)	8(5.56%)	5(3.47%)	6(4.17%)	7(4.86%)	17(11.8%)
Glove	116	17(14.66%)	34(29.31%)	14(12.07)	6(5.17%)	8(6.89%)	7(6.03%)	17(14.66%)	3(2.59%)	11(9.48%)
Lancet	130	21(16.15%)	47(36.15%)	14(10.77)	17(13.08%)	-	-	9(6.92%)	19(14.62%)	1(0.77%)
Liquid	130	25(19.23%)	22(16.92%)	16(12.31)	24(18.46%)	-	-	9(6.92%)	29(22.31%)	1(0.77%)
Total	520	92(17.69%)	152(29.23)	59(11.35)	55(10.58)	16(3.08)	12(2.31)	41(7.88%)	58(11.15%)	30(5.77%)

4.6. Prevalence of human pathogenic bacteria from biomedical wastes of JUSH

A total of 36 pathogens which were grouped to *Staphylococcus spp, Bacillus spp, Pseudomonas spp, Salmonella spp, Klebseilla sp, Shigella sp* and *Escherichia sp,* were positive from 80 total number of BMW samples. To the specific pathogenic bacteria 8, 7, 6, 6, 4, 3 and 2 numbers of *Salmonella sp, Klebseilla sp, S. aureus, Pseudomonas sp, Shigella sp* and *Escherichia sp,* were positive respectively from all BMWs samples. With this intension, there was no *Salmonella spp* detected from glove and lancet samples. Generally the highest numbers of pathogenic bacteria were detected from bandage whereas the smallest number of pathogenic bacteria from lancet sample (Fig. 1).

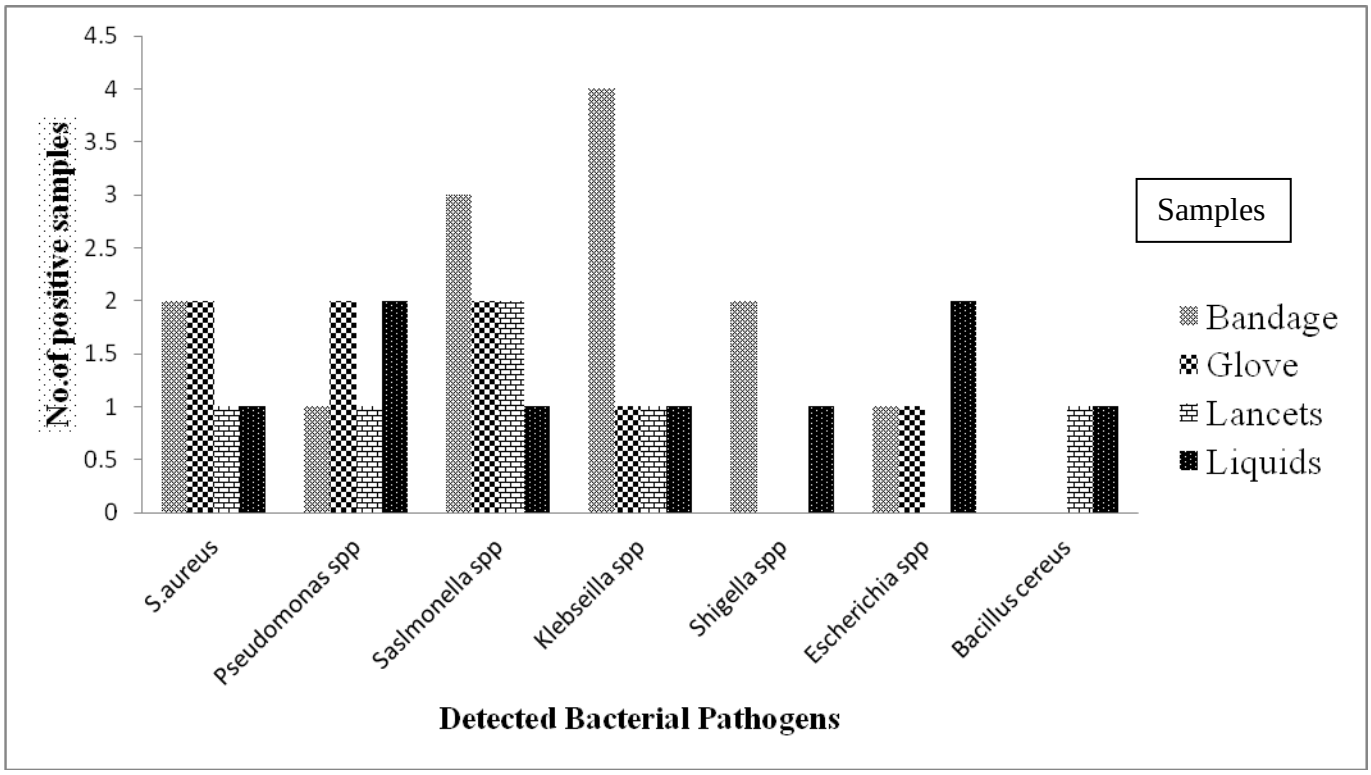

Fig. 3. Distribution of bacterial pathogens isolated from biomedical wastes of JUSH

4.7. Isolation of Pathogenic Fungi from Biomedical wastes of JUSH

From the total samples 51(21.25%) of pathogenic fungi were isolated. To the specific, 8, 7 and 2 of *Penicillium* sp, *Candida* sp, and *Aspergillus* sp were respectively isolated from bandage sample. From gloves, 4, *Penicillium* sp, and 3 *Aspergillus* sp *and Candida* sp were isolated. From lancets, 2, 1, and 1 of *Penicillium* sp *Aspergillus* sp and *Candida* sp were respectively isolated. Generally, the highest number of pathogenic fungi were *Penicillium* sp 23(28.75%) followed by *Candida sp* 16(20%) and *Aspergillus* sp 3 (Table 8).

Table 8 Fungal Pathogens isolated from BMWs of JUSH, (Jimma, 2019).

Pathogens	Sample	No of samples	Detected	Not detected
			Frequency (%)	Frequency (%)
Candida sp.	Bandage	20	7(35%)	13(65%)
	Glove	20	3(15%)	17(85%)
	Lancets	20	1(5%)	19(95%)
	Liquids	20	5(25%)	15(75%)
Aspergillus sp.	Bandage	20	2(10%)	18(90%)
	Glove	20	3(15%)	17(85%)
	Lancets	20	1(5%)	19(95%)
	Liquids	20	6(30%)	14(70%)
Penicillium sp.	Bandage	20	8(40%)	12(60%)
	Glove	20	4(20%)	16(80%)
	Lancets	20	2(10%)	18(90%)
	Liquids	20	9(45%)	11(55%)

4.8. Antimicrobial Susceptibility

4.8.1. Antibacterial Susceptibility of Gram Positive Pathogens

Out of the six (6) *S. aureus*, majorities 5(83.33%) of them were resistant to clindamycin and tetracycline. Moreover, majorities 5(83.33%) *Staphylococcus aureus* were susceptible to ciprofloxacin, Streptomycin and chloramphenicol, whereas 3(50%) of them susceptible to gentamycin and kanamycin (Table 9).

With regards to *Bacillus cereus,* all of them (2) were resistant to penicillin and tetracycline, whereas half (1) of them resistant to clindamycin, kanamycin and erythromycin. However, 2 of them were susceptible ciprofloxacin. While, 1 of them was susceptible to all the discs except to the clindamycin. The standard bacteria, *S. aureus* was susceptible to gentamycin and kanamycin, hence both of them (the standard bacteria (*S.aureus* ATCC25923) and the isolates) were highly susceptible to both gentamycin and kanamycin. However, there were no standard bacteria for *B. cereus* (Table 9).

Table 9 Antibacterial susceptibility of Gram positive spp, (Jimma, 2019).

Antimicrobial agent		***S. aureus***			***S.aurues***	***B. cereus***		
	Disc potency (µg/m)	Resistance Frequency (%)	Intermediate Frequency (%)	Sensitive Frequency (%)	**(ATCC25923)**	Resistance Frequency (%)	Intermediate Frequency (%)	Sensitive Frequency (%)
Chloramphenicol (C)	30	1(16.67)	-	5(83.33)	Susceptible	1(50)	-	1(50)
Ciprofloxacin (CIP)	5	-	1(16.67)	5(83.33)	Susceptible	-	-	2(100)
Clindamycin (DA)	2	5(83.33)	1(16.67)	-	Resistant	1(50)	1(50)	-
Erythromycin (E)	15	2(33.33)	3(50)	1(16.67)	Susceptible	1(50)	-	1(50)
Gentamycin (CN)	10	1(16.67)	2(33.33)	3(50)	Susceptible	-	1(50)	1(50)
Kanamycin (K)	30	1(16.67)	2(33.33)	3(50)	Susceptible	1(50)	-	1(50)
Penicillin G (P)	10	7(100%)	-	-	Resistant	2(100)	-	-
Streptomycin (S)	10	-	1(16.67)	5(83.33)	Susceptible	-	1(50)	1(50)
Tetracycline (TE)	30	5(83.33)	1(16.67)	-	Resistant	2(100)	-	-

The multi-drug resistance (MDR) patterns of *Staphylococcus aureus* revealed that, 5(83.33 %) to five antibiotics (DA/E/CN/P/TE) followed by 4(66.66%) of the isolates were resistant to 3 antibiotics (namely DA/ P/ TE and C/ P/TE combinations). The highest MDR in *Bacillus cereus* was observed to three and five each had 50% resistance (Table 10).

Table 10 MDR Patterns of Gram positive bacteria detected on BMWs of JUSH, (Jimma, 2019)

Types of bacteria	No of antimicrobial resistance	Patterns of resistance	No of isolates	Total
S. aureus	Three	DA/P/TE	3(50%)	4(66.66%)
		C/P/TE	1(16.67%)	
	Four	DA/E/K/P	1(16.67%)	1(16.67%)
	Five	DA/E/CN/P/TE	1(16.67%)	1(16.67%)
Bacillus cereus	Three	DA/P/TE	1(50%)	1(50%)
	Five	C/E/K/P/TE	1(50%)	1(50%)

Where, DA=Clindamycin, P=Penicillin, TE=Tetracycline, C=Chloramphenicol, E=Erythromycin, K=Kanamycin and CN= Gentamycin

4.8.2. Antimicrobial Susceptibility of Gram Negative Pathogens

Out of six (6) *Pseudomonas* sp, all of them (6) were resistant to ampicillin and 5 of them were resistant to tetracycline. However, 5 of them were susceptible to kanamycin. Out of eight (8) *Salmonella* sp 8 were resistant to ampicillin, 6 resistant to naldixic acid, streptomycin and tetracycline. However, 6 of them susceptible to norfloxacin and 5 to chloramphenicol. All of *Klebsiella sp* (7) were resistant to ampicillin but 5 susceptible to ciproflaxin. Out of three (3) *Shigella sp,* 3 were resistant to ampicillin and tetracycline, whereas, 1 of them resistant to chloramphenicol, but 2 susceptible to ciprofloxacin, naldixic acid and norflaxacin while 1 of them susceptible to gentamycin, chloramphenicol and streptomycin. All (4) of Escherichia spp were resistant to ampicillin, 3 of them were resistant to gentamycin and tetracycline, whereas 3 (Table 11).

Regarding to antimicrobial susceptibility of standard bacteria, *E.coli*(ATCC25922) was resistant to ciproflaxcin, gentamycin,streptomycin and Erytromycin, while *S.typhrium*(ATCC13311) was resistant to ciproflacin, Kanamycin,Naldic acid and gentamycin and *K.pneumonia* was resistant to gentamycin, kanamycin, Streptomycin and Erythromycin, respectively(Annex 4). To compare the antimicrobial susceptibility pattern of the *Shigella* spp, there was no standard bacterium. Generally, all standard bacteria were commonly resistant to Gentamycin and Erythromycin, respectively. On other hand, the standard bacteria, *P.aureginosa* (ATCC27253) was highly resistant to Naldic Acid and Streptomycin, while it was susceptible to Kanamycin and norfalacxin (Appendix 5).

Table 11 Antimicrobial susceptibility of Gram Negative pathogens isolated from BMWs of JUSH (Jimma, 2019)

Antimicrobial agent		*Pseudomonas spp*			*Salmonella spp*			*Klebsiella spp*			*Shigella spp*			*Escherichia spp*		
	Disc potency (µg/ml	R F (%)	I F (%)	S F (%)	R F (%)	I F (%)	S F (%)	R F (%)	I F (%)	S F (%)	R F (%)	I F (%)	S F (%)	R F (%)	I F (%)	S F (%)
Ampicillin (AMP)	30	6(100)	-	-	8(100)	-	-	7(100)	-	-	3(100)	-	-	4(10)	-	-
Chloramphenicol (C)	5	-	1(16.67)	5(83.33)	1(12.5)	2(25)	5(62.5)	-	1(14.28)	6(85.71)	1(33.33)	1(33.33)	1(33.33)	-	2(50)	2(50)
Ciprofloxacin (CIP)	5	-	-	6(100)	-	4(50)	3(37.5)	-	2(28.57)	5(71.43)	-	1(33.33)	2(66.67)	-	3(75)	1(25)
Gentamycin (CN)	15	-	-	6(100)	-	5(62.5)	3(37.5)	-	4(57.14)	3(42.85)	-	2(66.67)	1(33.33)	3(75)	-	1(25)
Kanamycin (K)	10	-	1(16.67)	5(83.33)	5(62.5)	3(37.5)	-	-	3(42.85)	4(57.14)	2(66.67)	-	1(33.33)	-	1(25)	3(75)
Naldixic Acid (NA)	30	4(66.67)	1(16.67)	1(16.67)	6(75)	1(12.5)	1(12.5)	2(28.57)	4(57.14)	1(14.28)	-	1(33.33)	2(66.67)	-	1(25)	3(75)
Norflaxacin (NOR)	10	-	-	6(100)	-	2(25)	6(75)	-	5(71.43)	2(28.57)	1(33.33)	-	2(66.67)	-	2(50)	1(25)
Streptomycin (S)	10	4(66.67)	-	2(33.33)	6(75)	2(25)	-	1(14.28)	1(14.28)	5(71.43)	-	2(66.67)	1(33.33)	1(25)	1(25)	2(50)
Tetracycline (TE)	30	5(83.33)	-	1(16.67)	6(75)	-	2(25)	3(42.85)	2(28.57)	2(28.57)	3(100)	-	-	-	3(75)	1(25)

Where, S= Susceptible I= Intermediate R= Resistance F= Frequency

The MDR profile of *Pseudomonas* sp, *Salmonella* sp, *Shigella* sp, and *Escherichia* sp were showed that the highest resistance 50 % of the isolates towards two antibiotics, 37.5% to five antibiotics, (66.65%) and (50%) towards two antibiotics respectively (Table 13).

Table 12 MDR patterns of Gram negative bacteria detected on BMWs of JUSH, (Jimma, 2019)

Type of detected bacteria	No. of antimicrobial resistance	Patterns of resistance	No of isolates %	Total %
Pseudomonas spp.	Two	AMP/S	1(16.67)	3(50%)
		AMP/TE	1(16.67)	
		AMP/NA	1(16.67)	
	Three	AMP/NA/S	1(16.67)	1(16.67%)
	Four	AMP/NA/S/TE	2(33.33%)	2(33.33%)
Salmonella spp.	Two	AMP/NA	1(12.5%)	1(12.5%)
	Three	AMP/NA/S	1(12.5%)	2(25%)
		AMP/K/TE	1(12.5%)	
	Four	AMP/K/S/TE	1(12.5%)	1(12.5%)
	Five	AMP/K/NA/S/TE	3(37.50%)	3(37.50%)
Klebsiella spp.	Two	AMP/S	1(14.28%)	2(28.57%)
		AMP/TE	1(14.28%)	
	Three	AMP/NA/TE	2(28.57%)	2(28.57%)
Shigella spp.	Two	AMP/K	1(33.33%)	2(66.66%)
		AMP/S	1(33.33%)	
	Five	AMP/C/K/NOR/S/TE	1(33.33%)	1(33.33%)
Escherichia spp	Two	AMP/CN	2(50%)	2(50%)
		AMP/CN		
	Three	AMP/CN/S	1(25%)	1(25%)

Where, Ampicillin = AMP, Chloramphenicol = C, Ciprofloxacin = CIP, Gentamycin = CN, Kanamycin =K, Nalidixic acid = NA, Norflaxacin = NOR, Streptomycin = S and Tetracycline = TE

5. Discussions

The socio-demographic characteristics of JUSH workers showed that, managements (8) professionals (137) and Sanitarians (134) were actively involved as role player. Similarly, health care professionals (doctors, nurses, labs, midwifes, HO and cleaners) were the direct stakeholders in the BMWM since they were many in number per their job category and mostly they are involved in either BMW generation/segregation or come in contact with the infectious waste more often during subsequent management than other staff (Teshiwal *et al*.,2018). This could be due to the direct consent between the health care workers and nosocomial infection transmission ways.

In the current study, 4 of the managements had 6 months to two years' experience in managing their workers. However, 52.98% of the sanitarians had 6 months to 2 years' experience while, 1.49% of the sanitarians had 3 to 4 years' experience. Experience is a critical issue that people get knowledge in detail on what they work and have linear connection between experience and training level of the cleaners and workers of the hospitals that contribute strong evidence for the distribution of hospital acquired infection.

According to the present study, 6 of the managements had detail knowledge of BMWM while only few sanitarians groups had information about good hygienic practices. As the study conducted in Nigeria about 45% study participants were had good knowledge (Itah and Ekpombok, 2004). Similarly, in Sri Lanka about 59.5% participants had detail knowledge (Samarakoon *et al*., 2011) and both studies are lower than the present result. This study revealed that, the education level of most of the sanitarians 33(99.25%) was at the certificate level and mostly the cleaners have no basic knowledge of BMW thereby involve in the occurrence of nosocomial infection.

The adequate knowledge; attitude and practice of health care workers are key factors for having successful BMWM system (Bassey *et al*., 2006). Regarding to the awareness of universal precaution rule, the managements had well informed and good awareness, but in contrast to this, 77.64 % of sanitarians were lack of awareness a about universal precaution rule. This may be due to lack of attention and follow-up by the concerned body. WHO has urged to implement the guideline to ensure safe management of wastes from health care activities (Chartier *et al*., 2014).

These gaps might be due to training access, national health sector strategy or academic knowledge difference. In current study, the mean count of AMB of bandage was 6.25 log CFU/cm^2 and liquid waste was 6.54 log CFU/ml. The mean counts of AMB, Entrobacteriaceae, staphylococci, coliform and moulds were also highest in bandage (6.25, 6.20, 6.16, 6.13, and 6.13 log CFU/cm^2) respectively. The highest microbial load could be due to the bandage direct contact with patient's injuries which was later disposed as hospital waste. The hospital wastes could have contributed immensely in the increased number of bacterial counts such as AMB and Enterobacteriaceae (Radhakrishna and Nagarajan, 2015). In the present study the microbes dominated the biomedical wastes were Entrococcus 152(29.23%) followed by Staphylococcus 92(17.69%) and Escherichia 62(11.92%) in general.

Regarding to antimicrobial susceptibility, in Gram positive pathogens such as *S.aureus* 5(83.33%) were susceptible to ciprofloxacin, Streptomycin and chloramphenicol, and 3(50%) of them were susceptible to gentamycin and streptomycin. In this study *S. aureus* were totally, 7(100%) resistant to Penicillin. The resistance of *S. aureus* to penicillin G could be due to the production of penicillinase enzyme (a type of ß-lactamase) that hydrolyzed the beta-lactam ring of penicillin (Lowy, 2003). In higher with the present study, Guta *et al*. (2014) reported that *S. aureus* (64.4%) were sensitive to gentamicin which was isolated from Hawassa University Referral Teaching Hospital. Concerning to the antimicrobial susceptibility pattern of the standard bacteria used in this study, all the standard isolates were highly resistant to Erythromycin and susceptible to Naldic acid, respectively.

The resistance of *Pseudomonas spp.*to gentamicin was 50% in exact agreement with the resistance level recorded in a study performed by Ngwuluka and associates (2009) in Nigeria. Gram negative pathogens such as Klebsiella, *E. coli* and *P. aeruginosa* were the most important bacteria responsible for post-operative wound infection in Hawassa University Referral Teaching Hospital (Guta *et al*., 2014).

Regarding to antimicrobial susceptibility of standard bacteria, *E.coli*(ATCC25922) was resistant to ciproflaxcin, gentamycin,streptomycin and Erytromycin, while *S.typhrium*(ATCC13311) was resistant to ciproflacin, Kanamycin,Naldic acid and gentamycin and *K.pneumonia* was resistant to gentamycin, kanamycin, Streptomycin and Erythromycin, respectively.

To compare the antimicrobial susceptibility pattern of the *Shigella* spp, there was no standard bacterium. Generally, all standard bacteria were commonly resistant to Gentamycin and Erythromycin, respectively. Generally, high rates of drug resistance to some commonly used antibiotics were observed in this study and this warrants attention to the problem. The persistence of antibiotic resistant bacteria and the ability to spread its genetic information in environment is largely determined by their capacity to survive under adverse conditions occasioned by biotic and a biotic factors (Grabaw and Prozesky, 1973).

The main risk for public health is that resistance genes that are transformed from environmental bacteria to human pathogens. As a result hospital waste dumping effluent could increases the number of resistant bacteria in the recipient sewers by both mechanisms of introduction and selection of resistant bacteria reported by some study multi drug resistance profile of bacteria isolated from biomedical waste dumping site soil (Abdulaziz, 2011).

Improper disposal of untreated hospital waste into rivers, drainages and roads especially in developing nations creates a major problem on public health and is of major concern (Sunmeet and Gangawane, 2017). The release of bacteria in to the environment such as through hospital wastewater, favors the exchange of genetic materials with previously non-resistant populations thereby increasing the dispersion of resistant capacity in the environment (Wegener *et al*., 1999). If the hospital effluents are not treated, concentrated forms of infectious agents and antibiotic resistant microbes are shed in to the environment resulting in the spread of antibiotic resistant genes and of diseases such as typhoid fever and gastroenteritis (Abah and Ohimain, 2010).

6. Conclusion

The hygienic status of JUSH was actually better. Solid BMWs were highly disposed followed by infectious wastes and chemical wastes but, treated by incineration method. However, the numbers of incinerators were not sufficient with respect to the amount and types of waste generated. The mean counts of microbes in all samples were above detectable level and their microbial analysis was grouped under nine bacterial genera in which the most dominant genus was entrococci followed by staphylococci. Among the pathogenic microbes two Gram positive and five Gram negative bacteria were detected from BMW samples. These pathogens like *S. aureus* and *B. cereus* were susceptible to gentamycin and ciprofloxacin but resistant to penicillin. On the other hand, *Salmonella sp. Pseudomonas* sp, *Klebseilla* sp, *Shigella* sp and *Escherichia* were susceptible to ciprofloxacin, gentamycin and kanamycin. However, they were resistant to ampicillin, tetracycline, erythromycin and naldixic acid.The MDR patterns of *S. aureus* revealed that, majorities of them were resistant to five antibiotics. But of *B. cereus* was observed to three and five antibiotics. The MDR profile of Gram negative isolates showed highest resistance towards two antibiotics.

7. Recommendations

From the present findings, the following recommendations were forwarded:

- The hygienic status of JUSH is better, to make it best, management and professionals should give training how to handle and dispose the solid wastes.
- Even though, the JUSH biomedical wastes treatment is to certain extent at standard level still some improvements like increasing the number of incinerators and transporting should be improved
- Infectious wastes were the main sources of nosocomial infection. Hence, the current method of disposing this waste has problems because the waste handlers simply dispose it in to an open hole, then managements should prepare special hole for infectious wastes
- The high microbial load of solid and liquid wastes can cause serious problems. So, enhancement of implementation of international guideline may reduce the risk. Hence, getting attention to wastes are mandatory.
- Currently, different antibiotics available in a market among these it is better to use chloramphenicol, gentamycin and ciprofloxacin for diseases caused by *S. aureus* and *B. cereus*. Ciprofloxacin, gentamycin, norflaxacin, kanamycin and chloramphenicol should be prescribed for *Salmonella sp, Pseudomonas sp, Klebseilla sp, Shigella sp,*and *Escherichia sp.*
- Morphological and biochemical methods of identification of the pathogens could not show the exact identity. Thus, molecular approach can overcome this drawback.

References

Abah, S. and Ohimain, E. (2010). Assessment of Dumpsite Rehabilitation Potential using the Integrated Risk Based Approach: A case study of Eneka, Nigeria. *World Applied Sciences Journal*, **4**: 436 - 442.

Abdulaziz, (2011). Review on hospital wastes and its possible treatments. *Academic Journal of Biological Science*, **3**: 55- 62.

Acco, M., Ferreira., F.S., Henriques, J.A. and Tondo, E.C. (2003). Identification of multiple strains of Staphyloccocus aureus colonizing nasal mucosa of food handlers. *Food Microbiology*, **20**: 489-493.

Ahmed, N., Gasmelseed, G., and Musa, A. (2014). Assessment of Medical Solid Waste Management in Khartoum State Hospitals, *Journal of Applied and Industrial Sciences*, **2**: 201-205.

Aina, I., Inger, K., Anders, F, and Roland, M. (2002). High Prevalence of Vancomycin-Resistant Enterococci in Swedish Sewage, *Applied and Environmental Microbiology*, p. 2838–2842.

Anitha, J. (2012). Isolation and Identification of Bacteria from Biomedical Waste. *International Journal of Pharmacy and Pharmaceutical Sciences*, **61**:78-84.

Anitha, J. and Jayraaj, I. (2012). Isolation and identification of bacteria from biomedical waste (BMW). *International Journal of Pharmacology Science*, **4**: 0975-1491.

Arjana, T., Tera, T., Smilja, K., and Vera, J. (2012). The Working Group of the Croatian Committee for Antibiotic Resistance Surveillance for *Antimicrobial Resistance in Croatia Emerging Infectious Diseases*, **8**:121-126.

Arvanitidou, M., Tsakris, D., Sofianou, R. and Katsouyannopoulos, V. (1998). Antimicrobial resistance and R-factor transfer of Salmonella isolated from chicken carcasses in Greek hospitals. *International Journal of Food Microbiology*, **40**:187-201.

Azage, M. and Kumie, A. (2010). Healthcare waste generation and its management system: the case of health centers in West Gojjam Zone, Amhra region, Ethiopia. *Ethiopian Journal of Health Development*, **24**: 119-26.

Bassey, B.,Benka-Coker, M., Aluyi, S. (2006). Characterization and management of solid medical wastes in the federal capital territory, Abuja Nigeria. *African Health Sciences*, **21**:136-147.

Bauer, A., Kirby W., Sherris J. and Jurck M. (1966). Antibiotic susceptibility testing by a standard single disc method. *American Journal of Clinical Pathology*, **45**:493-496.

Calva, J., Sifuentes-Osornio, J. and Ceron, C. (1996). Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico, *Antimicrobial Antigen Chemotherapy*, **40**:1699-1702.

Centers for Disease Control and Prevention, (2003). Guidelines for environmental infection control in health-care facilities: Recommendations of Center of Disease Control and the Healthcare Infection Control Practices Advisory Committee, **52**: 1– 4.

Chagas, T., Seki, L., Cury, J., Oliveira, J., Dávila, A. and Silva, D.(2011). Asensi: Multiresistance, beta-lactamase-encoding genes and bacterial diversity in hospital wastewater in Rio de Janeiro Brazil. *Journal of Applied Microbiology*, **111**: 572–581.

Chakraborty, S., Veeregowda, B., Gowda, L., Sannegowda, S., Tiwari, R. and Dhama, K. (2014). Biomedical waste management. *Advances in Animal and Veterinary Sciences*. **2**: 67-72

Chandra, H. (2009). Hospital Waste an Environmental Hazard and Its Management

Chartier, Y., Emmanuel J., Pieper U., Prüss A., Rushbrook P., Stringer R. (2014). Safe management of wastes from health-care activities. **2**: 46-82.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Cambridge University Press. Pp. 62.

Chitnis, V., Chitnis, S., Vaidya, K., Ravikant, S., Patil, S. and Chitnis, D. (2004). Bacterial population changes in hospital efluent treatment plant in central India. *Water Reservior*, **38**: 441-447

Cochran, W. (1977). Sampling techniques (3rd ed.). John Wiley & Sons. New York. USA.

Edwards, R. and Ewing. H. (1972). Identification of Enterobacteriaceae, 3rd ed. Burgess

Forbes, B., Sahm, D. and Weissfeld, A. (2007). *Bailey & Scott's Diagnostic Microbiology*. 12th edition, Mosby Elsevier. Texas, USA.

Gautam,V., Thapar, R., Sharma, M. (2010). Biomedical waste management: Incineration and environmental safety, Special Article, **28**: 191-192.

Gerald, M. and Russell, A. (1999). Antiseptics and Disinfectants: Activity, Action, and Resistance, Clinical Microbiology Reviews, **12**:147-179.

Goldstein, R., Micallef, S., Gibbs, S., Davis, J., He, X. and Sapkota, A. (2012). Methicillin-Resistant Staphylococcus aureus (Mannitol Salt Agar) detected at four U.S. Waste water treatment plants. *Environmental Health Perspective,* **120**:1551–1558.

Grabow, W. and Prozesky, O. (1973). Drug resistance of coliform bacteria in hospital and city sewage. Antimicrobial Agents Chemotherapy, **3**:175–180.

Gram HC (1884). Uber the isolated staining of schizomycetes in cutting and drying preparations ". Progress there. Medicine, **2**: 185–189.

Gregerson G. (1978). Rapid method for distinction of Gram negative from Gram positive bacteria. *European Journal of Applied Microbiology,* **5**:123-127.

Guta, M., Aragaw, K. and Merid, Y. (2014). Bacteria from infected surgical wounds and their antimicrobial resistance in Hawassa University Referral Teaching Hospital, Southern Ethiopia. *African Journal of Microbiology Research,* **8**: 1118-1124.

Hagen, D., Al-Humaidi, F., and Blake, M. (2001). Infectious waste surveys in a Saudi Arabian hospital: an important quality improvement tool. Am J Infection Control, **29**:198–202.

Hayleeyesus, S. and Cherinete, W. (2016). Health care waste generation and management in public healthcare facilities in Adama, Ethiopia. *Journal of Health and Pollution,* **6**: 64-73.

Hien, H. Drabo, M. and Ouédraogo, K. (2012). Healthcare associated infection in Burkina Faso: an assessment in a district hospital, Journal of Public Health in Africa; **3**:29-34

Hossain, M., Santhanam, A., Norulaini, N., Omar, A. (2011). Clinical solid waste management practices and its impact on human health and environment .A review. *Waste Management*, **31**:75466.

Hugh, M. and Leifson, E. (1953). The taxonomic significance fermentative versus oxidative gram negative bacteria. *Journal of Bacteriol*ogy, **66**:24-26.

Itah, A. and Ekpombok, M. (2004). Pollution status of swimming pools in south-south zone of south-eastern Nigeria using microbiological and physicochemical indices. *Southeast Asian Journal of Tropical Medicines and Public Health*, **35**: 488-493.

Jimma University Specialized Hospital (JUSH, 2019), Jimma University, record offices

John, L. (2012). An introduction to bacterial identification. Dichot key handout.

Johnson, T. and Case, C. (2007). Laboratory experiments in microbiology, 8th edition. San Francisco: Pearson Education's.

Katyayanee, N. (2017). Selection and Characterization of Potential Baker's Yeast from Indigenous Resources of Nepal. *Biotechnology Research International*, **1**: 1-10.

Keen, L.and Patrick, D. (2013). Tracking Change: a look at the ecological footprint of antibiotics and antimicrobial resistance. *Antibiotics Review*, **2**: 191-205

Khachatourians, G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic resistant bacteria, **159**:1129-1136.

Khan, H., Baig, F. and Mehboob, R. (2017). Nosocomial infections: epidemiology, prevention, control and surveillance. Asian Pacific Journal of Tropical Biomedicine, **7**:478-482.

Kumar, M., Singh, R. and Umesh, V. (2015). Awareness and practices about bio-medical waste among health care workers in tertiary care hospital of Haldwani, Nainital. *National Journal of Medical Research*, **159**: 72-3.

Kummerer, K. and Henninger, A. (2003). Promoting resistance by the emission of antibiotics fromhospitals and households into effluent, *Clinical Microbiology of Infection*, **9**:1203-1214.

Lee, J., Scheckler, P., William, E., Steele, L. and Christopher, E. (1998). Recommended Practices for Surveillance. Special Communication: *American Journal of Infection Control*, **26**: 277-288.

Linton, K., Richmond, M., Bevan, R., Gillespie, W. (1974). Antibiotic resistance and R factors in coliform bacilli isolated from hospital and domestic sewage. *Journal of Medical Microbiology*, **7**:91–103.

Lowy, F. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. *Journal of Clinical Investment*, **111**:1265–1273.

MacFaddin, J. (1980). Biochemical tests for identification of medical bacteria. 2nd edition, Williams and Wilkins, Baltimore.USA.

Mandal, S. and Dutta J., (2009). Integrated Bio-Medical Waste Management Plan for Patna City, Institute of Town Planners, India Journal, **6**: 01-25

Manyele, S. (2004). Medical waste in Tanzania: Current situation and the way forward. *African Journal of Environmental Assessment and Management*, **8**: 74-99.

Mark, C. and Meckest, M. (1982). Effect of UV Light Disinfection on Antibiotic-Resistant Coliforms in Wastewater Effluents. *Applied and Environmental Microbiology*, **43**: 371-377.

Mathur, P., Patan, S. and Shobhawat, S. (2012). Need of biomedical waste management system in hospitals, An emerging issue - A review. *Current World Environment*, **7**: 117-24.

Mesdaghinia, A., Naddafi, K., Nabizadeh, R., Saeedi, R. and Zamanzadeh, M. (2009). Wastewater Characteristics and Appropriate Method for Wastewater Management in the Hospitals. *Iranian Journal of Public Health*, **38**:34-40.

Moges, F., Endris, M. and Belyhun, Y. (2014). Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia, **7**:215-227.

Moore, J., Moore, E. and Millaretal, B. (2010). The presence of antibiotic resistant bacteria long the River Lagan, Agricultural Water Management, **1**:217–221.

Mulamattathil, S., Esterhuysen, F. and Pretorius, J. (2000). Antibiotic-resistant Gram-negative bacteria in a virtually closed water reticulation system,"*Journal of Applied Microbiology*, **88**: 930–937.

Nascimento, T., Januzzi, W., Leonel, M., Silva, L. and Diniz, C. (2009). Occurrence of clinically relevant bacteria in health service waste in a Brazilian sanitary landfill and antimicrobial susceptibility profile. *Review on Societies for Brazilians Medicines Tropical*, **42**: 60-79.

National Committee for Clinical Laboratory standards (2007). Performance standards for antimicrobial susceptibility testing Publishing Cooperation, Minneapolis 17th informational supplement.

Neely, A. and Orloff, M. (2001). Survival of some medically important fungi on hospital fabrics and plastics. *Journal of Clinical Microbiology*, **39**: 3360-3361.

Nemerow, N. (1978). Major Industrial Wastes. In Industrial Waste Pollution, Reading, Massachusetts, Addison Wesley, **11**:305-310.

Ngwuluka, N., Ochekpe, N., Dumosu, P. and John, S. (2009). Waste management in healthcare establishments within Jos Metropolis, Nigeria. *African Journal of environmental sciences tropical*, **3**:459–465.

Noman, E., Al-Gheethi A., Rahman, A., Nagao, H. and Kadir, M. (2016). Assessment of relevant fungal species in clinical solid wastes. *Environmental Sciences and Pollution Research*, **23**: 19806-19824.

Nuñez, L. & Moretton, J. (2007). Disinfectant-resistant bacteria in Buenos Aires city hospital wastewater: *Brazil. Journal of Microbiology*, **38**: 1123-1134.

Nyamogoba, H. and Obala, A. (2002). Nosocomial infections in developing countries: Cost effective control and prevention. *East African Medicines Journal*, **79**: 435-441.

Olowe, O., Olayemi, A., Eniola, K. and Adeyeba, O. (2004). Antibacterial activity of some selected disinfectants regularly used in hospitals. African Journal of Clinical an Expermintal microbiology.

Omar, Faruk SM, Masudul Azad, CAM, Nayeem, UK. (2014). Isolation of Cefixime Resistant *Salmonella* from Hospitals waste and Profiling Multi-drug Resistance Pattern of the Selected isolates. *International Resolution Journal of Biological Sci*ences, **3**: 86-92.

Pathak, S., Gaur, A. and Bhattacherjee, J. (1993). Distribution and antibiotic resistance among aerobic heterotrophic bacteria from rivers in relation to pollution. *Journal of Environmental Sciences and Health Assessments*, **28**:73–87.

Pauwels, B., and Verstraete, W. (2006). The treatment of hospital wastewater. An appraisal. *J. Water Health*, **4**: 405-416.

Radhakrishna, L. and Nagarajan, P. (2015). Isolation and Preliminary Characterization of Bacterial from Liquid Hospital Wastes. International Journal of Pharmacology Technology Restricts, **8**: 308-314.

Robert, W. (2011). Hospital Acquired Infections, Harrisons Principals of Internal Medicine. 16th Edition, **1**:775-781.

Rodney, M. Donlan, J. and William, C., (2002). Biofilms, Survival Mechanisms of Clinically Relevant Microorganisms. *Clinical Microbiology Revision*,**15**: 167–193.

Samarakoon, M. and Gunawardena, N. (2011). An evaluation of health care waste management in base hospitals of Colombo district. *Journal of the College of Community Physicians of Sri Lanka*, **16**: 15-20.

Saurabh, G. and Ram, B. (2006). Report: Biomedical waste management practices at Balrampur Hospital, Lucknow. India Waste Management & Research, **24**:584-591.

Sawalem, M., Selic, E. and Herbell, J. (2009). Hospital waste management in Libya: A case study. *Waste Management*, **29**:1370-5.

Schaeffer, A. and Fulton, M. (1933). A simplified method of staining endospore. Science, **77**: 194-1999.

Schwartz, T., Kohnen, W., Jansen, B. and Obst, U. (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water and drinking water biofilms, *Microbiology and Ecology*, **43**: 325-335.

Sharma, D., Pradhan, B. and Mishra, S. (2010). Multiple drug resistance in bacteria isolates from liquid wastes generated in central hospitals of Nepal, Katheran. *Universal Medical Journal*, **8**: 40-44.

Shields, P. and Cathcart, L. (2012). Motility test medium protocol.

Shlaes, D. (1997). Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance: Guidelines for the prevention of antimicrobial resistance in hospitals. *Infect Control Hospital Epidemiology*, **18**:275–291.

Singh, H., Rehman, R. and Bumb, S. (2014). Management of biomedical waste: a review. *International Journal Dental Medicines Resolution*, **1**:14-20.

Singh, V., Biswas, G. and Sharma, J. (2007). Biomedical Waste Management An Emerging Concern in Indian Hospitals Indian, *Journal of Forensic Medicine &Toxicology*, **1**:12-19.

Smith, K., Besser J., Hedberg C., Leano F., Bender J. and Wicklund J. (1998). Quinolone resistant *Campylobacter jejun* infections in Minnesota, *The New Engineering Journal of Medicines*, **3**:1525-1532.

Smyth R. (2005). Mannitol Salt Agar Cefoxitin Combination as a Screening Medium for Journal of Clinical Microbiology, **43**:3797-9.

Struelens, M. (1998). The epidemiology ofantimicrobial resistance in hospital-acquired infections: problems and possible solutions. *Bomedical Journal*, **317**:652–654.

Suma, G., Mulamattathil C., Bezuidenhout, D., Moses, M. and Njie, A. (2014). Journal of Pathogens: Isolation of Environmental Bacteria from Surface and Drinking Water in Mafikeng, South Africa, and Characterization Using Their Antibiotic Resistance Profiles. Hindawi Publishing Corporation, **21**:111-119.

Sunday, M. and Agbaji, E. (2012): The influence of hospital waste dumps and incinerator ash on the receiving environment, pelagia research library. Advance in applied sciences research; **3**: 2884-2889.

Sunmeet, M. and Gangawane, A. (2017). Knowledge, attitudes and practices of health-care personnel towards biomedical waste disposal management at Arbor Biotech Ltd, Mumbai. *International Journal of Innovative Research in Science and Engineering*; **3**: 307-16.

Susan, J., Rosser, K. and Hilar, K. (1993). Journal of Antimicrobial Chemotherapy, 44: 11–18.

Teshiwal D., Fatuma H., Kassew, A. and Aster, T. (2018). Assessment of Knowledge, Attitude, and Practice about Biomedical Waste Management and Associated Factors among the

Healthcare Professionals at Debre Markos Town Healthcare Facilities, Northwest *Ethiopia.Journal of Environmental and Public Health*, **10**: 132-139.

Tudor, T., Noonan, C. and Jenkin, L. (2005). Healthcare waste management: a case study from the National Health Service in Cornwall United Kingdom. Waste Management, **25**: 606–615.

USEPA, (2007). Stabilization Ponds, Constructed Wetlands, and Other Aquatic System. Onsite Wastewater Treatment Systems Technology, **7**:625-641

Vlkova, E., Rada, V., Popelarova, P., Trojanová, I. and Killer, J. (2006). Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. Livestock Sciences, **105**: 253-259.

Wegener, H, Aarestrup F, Gerner-Smidt P, Bager F. (1999). Transfer of resistant bacteria from animals to man Acta Vet Scand, **92**:51–58.

WHO, (2005). Management of solid healthcare waste at primary healthcare centers. A Decision Making Guide, Geneva.

WHO, (2012). A practical guide Prevention of hospital-acquired infections 2nd edition, World Health Organization, Department of Communicable Disease, Surveillance and Response.

Windfeld, Elliott Steen, Brooks, M.S.-L. (2015). Medical waste management – a review. *Journal of Environmental Management*, **163**:98–108.

Yadav, C., Devkota S. and Aryal S. (2002). Healthcare Waste Management Training Manual for Medical Professionals, Kathmandu: World Health Organization/ Nepal Health Research Council.

Yang, C., Lin M., Liao P., Yeh H., Chang B., Tang T., Cheng C., Sung and Liou M. (2009). Comparison of antimicrobial resistance patterns between clinical and wastewater strains in a regional hospital in Taiwan. *Lettest Applied Microbiology*, **48**:560–565.

Ziebuhr, W., Hennig S., Eckart M., Kr¨anzler H., Batzilla C. and Kozitskaya S. (2006). Nosocomial infections by Staphylococcus epidermidis: How a commensal bacterium turns into a pathogen. *International Journal of Antimicrobial Agents*, **28**:14–20.

APPENDICES

A questionnaire is designed for the purpose of obtaining knowledge about the present waste generation and management strategy being followed in the Jimma University specialized hospitals and determining the various factors which restrict the proper management and disposal of waste being generated in various units at the hospital.

Appendix 1 Socio-demographic Characteristics of the JUSH workers

S.N	Background information	Managers		Professionals		Sanitarians	
		Frequency	%	Frequency	%	Frequency	%
	Sex						
	Female						
1	Male						
	Educational Status						
	Sub specialist						
	Specialist						
	Dr.						
	Nurses						
	Intern						
	Diploma						
	Certificate						
2	Other						
	Department						
	Accident & Emergency						
	Orthopedic						
	Gen. Surgery						
	Specialized Surgical						
	Anesthesia Theatres						
	Rehabilitative Services						
	Dental						
3	Others						
	Experience						
	<6 months						
	6 months - 2 Years						
	3- 4 Years						
4	>5 years						

Appendix 2 Transmission ways of nosocomial infection in JUSH

Ways of nosocomial infection transmission	Yes		No	
	Frequency	%	Frequency	%
Disinfection of white coat				
Population of a patients in the room				
Contact b/n patients and waste baskets				
Presence of re-infected patient				
Hand shaking of patients				
Using masks always				
Specific tool to dispose cough droplets for each patient				
Presence of flies, mosquitoes and ticks in patient room				
BMW common for infectious disease in JUSH context				

Appendix 3 Management and sanitarians response on BMWs handling in JUSH, (Jimma, 2019)

BMWM management or handling	Managements (N=8)		Sanitarians (N=134)	
	Frequency	%	Frequency	%
Waste handlers got training on BWMs				
Yes				
No				
Awareness of Universal Precaution Rule				
Yes				
No				
The hygienic condition of JUSH				
Best				
Good				
Fair				
Means to transport the wastes mostly				
Wheeled Trolleys				
Carts				
Containers				
Enough number of incinerators				
Yes				
No				
Frequency of solid waste incineration per a day				
Once				
Twice				
Three times				
Procedure being applied for the waste Management				
International				
National				
Institutional procedure				
Hand washing facility near the waste depositing site				
Yes				
No				
Having detail knowledge about BMWM				
Yes				
No				
Types of BMWM mainly generated				
Infectious wastes (Blood and body fluids)				
Anatomical wastes (human tissues, body parts)				
Sharp wastes				
Chemical wastes reagents, solvents)				
Pharmaceutical wastes (outdated meds)				
Radioactive wastes and genotoxic wastes				
Papers/Food stuff				
Solid wastes (bandages, gloves and plastics)				

Appendix 4 Morphological and biochemical characterization of the isolates

S/N	Sample	Code	Gram rxn	Shape	Aerobic growth	An Aerobic growth	Endospore	Motility	Catalase rxn	Oxidase rxn	O/F test	Genus or Family level
1	Bandage	JUSH7	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
2	Bandage	JUSH401	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
3	Bandage	JUSH409	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
4	Bandage	JUSH418	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
5	Bandage	JUSH423	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
6	Bandage	JUSH430	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
7	Glove	JUSH446	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
8	Glove	JUSH133	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
9	Glove	JUSH139	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
10	Glove	JUSH145	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
11	Glove	JUSH147	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
12	Glove	JUSH150	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
13	Glove	JUSH153	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
14	Glove	JUSH156	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
15	Glove	JUSH158	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
16	Glove	JUSH164	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
17	Glove	JUSH166	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
18	Glove	JUSH172	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
19	Glove	JUSH175	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
20	Glove	JUSH177	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
21	Glove	JUSH180	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
22	Glove	JUSH183	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
23	Glove	JUSH185	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
24	Lancet	JUSH188	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
25	Lancet	JUSH191	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus

26	Lancet	JUSH193	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
27	Lancet	JUSH195	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
28	Lancet	JUSH197	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
29	Lancet	JUSH199	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
30	Lancet	JUSH204	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
31	Lancet	JUSH206	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
32	Lancet	JUSH470	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
33	Lancet	JUSH480	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
34	Lancet	JUSH489	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
35	Liquid	JUSH359	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
36	Liquid	JUSH367	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
37	Liquid	JUSH377	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
38	Liquid	JUSH385	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
39	Liquid	JUSH394	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
40	Liquid	JUSH497	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
41	Liquid	JUSH504	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
42	Liquid	JUSH509	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
43	Liquid	JUSH512	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
44	Liquid	JUSH520	P	Rod	P	P	P	P/N	P	P/N	N	Bacillus
45	Bandage	JUSH2	P	CCh	P	P	N	N	N	N	N	Entrococcus
46	Bandage	JUSH10	P	CCh	P	P	N	N	N	N	N	Entrococcus
47	Bandage	JUSH13	P	CCh	P	P	N	N	N	N	N	Entrococcus
48	Bandage	JUSH16	P	CCh	P	P	N	N	N	N	N	Entrococcus
49	Bandage	JUSH18	P	CCh	P	P	N	N	N	N	N	Entrococcus
50	Bandage	JUSH20	P	CCh	P	P	N	N	N	N	N	Entrococcus
51	Bandage	JUSH25	P	CCh	P	P	N	N	N	N	N	Entrococcus
52	Bandage	JUSH27	P	CCh	P	P	N	N	N	N	N	Entrococcus
53	Bandage	JUSH32	P	CCh	P	P	N	N	N	N	N	Entrococcus
54	Bandage	JUSH34	P	CCh	P	P	N	N	N	N	N	Entrococcus
55	Bandage	JUSH35	P	CCh	P	P	N	N	N	N	N	Entrococcus
56	Bandage	JUSH37	P	CCh	P	P	N	N	N	N	N	Entrococcus
57	Bandage	JUSH39	P	CCh	P	P	N	N	N	N	N	Entrococcus
58	Bandage	JUSH40	P	CCh	P	P	N	N	N	N	N	Entrococcus
59	Bandage	JUSH42	P	CCh	P	P	N	N	N	N	N	Entrococcus
60	Bandage	JUSH44	P	CCh	P	P	N	N	N	N	N	Entrococcus
61	Bandage	JUSH45	P	CCh	P	P	N	N	N	N	N	Entrococcus

62	Bandage	JUSH47	P	CCh	P	P	N	N	N	N	N	Entrococcus
63	Bandage	JUSH49	P	CCh	P	P	N	N	N	N	N	Entrococcus
64	Bandage	JUSH50	P	CCh	P	P	N	N	N	N	N	Entrococcus
65	Bandage	JUSH52	P	CCh	P	P	N	N	N	N	N	Entrococcus
66	Bandage	JUSH54	P	CCh	P	P	N	N	N	N	N	Entrococcus
67	Bandage	JUSH55	P	CCh	P	P	N	N	N	N	N	Entrococcus
68	Bandage	JUSH58	P	CCh	P	P	N	N	N	N	N	Entrococcus
69	Bandage	JUSH59	P	CCh	P	P	N	N	N	N	N	Entrococcus
70	Bandage	JUSH61	P	CCh	P	P	N	N	N	N	N	Entrococcus
71	Bandage	JUSH62	P	CCh	P	P	N	N	N	N	N	Entrococcus
72	Bandage	JUSH64	P	CCh	P	P	N	N	N	N	N	Entrococcus
73	Bandage	JUSH65	P	CCh	P	P	N	N	N	N	N	Entrococcus
74	Bandage	JUSH67	P	CCh	P	P	N	N	N	N	N	Entrococcus
75	Bandage	JUSH68	P	CCh	P	P	N	N	N	N	N	Entrococcus
76	Bandage	JUSH70	P	CCh	P	P	N	N	N	N	N	Entrococcus
77	Bandage	JUSH71	P	CCh	P	P	N	N	N	N	N	Entrococcus
78	Bandage	JUSH73	P	CCh	P	P	N	N	N	N	N	Entrococcus
79	Bandage	JUSH74	P	CCh	P	P	N	N	N	N	N	Entrococcus
80	Bandage	JUSH75	P	CCh	P	P	N	N	N	N	N	Entrococcus
81	Bandage	JUSH77	P	CCh	P	P	N	N	N	N	N	Entrococcus
82	Bandage	JUSH78	P	CCh	P	P	N	N	N	N	N	Entrococcus
83	Bandage	JUSH80	P	CCh	P	P	N	N	N	N	N	Entrococcus
84	Bandage	JUSH82	P	CCh	P	P	N	N	N	N	N	Entrococcus
85	Bandage	JUSH84	P	CCh	P	P	N	N	N	N	N	Entrococcus
86	Bandage	JUSH86	P	CCh	P	P	N	N	N	N	N	Entrococcus
87	Bandage	JUSH88	P	CCh	P	P	N	N	N	N	N	Entrococcus
88	Bandage	JUSH90	P	CCh	P	P	N	N	N	N	N	Entrococcus
89	Bandage	JUSH92	P	CCh	P	P	N	N	N	N	N	Entrococcus
90	Bandage	JUSH93	P	CCh	P	P	N	N	N	N	N	Entrococcus
91	Bandage	JUSH96	P	CCh	P	P	N	N	N	N	N	Entrococcus
92	Bandage	JUSH97	P	CCh	P	P	N	N	N	N	N	Entrococcus
93	Bandage	JUSH98	P	CCh	P	P	N	N	N	N	N	Entrococcus
94	Glove	JUSH101	P	CCh	P	P	N	N	N	N	N	Entrococcus
95	Glove	JUSH103	P	CCh	P	P	N	N	N	N	N	Entrococcus
96	Glove	JUSH104	P	CCh	P	P	N	N	N	N	N	Entrococcus
97	Glove	JUSH106	P	CCh	P	P	N	N	N	N	N	Entrococcus

98	Glove	JUSH109	P	CCh	P	P	N	N	N	N	N	Entrococcus
99	Glove	JUSH112	P	CCh	P	P	N	N	N	N	N	Entrococcus
100	Glove	JUSH115	P	CCh	P	P	N	N	N	N	N	Entrococcus
101	Glove	JUSH117	P	CCh	P	P	N	N	N	N	N	Entrococcus
102	Glove	JUSH121	P	CCh	P	P	N	N	N	N	N	Entrococcus
103	Glove	JUSH123	P	CCh	P	P	N	N	N	N	N	Entrococcus
104	Glove	JUSH126	P	CCh	P	P	N	N	N	N	N	Entrococcus
105	Glove	JUSH129	P	CCh	P	P	N	N	N	N	N	Entrococcus
106	Glove	JUSH132	P	CCh	P	P	N	N	N	N	N	Entrococcus
107	Glove	JUSH134	P	CCh	P	P	N	N	N	N	N	Entrococcus
108	Glove	JUSH136	P	CCh	P	P	N	N	N	N	N	Entrococcus
109	Glove	JUSH138	P	CCh	P	P	N	N	N	N	N	Entrococcus
110	Glove	JUSH141	P	CCh	P	P	N	N	N	N	N	Entrococcus
111	Glove	JUSH144	P	CCh	P	P	N	N	N	N	N	Entrococcus
112	Glove	JUSH146	P	CCh	P	P	N	N	N	N	N	Entrococcus
113	Glove	JUSH149	P	CCh	P	P	N	N	N	N	N	Entrococcus
114	Glove	JUSH151	P	CCh	P	P	N	N	N	N	N	Entrococcus
115	Glove	JUSH155	P	CCh	P	P	N	N	N	N	N	Entrococcus
116	Glove	JUSH157	P	CCh	P	P	N	N	N	N	N	Entrococcus
117	Glove	JUSH160	P	CCh	P	P	N	N	N	N	N	Entrococcus
118	Glove	JUSH163	P	CCh	P	P	N	N	N	N	N	Entrococcus
119	Glove	JUSH165	P	CCh	P	P	N	N	N	N	N	Entrococcus
120	Glove	JUSH168	P	CCh	P	P	N	N	N	N	N	Entrococcus
121	Glove	JUSH171	P	CCh	P	P	N	N	N	N	N	Entrococcus
122	Glove	JUSH173	P	CCh	P	P	N	N	N	N	N	Entrococcus
123	Glove	JUSH176	P	CCh	P	P	N	N	N	N	N	Entrococcus
124	Glove	JUSH179	P	CCh	P	P	N	N	N	N	N	Entrococcus
125	Glove	JUSH182	P	CCh	P	P	N	N	N	N	N	Entrococcus
126	Glove	JUSH184	P	CCh	P	P	N	N	N	N	N	Entrococcus
127	Glove	JUSH186	P	CCh	P	P	N	N	N	N	N	Entrococcus
128	Lancet	JUSH189	P	CCh	P	P	N	N	N	N	N	Entrococcus
129	Lancet	JUSH192	P	CCh	P	P	N	N	N	N	N	Entrococcus
130	Lancet	JUSH196	P	CCh	P	P	N	N	N	N	N	Entrococcus
131	Lancet	JUSH198	P	CCh	P	P	N	N	N	N	N	Entrococcus
132	Lancet	JUSH201	P	CCh	P	P	N	N	N	N	N	Entrococcus
133	Lancet	JUSH203	P	CCh	P	P	N	N	N	N	N	Entrococcus

134	Lancet	JUSH205	P	CCh	P	P	N	N	N	N	N	Entrococcus
135	Lancet	JUSH208	P	CCh	P	P	N	N	N	N	N	Entrococcus
136	Lancet	JUSH210	P	CCh	P	P	N	N	N	N	N	Entrococcus
137	Lancet	JUSH212	P	CCh	P	P	N	N	N	N	N	Entrococcus
138	Lancet	JUSH214	P	CCh	P	P	N	N	N	N	N	Entrococcus
139	Lancet	JUSH217	P	CCh	P	P	N	N	N	N	N	Entrococcus
140	Lancet	JUSH218	P	CCh	P	P	N	N	N	N	N	Entrococcus
141	Lancet	JUSH220	P	CCh	P	P	N	N	N	N	N	Entrococcus
142	Lancet	JUSH222	P	CCh	P	P	N	N	N	N	N	Entrococcus
143	Lancet	JUSH225	P	CCh	P	P	N	N	N	N	N	Entrococcus
144	Lancet	JUSH227	P	CCh	P	P	N	N	N	N	N	Entrococcus
145	Lancet	JUSH229	P	CCh	P	P	N	N	N	N	N	Entrococcus
146	Lancet	JUSH231	P	CCh	P	P	N	N	N	N	N	Entrococcus
147	Lancet	JUSH233	P	CCh	P	P	N	N	N	N	N	Entrococcus
148	Lancet	JUSH235	P	CCh	P	P	N	N	N	N	N	Entrococcus
149	Lancet	JUSH237	P	CCh	P	P	N	N	N	N	N	Entrococcus
150	Lancet	JUSH239	P	CCh	P	P	N	N	N	N	N	Entrococcus
151	Lancet	JUSH241	P	CCh	P	P	N	N	N	N	N	Entrococcus
152	Lancet	JUSH242	P	CCh	P	P	N	N	N	N	N	Entrococcus
153	Lancet	JUSH244	P	CCh	P	P	N	N	N	N	N	Entrococcus
154	Lancet	JUSH246	P	CCh	P	P	N	N	N	N	N	Entrococcus
155	Lancet	JUSH249	P	CCh	P	P	N	N	N	N	N	Entrococcus
156	Lancet	JUSH251	P	CCh	P	P	N	N	N	N	N	Entrococcus
157	Lancet	JUSH252	P	CCh	P	P	N	N	N	N	N	Entrococcus
158	Lancet	JUSH256	P	CCh	P	P	N	N	N	N	N	Entrococcus
159	Lancet	JUSH257	P	CCh	P	P	N	N	N	N	N	Entrococcus
160	Lancet	JUSH259	P	CCh	P	P	N	N	N	N	N	Entrococcus
161	Lancet	JUSH263	P	CCh	P	P	N	N	N	N	N	Entrococcus
162	Lancet	JUSH265	P	CCh	P	P	N	N	N	N	N	Entrococcus
163	Lancet	JUSH267	P	CCh	P	P	N	N	N	N	N	Entrococcus
164	Lancet	JUSH269	P	CCh	P	P	N	N	N	N	N	Entrococcus
165	Lancet	JUSH272	P	CCh	P	P	N	N	N	N	N	Entrococcus
166	Lancet	JUSH275	P	CCh	P	P	N	N	N	N	N	Entrococcus
167	Lancet	JUSH277	P	CCh	P	P	N	N	N	N	N	Entrococcus
168	Lancet	JUSH279	P	CCh	P	P	N	N	N	N	N	Entrococcus
169	Lancet	JUSH281	P	CCh	P	P	N	N	N	N	N	Entrococcus

170	Lancet	JUSH284	P	CCh	P	P	N	N	N	N	N	Entrococcus
171	Lancet	JUSH286	P	CCh	P	P	N	N	N	N	N	Entrococcus
172	Lancet	JUSH288	P	CCh	P	P	N	N	N	N	N	Entrococcus
173	Lancet	JUSH291	P	CCh	P	P	N	N	N	N	N	Entrococcus
174	Lancet	JUSH294	P	CCh	P	P	N	N	N	N	N	Entrococcus
175	Liquid	JUSH297	P	CCh	P	P	N	N	N	N	N	Entrococcus
176	Liquid	JUSH298	P	CCh	P	P	N	N	N	N	N	Entrococcus
177	Liquid	JUSH301	P	CCh	P	P	N	N	N	N	N	Entrococcus
178	Liquid	JUSH305	P	CCh	P	P	N	N	N	N	N	Entrococcus
179	Liquid	JUSH307	P	CCh	P	P	N	N	N	N	N	Entrococcus
180	Liquid	JUSH309	P	CCh	P	P	N	N	N	N	N	Entrococcus
181	Liquid	JUSH312	P	CCh	P	P	N	N	N	N	N	Entrococcus
182	Liquid	JUSH314	P	CCh	P	P	N	N	N	N	N	Entrococcus
183	Liquid	JUSH317	P	CCh	P	P	N	N	N	N	N	Entrococcus
184	Liquid	JUSH320	P	CCh	P	P	N	N	N	N	N	Entrococcus
185	Liquid	JUSH322	P	CCh	P	P	N	N	N	N	N	Entrococcus
186	Liquid	JUSH326	P	CCh	P	P	N	N	N	N	N	Entrococcus
187	Liquid	JUSH329	P	CCh	P	P	N	N	N	N	N	Entrococcus
188	Liquid	JUSH332	P	CCh	P	P	N	N	N	N	N	Entrococcus
189	Liquid	JUSH334	P	CCh	P	P	N	N	N	N	N	Entrococcus
190	Liquid	JUSH336	P	CCh	P	P	N	N	N	N	N	Entrococcus
191	Liquid	JUSH338	P	CCh	P	P	N	N	N	N	N	Entrococcus
192	Liquid	JUSH340	P	CCh	P	P	N	N	N	N	N	Entrococcus
193	Liquid	JUSH343	P	CCh	P	P	N	N	N	N	N	Entrococcus
194	Liquid	JUSH347	P	CCh	P	P	N	N	N	N	N	Entrococcus
195	Liquid	JUSH349	P	CCh	P	P	N	N	N	N	N	Entrococcus
196	Liquid	JUSH352	P	CCh	P	P	N	N	N	N	N	Entrococcus
197	Bandage	JUSH3	N	Rod	p	p	N	P	P	N	P	Escherichia
198	Bandage	JUSH24	N	Rod	p	p	N	P	P	N	P	Escherichia
199	Bandage	JUSH33	N	Rod	p	p	N	P	P	N	P	Escherichia
200	Bandage	JUSH38	N	Rod	p	p	N	P	P	N	P	Escherichia
201	Bandage	JUSH43	N	Rod	p	p	N	P	P	N	P	Escherichia
202	Bandage	JUSH48	N	Rod	p	p	N	P	P	N	P	Escherichia
203	Bandage	JUSH56	N	Rod	p	p	N	P	P	N	P	Escherichia
204	Bandage	JUSH69	N	Rod	p	p	N	P	P	N	P	Escherichia
205	Bandage	JUSH81	N	Rod	p	p	N	P	P	N	P	Escherichia

206	Bandage	JUSH89	N	Rod	p	p	N	P	P	N	P	Escherichia
207	Bandage	JUSH95	N	Rod	p	p	N	P	P	N	P	Escherichia
208	Bandage	JUSH403	N	Rod	p	p	N	P	P	N	P	Escherichia
209	Bandage	JUSH411	N	Rod	p	p	N	P	P	N	P	Escherichia
210	Bandage	JUSH419	N	Rod	p	p	N	P	P	N	P	Escherichia
211	Bandage	JUSH429	N	Rod	p	p	N	P	P	N	P	Escherichia
212	Glove	JUSH443	N	Rod	p	p	N	P	P	N	P	Escherichia
213	Glove	JUSH450	N	Rod	p	p	N	P	P	N	P	Escherichia
214	Glove	JUSH459	N	Rod	p	p	N	P	P	N	P	Escherichia
215	Glove	JUSH105	N	Rod	p	p	N	P	P	N	P	Escherichia
216	Glove	JUSH108	N	Rod	p	p	N	P	P	N	P	Escherichia
217	Glove	JUSH120	N	Rod	p	p	N	P	P	N	P	Escherichia
218	Glove	JUSH128	N	Rod	p	p	N	P	P	N	P	Escherichia
219	Glove	JUSH137	N	Rod	p	p	N	P	P	N	P	Escherichia
220	Glove	JUSH143	N	Rod	p	p	N	P	P	N	P	Escherichia
221	Glove	JUSH152	N	Rod	p	p	N	P	P	N	P	Escherichia
222	Glove	JUSH161	N	Rod	p	p	N	P	P	N	P	Escherichia
223	Glove	JUSH169	N	Rod	p	p	N	P	P	N	P	Escherichia
224	Glove	JUSH170	N	Rod	p	p	N	P	P	N	P	Escherichia
225	Glove	JUSH178	N	Rod	p	p	N	P	P	N	P	Escherichia
226	Lancet	JUSH190	N	Rod	p	p	N	P	P	N	P	Escherichia
227	Lancet	JUSH200	N	Rod	p	p	N	P	P	N	P	Escherichia
228	Lancet	JUSH207	N	Rod	p	p	N	P	P	N	P	Escherichia
229	Lancet	JUSH224	N	Rod	p	p	N	P	P	N	P	Escherichia
230	Lancet	JUSH232	N	Rod	p	p	N	P	P	N	P	Escherichia
231	Lancet	JUSH240	N	Rod	p	p	N	P	P	N	P	Escherichia
232	Lancet	JUSH248	N	Rod	p	p	N	P	P	N	P	Escherichia
233	Lancet	JUSH255	N	Rod	p	p	N	P	P	N	P	Escherichia
234	Lancet	JUSH264	N	Rod	p	p	N	P	P	N	P	Escherichia
235	Lancet	JUSH273	N	Rod	p	p	N	P	P	N	P	Escherichia
236	Lancet	JUSH280	N	Rod	p	p	N	P	P	N	P	Escherichia
237	Lancet	JUSH289	N	Rod	p	p	N	P	P	N	P	Escherichia
238	Lancet	JUSH476	N	Rod	p	p	N	P	P	N	P	Escherichia
239	Lancet	JUSH485	N	Rod	p	p	N	P	P	N	P	Escherichia
240	Liquid	JUSH296	N	Rod	p	p	N	P	P	N	P	Escherichia
241	Liquid	JUSH304	N	Rod	p	p	N	P	P	N	P	Escherichia

242	Liquid	JUSH311	N	Rod	p	p	N	P	P	N	P	Escherichia
243	Liquid	JUSH319	N	Rod	p	p	N	P	P	N	P	Escherichia
244	Liquid	JUSH327	N	Rod	p	p	N	P	P	N	P	Escherichia
245	Liquid	JUSH333	N	Rod	p	p	N	P	P	N	P	Escherichia
246	Liquid	JUSH337	N	Rod	p	p	N	P	P	N	P	Escherichia
247	Liquid	JUSH345	N	Rod	p	p	N	P	P	N	P	Escherichia
248	Liquid	JUSH348	N	Rod	p	p	N	P	P	N	P	Escherichia
249	Liquid	JUSH355	N	Rod	p	p	N	P	P	N	P	Escherichia
250	Liquid	JUSH363	N	Rod	p	p	N	P	P	N	P	Escherichia
251	Liquid	JUSH372	N	Rod	p	p	N	P	P	N	P	Escherichia
252	Liquid	JUSH381	N	Rod	p	p	N	P	P	N	P	Escherichia
253	Liquid	JUSH387	N	Rod	p	p	N	P	P	N	P	Escherichia
254	Liquid	JUSH395	N	Rod	p	p	N	P	P	N	P	Escherichia
255	Liquid	JUSH492	N	Rod	p	p	N	P	P	N	P	Escherichia
256	Liquid	JUSH500	N	Rod	p	p	N	P	P	N	P	Escherichia
257	Liquid	JUSH508	N	Rod	p	p	N	P	P	N	P	Escherichia
258	Liquid	JUSH516	N	Rod	p	p	N	P	P	N	P	Escherichia
259	Bandage	JUSH5	N	Rod	P	P	N	N	P	N	P	Kliepsiella
260	Bandage	JUSH15	N	Rod	P	P	N	N	P	N	P	Kliepsiella
261	Bandage	JUSH17	N	Rod	P	P	N	N	P	N	P	Kliepsiella
262	Bandage	JUSH26	N	Rod	P	P	N	N	P	N	P	Kliepsiella
263	Bandage	JUSH28	N	Rod	P	P	N	N	P	N	P	Kliepsiella
264	Bandage	JUSH31	N	Rod	P	P	N	N	P	N	P	Kliepsiella
265	Bandage	JUSH87	N	Rod	P	P	N	N	P	N	P	Kliepsiella
266	Bandage	JUSH435	N	Rod	P	P	N	N	P	N	P	Kliepsiella
267	Glove	JUSH465	N	Rod	P	P	N	N	P	N	P	Kliepsiella
268	Glove	JUSH467	N	Rod	P	P	N	N	P	N	P	Kliepsiella
269	Glove	JUSH468	N	Rod	P	P	N	N	P	N	P	Kliepsiella
270	Glove	JUSH102	N	Rod	P	P	N	N	P	N	P	Kliepsiella
271	Glove	JUSH111	N	Rod	P	P	N	N	P	N	P	Kliepsiella
272	Glove	JUSH114	N	Rod	P	P	N	N	P	N	P	Kliepsiella
273	Glove	JUSH119	N	Rod	P	P	N	N	P	N	P	Kliepsiella
274	Glove	JUSH124	N	Rod	P	P	N	N	P	N	P	Kliepsiella
275	Bandage	JUSH8	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
276	Bandage	JUSH397	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
277	Bandage	JUSH405	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas

278	Bandage	JUSH413	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
279	Bandage	JUSH420	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
280	Bandage	JUSH425	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
281	Bandage	JUSH431	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
282	Glove	JUSH441	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
283	Glove	JUSH444	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
284	Glove	JUSH452	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
285	Lancet	JUSH209	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
286	Lancet	JUSH211	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
287	Lancet	JUSH216	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
288	Lancet	JUSH221	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
289	Lancet	JUSH228	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
290	Lancet	JUSH236	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
291	Lancet	JUSH243	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
292	Lancet	JUSH253	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
293	Lancet	JUSH262	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
294	Lancet	JUSH268	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
295	Lancet	JUSH276	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
296	Lancet	JUSH278	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
297	Lancet	JUSH283	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
298	Lancet	JUSH287	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
299	Lancet	JUSH292	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
300	Lancet	JUSH295	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
301	Lancet	JUSH473	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
302	Lancet	JUSH479	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
303	Lancet	JUSH488	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
304	Liquid	JUSH300	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
305	Liquid	JUSH303	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
306	Liquid	JUSH306	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
307	Liquid	JUSH310	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
308	Liquid	JUSH315	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
309	Liquid	JUSH321	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
310	Liquid	JUSH325	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
311	Liquid	JUSH328	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
312	Liquid	JUSH330	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
313	Liquid	JUSH335	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas

314	Liquid	JUSH341	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
315	Liquid	JUSH342	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
316	Liquid	JUSH350	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
317	Liquid	JUSH351	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
318	Liquid	JUSH356	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
319	Liquid	JUSH358	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
320	Liquid	JUSH360	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
321	Liquid	JUSH365	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
322	Liquid	JUSH369	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
323	Liquid	JUSH373	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
324	Liquid	JUSH376	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
325	Liquid	JUSH378	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
326	Liquid	JUSH383	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
327	Liquid	JUSH386	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
328	Liquid	JUSH388	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
329	Liquid	JUSH391	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
330	Liquid	JUSH495	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
331	Liquid	JUSH503	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
332	Liquid	JUSH513	P	Rod	P	N	P	P/N	P	P/N	N	Pseudomonas
333	Bandage	JUSH4	N	Rod	P	P	P	P	P	N	P	Salmonella
334	Bandage	JUSH398	N	Rod	P	P	P	P	P	N	P	Salmonella
335	Bandage	JUSH402	N	Rod	P	P	P	P	P	N	P	Salmonella
336	Bandage	JUSH406	N	Rod	P	P	P	P	P	N	P	Salmonella
337	Bandage	JUSH410	N	Rod	P	P	P	P	P	N	P	Salmonella
338	Bandage	JUSH414	N	Rod	P	P	P	P	P	N	P	Salmonella
339	Bandage	JUSH415	N	Rod	P	P	P	P	P	N	P	Salmonella
340	Bandage	JUSH421	N	Rod	P	P	P	P	P	N	P	Salmonella
341	Glove	JUSH440	N	Rod	P	P	P	P	P	N	P	Salmonella
342	Glove	JUSH445	N	Rod	P	P	P	P	P	N	P	Salmonella
343	Glove	JUSH448	N	Rod	P	P	P	P	P	N	P	Salmonella
344	Glove	JUSH451	N	Rod	P	P	P	P	P	N	P	Salmonella
345	Glove	JUSH456	N	Rod	P	P	P	P	P	N	P	Salmonella
346	Glove	JUSH460	N	Rod	P	P	P	P	P	N	P	Salmonella
347	Lancet	JUSH213	N	Rod	P	P	P	P	P	N	P	Salmonella
348	Lancet	JUSH219	N	Rod	P	P	P	P	P	N	P	Salmonella
349	Lancet	JUSH226	N	Rod	P	P	P	P	P	N	P	Salmonella

350	Lancet	JUSH234	N	Rod	P	P	P	P	P	N	P	Salmonella
351	Lancet	JUSH245	N	Rod	P	P	P	P	P	N	P	Salmonella
352	Lancet	JUSH250	N	Rod	P	P	P	P	P	N	P	Salmonella
353	Lancet	JUSH258	N	Rod	P	P	P	P	P	N	P	Salmonella
354	Lancet	JUSH260	N	Rod	P	P	P	P	P	N	P	Salmonella
355	Lancet	JUSH270	N	Rod	P	P	P	P	P	N	P	Salmonella
356	Lancet	JUSH271	N	Rod	P	P	P	P	P	N	P	Salmonella
357	Lancet	JUSH285	N	Rod	P	P	P	P	P	N	P	Salmonella
358	Lancet	JUSH293	N	Rod	P	P	P	P	P	N	P	Salmonella
359	Lancet	JUSH474	N	Rod	P	P	P	P	P	N	P	Salmonella
360	Lancet	JUSH478	N	Rod	P	P	P	P	P	N	P	Salmonella
361	Lancet	JUSH482	N	Rod	P	P	P	P	P	N	P	Salmonella
362	Lancet	JUSH486	N	Rod	P	P	P	P	P	N	P	Salmonella
363	Lancet	JUSH487	N	Rod	P	P	P	P	P	N	P	Salmonella
364	Liquid	JUSH302	N	Rod	P	P	P	P	P	N	P	Salmonella
365	Liquid	JUSH308	N	Rod	P	P	P	P	P	N	P	Salmonella
366	Liquid	JUSH318	N	Rod	P	P	P	P	P	N	P	Salmonella
367	Liquid	JUSH323	N	Rod	P	P	P	P	P	N	P	Salmonella
368	Liquid	JUSH344	N	Rod	P	P	P	P	P	N	P	Salmonella
369	Liquid	JUSH353	N	Rod	P	P	P	P	P	N	P	Salmonella
370	Liquid	JUSH357	N	Rod	P	P	P	P	P	N	P	Salmonella
371	Liquid	JUSH361	N	Rod	P	P	P	P	P	N	P	Salmonella
372	Liquid	JUSH364	N	Rod	P	P	P	P	P	N	P	Salmonella
373	Liquid	JUSH366	N	Rod	P	P	P	P	P	N	P	Salmonella
374	Liquid	JUSH370	N	Rod	P	P	P	P	P	N	P	Salmonella
375	Liquid	JUSH374	N	Rod	P	P	P	P	P	N	P	Salmonella
376	Liquid	JUSH375	N	Rod	P	P	P	P	P	N	P	Salmonella
377	Liquid	JUSH379	N	Rod	P	P	P	P	P	N	P	Salmonella
378	Liquid	JUSH382	N	Rod	P	P	P	P	P	N	P	Salmonella
379	Liquid	JUSH390	N	Rod	P	P	P	P	P	N	P	Salmonella
380	Liquid	JUSH392	N	Rod	P	P	P	P	P	N	P	Salmonella
381	Liquid	JUSH493	N	Rod	P	P	P	P	P	N	P	Salmonella
382	Liquid	JUSH496	N	Rod	P	P	P	P	P	N	P	Salmonella
383	Liquid	JUSH501	N	Rod	P	P	P	P	P	N	P	Salmonella
384	Liquid	JUSH505	N	Rod	P	P	P	P	P	N	P	Salmonella
385	Liquid	JUSH510	N	Rod	P	P	P	P	P	N	P	Salmonella

386	Liquid	JUSH511	N	Rod	P	P	P	P	P	N	P	Salmonella
387	Liquid	JUSH517	N	Rod	P	P	P	P	P	N	P	Salmonella
388	Bandage	JUSH6	N	Rod	P	P	N	N	P	N	P	Shigella
389	Bandage	JUSH436	N	Rod	P	P	N	N	P	N	P	Shigella
390	Bandage	JUSH437	N	Rod	P	P	N	N	P	N	P	Shigella
391	Bandage	JUSH438	N	Rod	P	P	N	N	P	N	P	Shigella
392	Bandage	JUSH439	N	Rod	P	P	N	N	P	N	P	Shigella
393	Glove	JUSH461	N	Rod	P	P	N	N	P	N	P	Shigella
394	Glove	JUSH462	N	Rod	P	P	N	N	P	N	P	Shigella
395	Glove	JUSH463	N	Rod	P	P	N	N	P	N	P	Shigella
396	Glove	JUSH464	N	Rod	P	P	N	N	P	N	P	Shigella
397	Glove	JUSH466	N	Rod	P	P	N	N	P	N	P	Shigella
398	Glove	JUSH469	N	Rod	P	P	N	N	P	N	P	Shigella
399	Bandage	JUSH1	P	CCl	p	p	N	N	P	N	P	Staphylococcus
400	Bandage	JUSH12	P	CCl	p	p	N	N	P	N	P	Staphylococcus
401	Bandage	JUSH22	P	CCl	p	p	N	N	P	N	P	Staphylococcus
402	Bandage	JUSH29	P	CCl	p	p	N	N	P	N	P	Staphylococcus
403	Bandage	JUSH41	P	CCl	p	p	N	N	P	N	P	Staphylococcus
404	Bandage	JUSH46	P	CCl	p	p	N	N	P	N	P	Staphylococcus
405	Bandage	JUSH51	P	CCl	p	p	N	N	P	N	P	Staphylococcus
406	Bandage	JUSH57	P	CCl	p	p	N	N	P	N	P	Staphylococcus
407	Bandage	JUSH63	P	CCl	p	p	N	N	P	N	P	Staphylococcus
408	Bandage	JUSH72	P	CCl	p	p	N	N	P	N	P	Staphylococcus
409	Bandage	JUSH79	P	CCl	p	p	N	N	P	N	P	Staphylococcus
410	Bandage	JUSH85	P	CCl	p	p	N	N	P	N	P	Staphylococcus
411	Bandage	JUSH94	P	CCl	p	p	N	N	P	N	P	Staphylococcus
412	Bandage	JUSH99	P	CCl	p	p	N	N	P	N	P	Staphylococcus
413	Bandage	JUSH396	P	CCl	p	p	N	N	P	N	P	Staphylococcus
414	Bandage	JUSH399	P	CCl	p	p	N	N	P	N	P	Staphylococcus
415	Bandage	JUSH404	P	CCl	p	p	N	N	P	N	P	Staphylococcus
416	Bandage	JUSH407	P	CCl	p	p	N	N	P	N	P	Staphylococcus
417	Bandage	JUSH412	P	CCl	p	p	N	N	P	N	P	Staphylococcus
418	Bandage	JUSH416	P	CCl	p	p	N	N	P	N	P	Staphylococcus
419	Bandage	JUSH422	P	CCl	p	p	N	N	P	N	P	Staphylococcus
420	Bandage	JUSH424	P	CCl	p	p	N	N	P	N	P	Staphylococcus
421	Bandage	JUSH426	P	CCl	p	p	N	N	P	N	P	Staphylococcus

422	Bandage	JUSH427	P	CCl	p	p	N	N	P	N	P	Staphylococcus
423	Bandage	JUSH428	P	CCl	p	p	N	N	P	N	P	Staphylococcus
424	Bandage	JUSH432	P	CCl	p	p	N	N	P	N	P	Staphylococcus
425	Bandage	JUSH433	P	CCl	p	p	N	N	P	N	P	Staphylococcus
426	Bandage	JUSH434	P	CCl	p	p	N	N	P	N	P	Staphylococcus
427	Glove	JUSH442	P	CCl	p	p	N	N	P	N	P	Staphylococcus
428	Glove	JUSH447	P	CCl	p	p	N	N	P	N	P	Staphylococcus
429	Glove	JUSH449	P	CCl	p	p	N	N	P	N	P	Staphylococcus
430	Glove	JUSH453	P	CCl	p	p	N	N	P	N	P	Staphylococcus
431	Glove	JUSH454	P	CCl	p	p	N	N	P	N	P	Staphylococcus
432	Glove	JUSH457	P	CCl	p	p	N	N	P	N	P	Staphylococcus
433	Glove	JUSH458	P	CCl	p	p	N	N	P	N	P	Staphylococcus
434	Glove	JUSH110	P	CCl	p	p	N	N	P	N	P	Staphylococcus
435	Glove	JUSH118	P	CCl	p	p	N	N	P	N	P	Staphylococcus
436	Glove	JUSH125	P	CCl	p	p	N	N	P	N	P	Staphylococcus
437	Glove	JUSH130	P	CCl	p	p	N	N	P	N	P	Staphylococcus
438	Glove	JUSH140	P	CCl	p	p	N	N	P	N	P	Staphylococcus
439	Glove	JUSH148	P	CCl	p	p	N	N	P	N	P	Staphylococcus
440	Glove	JUSH154	P	CCl	p	p	N	N	P	N	P	Staphylococcus
441	Glove	JUSH162	P	CCl	p	p	N	N	P	N	P	Staphylococcus
442	Glove	JUSH174	P	CCl	p	p	N	N	P	N	P	Staphylococcus
443	Glove	JUSH181	P	CCl	p	p	N	N	P	N	P	Staphylococcus
444	Lancet	JUSH187	P	CCl	p	p	N	N	P	N	P	Staphylococcus
445	Lancet	JUSH194	P	CCl	p	p	N	N	P	N	P	Staphylococcus
446	Lancet	JUSH202	P	CCl	p	p	N	N	P	N	P	Staphylococcus
447	Lancet	JUSH215	P	CCl	p	p	N	N	P	N	P	Staphylococcus
448	Lancet	JUSH223	P	CCl	p	p	N	N	P	N	P	Staphylococcus
449	Lancet	JUSH230	P	CCl	p	p	N	N	P	N	P	Staphylococcus
450	Lancet	JUSH238	P	CCl	p	p	N	N	P	N	P	Staphylococcus
451	Lancet	JUSH247	P	CCl	p	p	N	N	P	N	P	Staphylococcus
452	Lancet	JUSH254	P	CCl	p	p	N	N	P	N	P	Staphylococcus
453	Lancet	JUSH261	P	CCl	p	p	N	N	P	N	P	Staphylococcus
454	Lancet	JUSH266	P	CCl	p	p	N	N	P	N	P	Staphylococcus
455	Lancet	JUSH274	P	CCl	p	p	N	N	P	N	P	Staphylococcus
456	Lancet	JUSH282	P	CCl	p	p	N	N	P	N	P	Staphylococcus
457	Lancet	JUSH290	P	CCl	p	p	N	N	P	N	P	Staphylococcus

458	Lancet	JUSH471	P	CCl	p	p	N	N	P	N	P	Staphylococcus
459	Lancet	JUSH475	P	CCl	p	p	N	N	P	N	P	Staphylococcus
460	Lancet	JUSH477	P	CCl	p	p	N	N	P	N	P	Staphylococcus
461	Lancet	JUSH481	P	CCl	p	p	N	N	P	N	P	Staphylococcus
462	Lancet	JUSH483	P	CCl	p	p	N	N	P	N	P	Staphylococcus
463	Lancet	JUSH484	P	CCl	p	p	N	N	P	N	P	Staphylococcus
464	Lancet	JUSH490	P	CCl	p	p	N	N	P	N	P	Staphylococcus
465	Liquid	JUSH299	P	CCl	p	p	N	N	P	N	P	Staphylococcus
466	Liquid	JUSH313	P	CCl	p	p	N	N	P	N	P	Staphylococcus
467	Liquid	JUSH316	P	CCl	p	p	N	N	P	N	P	Staphylococcus
468	Liquid	JUSH324	P	CCl	p	p	N	N	P	N	P	Staphylococcus
469	Liquid	JUSH331	P	CCl	p	p	N	N	P	N	P	Staphylococcus
470	Liquid	JUSH339	P	CCl	p	p	N	N	P	N	P	Staphylococcus
471	Liquid	JUSH346	P	CCl	p	p	N	N	P	N	P	Staphylococcus
472	Liquid	JUSH354	P	CCl	p	p	N	N	P	N	P	Staphylococcus
473	Liquid	JUSH362	P	CCl	p	p	N	N	P	N	P	Staphylococcus
474	Liquid	JUSH371	P	CCl	p	p	N	N	P	N	P	Staphylococcus
475	Liquid	JUSH380	P	CCl	p	p	N	N	P	N	P	Staphylococcus
476	Liquid	JUSH384	P	CCl	p	p	N	N	P	N	P	Staphylococcus
477	Liquid	JUSH389	P	CCl	p	p	N	N	P	N	P	Staphylococcus
478	Liquid	JUSH393	P	CCl	p	p	N	N	P	N	P	Staphylococcus
479	Liquid	JUSH491	P	CCl	p	p	N	N	P	N	P	Staphylococcus
480	Liquid	JUSH494	P	CCl	p	p	N	N	P	N	P	Staphylococcus
481	Liquid	JUSH498	P	CCl	p	p	N	N	P	N	P	Staphylococcus
482	Liquid	JUSH499	P	CCl	p	p	N	N	P	N	P	Staphylococcus
483	Liquid	JUSH502	P	CCl	p	p	N	N	P	N	P	Staphylococcus
484	Liquid	JUSH506	P	CCl	p	p	N	N	P	N	P	Staphylococcus
485	Liquid	JUSH507	P	CCl	p	p	N	N	P	N	P	Staphylococcus
486	Liquid	JUSH514	P	CCl	p	p	N	N	P	N	P	Staphylococcus
487	Liquid	JUSH515	P	CCl	p	p	N	N	P	N	P	Staphylococcus
488	Liquid	JUSH518	P	CCl	p	p	N	N	P	N	P	Staphylococcus
489	Liquid	JUSH519	P	CCl	p	p	N	N	P	N	P	Staphylococcus
490	Bandage	JUSH9	P	CCh	P	P	N	N	N	N	P	Streptococcus
491	Bandage	JUSH11	P	CCh	P	P	N	N	N	N	P	Streptococcus
492	Bandage	JUSH14	P	CCh	P	P	N	N	N	N	P	Streptococcus
493	Bandage	JUSH19	P	CCh	P	P	N	N	N	N	P	Streptococcus

494	Bandage	JUSH21	P	CCh	P	P	N	N	N	N	P	Streptococcus
495	Bandage	JUSH23	P	CCh	P	P	N	N	N	N	P	Streptococcus
496	Bandage	JUSH30	P	CCh	P	P	N	N	N	N	P	Streptococcus
497	Bandage	JUSH36	P	CCh	P	P	N	N	N	N	P	Streptococcus
498	Bandage	JUSH53	P	CCh	P	P	N	N	N	N	P	Streptococcus
499	Bandage	JUSH60	P	CCh	P	P	N	N	N	N	P	Streptococcus
500	Bandage	JUSH66	P	CCh	P	P	N	N	N	N	P	Streptococcus
501	Bandage	JUSH76	P	CCh	P	P	N	N	N	N	P	Streptococcus
502	Bandage	JUSH83	P	CCh	P	P	N	N	N	N	P	Streptococcus
503	Bandage	JUSH91	P	CCh	P	P	N	N	N	N	P	Streptococcus
504	Bandage	JUSH100	P	CCh	P	P	N	N	N	N	P	Streptococcus
505	Bandage	JUSH400	P	CCh	P	P	N	N	N	N	P	Streptococcus
506	Bandage	JUSH408	P	CCh	P	P	N	N	N	N	P	Streptococcus
507	Bandage	JUSH417	P	CCh	P	P	N	N	N	N	P	Streptococcus
508	Glove	JUSH455	P	CCh	P	P	N	N	N	N	P	Streptococcus
509	Glove	JUSH107	P	CCh	P	P	N	N	N	N	P	Streptococcus
510	Glove	JUSH113	P	CCh	P	P	N	N	N	N	P	Streptococcus
511	Glove	JUSH116	P	CCh	P	P	N	N	N	N	P	Streptococcus
512	Glove	JUSH122	P	CCh	P	P	N	N	N	N	P	Streptococcus
513	Glove	JUSH127	P	CCh	P	P	N	N	N	N	P	Streptococcus
514	Glove	JUSH131	P	CCh	P	P	N	N	N	N	P	Streptococcus
515	Glove	JUSH135	P	CCh	P	P	N	N	N	N	P	Streptococcus
516	Glove	JUSH142	P	CCh	P	P	N	N	N	N	P	Streptococcus
517	Glove	JUSH159	P	CCh	P	P	N	N	N	N	P	Streptococcus
518	Glove	JUSH167	P	CCh	P	P	N	N	N	N	P	Streptococcus
519	Lancet	JUSH472	P	CCh	P	P	N	N	N	N	P	Streptococcus
520	Liquid	JUSH368	P	CCh	P	P	N	N	N	N	P	Streptococcus

Where; CCl= Coccus (clusters), CCh =Coccus (chains), P= positive, N= negative and JUSH = Jimma University Specialized Hospital.

Appendix 5 Antimicrobial susceptibility pattern of standard bacteria

Antimicrobial agent	Drug potency	Standard bacteria				
		E. coli (ATCC25922)	*S. aurues* (ATCC25923)	*K. pneumonia* (ATCC700603)	*S. typhimurium* (ATCC13311)	*P.auroginosa* (ATCC27253)
Ampicillin (AMP)	30	R	S	R	R	R
Chloramphenicol (C)	5	S	S	S	S	S
Ciprofloxacin (CIP)	5	R	S	S	R	S
Gentamycin (CN)	15	R	S	S	S	R
Kanamycin (K)	10	S	S	R	R	R
Naldixic Acid (NA)	30	S	S	S	R	S
Norflaxacin (NOR)	10	S	S	S	S	S
Streptomycin (S)	10	R	S	S	S	R
Tetracycline (TE)	30	R	R	S	R	S
Clindamycin (DA)	2	R	S	R	S	R
Erythromycin (E)	15	R	R	R	R	R
Penicillin G (P)	10	R	R	R	R	R

Where, R=resistant and S= susceptible

NB: Intermediate zone was accepted as resistant or susceptible based on its nearer value for the sake of analysis.

Appendix 6 The minimum and maximum mean counts of all samples

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
AMB	Bandage	20	6.2485	.26753	.05982	6.1233	6.3737	5.54	6.53
	Lancet	20	5.9200	.83288	.18624	5.5302	6.3098	4.19	6.55
	Glove	20	6.2305	.72965	.16316	5.8890	6.5720	4.18	7.29
	Liquid	20	6.5405	.28003	.06262	6.4094	6.6716	6.02	6.91
	Total	80	6.2349	.61624	.06890	6.0977	6.3720	4.18	7.29
Entro	Bandage	20	6.2025	.24253	.05423	6.0890	6.3160	5.55	6.50
	Lancet	20	5.8310	.74512	.16661	5.4823	6.1797	4.07	6.34
	Glove	20	6.0565	.74469	.16652	5.7080	6.4050	3.42	6.69
	Liquid	20	6.2020	.78151	.17475	5.8362	6.5678	2.95	6.64
	Total	80	6.0730	.67179	.07511	5.9235	6.2225	2.95	6.69
Coliform	Bandage	20	6.1560	.21503	.04808	6.0554	6.2566	5.51	6.37
	Lancet	20	5.3065	.82474	.18442	5.5205	6.2925	3.29	6.45
	Glove	20	5.9370	.74714	.16707	5.5873	6.2867	3.50	6.38
	Liquid	20	6.0295	.95726	.21405	5.5815	6.4775	2.04	6.49
	Total	80	6.0073	.73410	.08207	5.8439	6.1706	2.04	6.49
Staph	Bandage	20	6.1345	.21360	.04776	6.0345	6.2345	5.52	6.35
	Lancet	20	5.3805	.81343	.18189	4.9998	5.7612	3.43	6.22
	Glove	20	5.7320	.93567	.20922	5.2941	6.1699	3.31	6.26
	Liquid	20	5.9860	.94365	.21101	5.5444	6.4276	2.07	6.43
	Total	80	5.8083	.82315	.09203	5.6251	5.9914	2.07	6.43
Moulds and yeast	Bandage	20	6.1260	.15243	.03409	6.0547	6.1973	5.52	6.28
	Lancet	20	5.4765	.78383	.17527	5.1097	5.8433	3.26	6.27
	Glove	20	5.5215	1.05285	.23542	5.0288	6.0142	3.20	6.32
	Liquid	20	5.9140	1.03513	.23146	5.4295	6.3985	1.56	6.33
	Total	80	5.7595	.86736	.09697	5.5665	5.9525	1.56	6.33

Appendix 7 ANOVA analysis of all isolates from all samples

		Sum of Squares	Df	Mean Square	F	Sig.
AMB	Between Groups	3.855	3	1.285	3.735	.015
	Within Groups	26.145	76	.344		
	Total	30.000	79			
Entro	Between Groups	1.845	3	.615	1.382	.255
	Within Groups	33.808	76	.445		
	Total	35.652	79			
Coliform	Between Groups	.754	3	.251	.457	.713
	Within Groups	41.819	76	.550		
	Total	42.573	79			
Staph	Between Groups	6.536	3	2.179	3.524	.019
	Within Groups	46.992	76	.618		
	Total	53.528	79			
Moulds and yeast	Between Groups	5.899	3	1.966	2.791	.046
	Within Groups	53.535	76	.704		
	Total	59.433	79			